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# 34th R3-Nordic Contamination Control Symposium

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# 34TH R3-NORDIC CONTAMINATION CONTROL SYMPOSIUM

Turku, Finland, June 2–4, 2003

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# PREFACE

$R^3$-Nordic, the Nordic Association for Contamination Control, is a non-profit, independent association for the promotion of new technologies in contamination control in the Nordic countries. The venue of the annual symposium is Turku Polytechnic, which is in the Turku Science Park. This area is the core area for innovative action in bioscience in the Turku region, it is also called BioTurku.

The aim of the annual $R^3$-Nordic Symposium is to provide knowledge of contamination control and clean room technology dealing with topics in the pharmaceutical, food and microelectronic industries. The topics at the 34th $R^3$-Nordic Contamination Control Symposium are:

- contamination control
- clean room technology and management
- regulations and standards in clean rooms
- clean room clothing
- isolation applications
- $R^3$ technology and air handling
- environmental monitoring in production
- process design
- production hygiene
- cleanability
- cleaning and disinfection
- risk assessment
- risk management in packaging material production
- quality systems
- contamination control
- occupational safety
- production of pharmaceuticals, biopharmaceuticals, biomedicines and vaccines.

The persons involved in the Programme Committee are Raimo Pärssinen, Irma Isotalo, Jaakko Lenkkeri, Antti Mikkola, Eva Sairio, Satu Salo and Gun Wirtanen. Jarmo Saari was involved in the organisation before he got his new job to Germany. The editors of the proceedings as well as the whole Programme Committee would like to express their gratitude to the speakers for preparing the abstracts published in the January issue (32 [2003] 1:18–24) of the journal

Renhetsteknik as well as the full papers or extended abstracts published in these proceedings. We wish that this event will be fruitful in giving new ideas to all participants.

*Programme Committee*

# *Välkommen ! – Tervetuloa ! – Welcome !*

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## SYMPOSIUM PROGRAMME

# PLENARY SESSION ON CONTAMINATION CONTROL

# ASEPTIC PROCESSING AND STERILITY ASSURANCE – RE-EXAMINATION OF FAMILIAR TERMS AND THEIR RELEVANCE IN PROCESS CONTROL AND PRODUCT SAFETY

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## Abstract

There is a lack of scientific clarity and consistency in the manner in which the word *sterile* is used in the pharmaceutical industry. The word *sterile* is used to characterize both products manufactured by aseptic processing as well as those manufactured by terminal sterilization. Nearly two decades ago there was an active debate concerning the appropriateness of using the word *sterile* in conjunction with products that were manufactured aseptically. However, over the years industry and the regulatory community have failed to consider the important technical distinction that exists between aseptic manufacturing and terminal sterilization. Many people, including supposed experts, have taken the position that it was essential to prove absolute sterility in aseptic processing. This has resulted in some unfortunate regulatory policy and in some cases these policies may have unwittingly decreased rather than increased product safety and process control. It is time to ask if a new understanding of the word sterile is necessary for industry and our regulators to avoid technical and scientific pitfalls. The term sterility assurance, for example, has quite possibly outlived its usefulness. Perhaps with respect to aseptic processing it would be advantageous to encourage regulatory guidelines and standards to be written with a goal of end-user safety in mind rather than using sterility. Safety is an attribute that can be quantified and which can be assessed using various risk analysis methods in conjunction with hard data from end-user experience with aseptically manufactured product. Sterility on the other hand is an absolute which can not be effectively and unequivocally proved in aseptic processing.

# 1. The fundamental problem of measurement of the attribute known as “sterility”

There is a widely held belief that we must be able to determine whether or not an aseptically manufactured product lot is sterile. Unfortunately, sterility can be termed a negative absolute and there is, realistically, no way to prove that microbes are unequivocally absent from a product even those that are terminally sterilized. In a roundtable discussion at a PDA meeting a number of years, former FDA staff member and PDA Executive Director Ed Fry, commented that while it was understood that microbes may be found in environmental monitoring and that the occasional media fill positive was likely, from a legal perspective each and every unit in each and every lot was expected to be sterile.

Certainly, Mr. Fry’s statement was and still is valid in many respects. The USP Sterility Test is the reference test for sterility in the United States and should a FDA laboratory requisition samples from a given lot of product, and subject those units to the sterility test the expectation is that each of those product units must pass the sterility test. So, we could summarize by saying that in the event any unit in any lot is tested for sterility, it must be sterile or the entire lot will be considered non-sterile from a regulatory/legal perspective.

Of course, realistically most lots are subjected only to a lot release sterility test in which consists of 20 units drawn from throughout the lot. The statistical implications of such a limited number of samples regarding the actual ability to prove sterility are well known, and require no further comment here. Clearly, the expectation is that each unit subjected to a sterility test, whether in a lot release assay or a reference test must be sterile. This expectation fits with the legal requirement that each tested container in each lot must be sterile. However, since the sterility test is destructive and cannot be applied to each container in each lot, we have to infer that all the units in each lot would be sterile if subjected to the sterility test. In other words we have to make a scientifically informed value judgment.

It seems reasonable to ask whether or not the sterility test is capable of proving sterility on a unit by unit basis. In order to unequivocally prove sterility, the statistical limitations inherent in the sterility test notwithstanding, the test would have to be able to find as few as one viable organism of any type in any tested

unit. However, the growth promotion tests done on media and the bacteriostasis and fungistasis tests done to validate the sterility test call for inoculation levels considerably higher than one. This is because there is no effective way to prepare an inoculum that is known, beyond doubt, to contain a single organism. Therefore, it is quite likely that the sterility test does not have sufficient resolution to detect the presence of a single organism.

However, even if it did have that sensitivity with respect to certain organisms, it would be quite a different matter altogether to demonstrate that a sterility test had the ability to detect one viable cell of any known genera and species. The sterility test utilized general purpose media, presumably because the originators of the test wisely realized that it's true value lies in proving that a preparation is microbiological pure enough, rather than absolutely sterile. For example, a product could contain a million plaque forming units of influenza virus per dose and still pass the pharmacopeial sterility test. Thus, the widely discussed sampling statistical limitations of the sterility test represent only one problem. The sterility test is also limited with respect to its ability to reliably detect low levels of microorganisms and it is further limited with respect to its ability to recover all potentially viable to say nothing of pathogenic organisms that could be present.

It seems obvious then that while the ability to pass a sterility test either at the time of product release or in a post release laboratory evaluation is a clear legal requirement, the ability of a lot to pass this test does not prove that the product is microbiologically sterile, only that it is legally sterile. It is quite likely in fact, that a wise and capable attorney, armed with some knowledge of microbiology and medicine and supported by well-informed expert witnesses could quite effectively argue that there is no way to prove or disprove that any unit in any lot is truly sterile. It is simply not possible to prove the complete absence of something in a material.

Therefore, what should be our objective if we prove absolute sterility? It seems logical that the answer must be safety. In order to better understand how we might approach the measurement of safety, let us look at a few important definitions.

# 2. Sterility, viability, pathogenicity and virulence

These four terms are well-understood by infectious disease specialists and medical microbiologists, but it is possible that they are less well understood by workers the health care product manufacturing industry, and by those who regulate these industries. I think it is equally clear that many individuals who attempt to write microbiological standards for pharmaceutical manufacturing do not understand these concepts as well as they might. I submit that without a clear understanding of these terms and related concepts it is impossible to conduct risk analysis with respect to microbiological safety.

When asked to define sterility and aseptic, pharmaceutical scientists including microbiologists typically give a variety of answers. In most cases, pharmaceutical scientists appear to consider the words sterile and aseptic to be synonymous. Actually, aseptic and sterile have distinctly different meanings, in fact, there are very clear scientific distinctions between these words. Only when we acknowledge the obvious technical differences between these words will be find a path to more rational standard setting and regulation.

## 2.1 Sharp's Axioms

Recently, Mr. John Sharp in an article in the European Journal of Parenteral and Pharmaceutical Sciences drew the following conclusions, which he termed "basic axioms". The axioms he proposed are:

1. Sterility is the complete absence of living organisms.
2. Sterility is an absolute. There are no degrees of sterility.
3. A distinction between sterile products and aseptic products is an invalid one. The term sterile may properly be applied to a product. Aseptic is one that is applied to a process.
4. It is not pathogenic organisms alone that can cause harm. Patients can be, and have been seriously damaged by the injection of organisms normally considered to be non-pathogenic.
5. Great care must be taken to exclude, remove or destroy *all* microorganisms, with the highest level of probability.
6. Aseptic processing is not a method of sterilization.

7. The concept of “sterility assurance level” has no meaning in the context of aseptic processing.
8. Whenever possible, sterile products should be terminally sterilized.

I believe that Mr. Sharp has managed to accurately portray the regulatory position that has evolved over the years regarding aseptic processing. Unfortunately, this position is convoluted, in many ways contradictory, and nearly impossible to implement. In order to understand these issues more clearly let us examine the underlying principles from a microbiological perspective.

## 2.2 A critical look at microbiological concepts pertinent to sterile product manufacturing

Frequently when we discuss sterile and aseptic I hear it said that the differences in meaning between these words are of a strictly semantic nature, in case of Mr. Sharp’s article he claims that asepsis is a process while sterility may be applied to a product. One definition found in Webster New Collegiate Dictionary for general semantics is, “The language used in political propaganda or advertising to achieve a desired effect on an audience.” This is exactly what I believe the conventional regulatory positions regarding both sterility and asepsis have done, and as an unfortunate side effect they have managed to distort the legal requirements as well. My objective today is not to propagandize the audience but rather to facilitate a common understanding. In order to accomplish the goal of common understanding we must examine the scientific nature of microbial contamination and risk.

It is vital if we are to have harmonized and universally applicable standards and we as an industry must first agree upon a common lexicon. The 1987 *FDA Guideline on Sterile Drug Products Produced by Aseptic Processing and the recent “concept paper”* imply in their title that aseptic processing can achieve a truly sterile result. Let us examine this implication further by looking at the definitions microbiologists apply to the word “sterile”.

Not surprisingly, that while there is substantial agreement on the meaning of the word sterile, even the definition of this supposedly absolute term is not without conflict. In Jawetz, Melnick and Adelberg’s Medical Microbiology sterile is defined as; “*Free of life of every kind. Sterilization may be accomplished by*

*filtration (in the case of liquids or air) or by treatment with microbiocidal agents. Since the criterion of death for microorganisms is the ability to reproduce, sterile material may contain intact metabolizing microbial cells."* It can be argued that the author of this definition is having it both ways since they clearly state sterile means free of life and then go on to define a condition in which metabolizing organisms are present. I prefer to conclude that the author has simply realized that the continuation of life requires the ability to reproduce. However, one can see the problem this definition poses for the detection of a sterile condition. If metabolic activity alone is not an indicator of non-sterility this suggest that test methods that rely on biochemical activity alone cannot prove or disprove that living (reproducing) organisms are present. It is inarguably true that no organism lacking the ability to reproduce ever initiating an infection.

Zinsser's Medical Microbiology test defines sterilization as *"an absolute (term) that implies the total inactivation of all forms of microbial life in terms of the organism's ability to reproduce."* These authors perhaps wisely, steered clear of the metabolism issue and simply based their definition upon the ability to reproduce.

Microbiology by Davis et al, a common text used in graduate microbiology and medical schools defines sterile thusly; *"The use of either physical or chemical agents to eliminate all viable microorganisms."* This definition avoids the debate about metabolism and growth but introduces the term viability instead. Viability is then defined as the, "ability of the organism to propagate indefinitely." Hence, their definition is once again based upon the ability of an organism to reproduce.

Although other microbiology texts and even dictionaries offer slightly different definitions of sterility, all in are general agreement that the destruction of an organism's ability to replicate is central to the concept of sterility. This is logical, because as previously noted organisms that lack the ability to reproduce are incapable of causing an infectious disease. Interestingly, it follows from this line of reasoning that viable but non-culturable organisms are in fact sterile, unless they exist in conditions in which they can grow. The description "viable but non-culturable" is an oxymoron unless one expands the definition of viable to include metabolic activity, which would is not appropriate because an organism lacking the ability to replicate can neither be recovered and grown nor cause infection.

Thus, metabolic activity alone is an unreliable indicator of either the attribute of viability or the condition of sterility.

The ruling out of metabolic activity as an indicator of viability and thus sterility is essential. Among the vast array of microorganisms that live in the environment and cause human and animal disease are a large number of obligate intracellular parasites, most notably viruses. These parasites are lack metabolic activity and take over the metabolic and macromolecular synthesis machinery of their host in order to effect replication. Therefore, a material could be both free of detectable growth on all bacterial or mycological media, without metabolic activity and still be non-sterile.

If we consider the term sterility a bit more critically, we can see that the definition while absolute could be situational. While viable organisms may be present in a material, they would only be able to replicate if appropriate growth conditions were present. In terms of infectious disease the term "virulence" is used to describe the ability of an organism or virus to replicate in a susceptible host and cause infection. Virulence is according to Zinsser's Microbiology, *"is a quantitative measure of the degree of pathogenicity of a particular microorganism. Virulence is usually measured by the numbers of micro-organisms necessary to kill or alter a particular organism under standardized test conditions."*

Virulence continues to be, an area of very active research within the general study of host-parasite relationships. Virulence depends not only upon the route of exposure and the number of organisms to which a host is exposed, but also upon the general health of the organism studied. Virulence is far too complicated a subject to be covered in depth in a short article, but some general concepts can be introduced. First, of the many thousands of microorganisms known only about 300 have been reported to have the ability to produce disease. Second, Finlay and Falkow reported in Microbiological Reviews that intradermal inoculation of healthy volunteers with virulent bacteria could require as many as 5–10 million organisms to initiate an infection. Even extremely virulent organisms such as *Franscisella tularensis* may require several organisms to initiate infection. Studies on viruses have shown that typically several hundred to several thousand infectious virions are required to initiate an infection. It must be noted that the organism, even in a weakened host, faces a very hostile environment. Thus, it is not surprising that for example, that last years attacks

with *B. anthracis*, even though they involved millions of specially purified spores designed to disseminate in the environment, produced relatively little morbidity and mortality.

These data concerning microbial virulence do not mean that the healthcare industry in general or the pharmaceutical industry specifically can or should take a cavalier attitude toward the dangers of microbial contamination. Rather, they suggest that it is possible for industry and government to take a more scientific and realistic approach to risk analysis and control. The question that remains is what control requirements are scientifically logical and appropriate for aseptically manufactured products?

There is considerable ambivalence in the regulations concerning the ability of "aseptic" manufacturers to product "sterile" products. For example, while numerous statements can be found in government regulations and guidelines that a target of zero contamination in media fills is appropriate, one cannot find any guideline or standard that completely rules out the likelihood that some contaminated units may, at least occasionally be found. Contamination, must of course indicate the potential of a non-sterile outcome in production, or put another way actual practice.

In Technical Report No. 22 entitled *Process Simulation Testing for Aseptically Filled Products* it is stated in the section on acceptance criteria and interpretation of results that, *"Despite the number of units filled during a process simulation test or the number of positives allowed the ultimate goal for the number of positives for any process simulation should be zero." A sterile product is, after all one that contains no viable organisms. There are, however, numerous technical problems in achieving this goal…. Thus, a limit of some low number other than zero is often chosen."* The PDA position is reasonable and by and large scientifically defensible in that it recognizes that a media fill test cannot really demonstrate the complete absence of viable organisms; it can only show that there are not enough organisms present to initiate growth on the chosen medium.

Of course media fills form only one of the lines of proof required to demonstrate adequate process control in aseptic processing. Environmental monitoring forms another supporting leg and one that has taken on more significance in recent years. Increasingly, an attitude that environmental monitoring can be used as a

kind of secondary sterility test has come to the fore. This attitude has been manifested not only in inspectional outcomes, but to one degree or another in proposed guidelines and standards. Of course, environmental monitoring has the same limitations; albeit somewhat different in nature, as sterility testing and media fill testing. Like medial fill testing and sterility testing, environmental monitoring is dependent upon growth and recovery of viable organisms. Data indicate that there is considerable variability associated with environmental sampling and that this variability is the result, of among other things, sensitivity, spectrum of recovery, sampling efficiency and perhaps worst of all adventitious contamination. Environmental monitoring, like sterility testing, relies on its own set of aseptic manipulations and therefore must have a persistent and largely unknown level of adventitious contamination.

## 3. Measurement of process safety

In order to gain what are hoped to be better insights into aseptic manufacturing process control, industry has been pressured to dramatically increase environmental monitoring while at the same time lowering alert and action levels. The notion behind this pressure seems to be that more monitoring results in an improve ability to ensure sterility. Because, monitoring requires more interventions into the critical zone, industry is faced with conflicting requirements and expectations. On the one hand, we are told that all interventions must be challenged in media fill studies, presumably because of the risk they entail to asepsis. However, on the other hand industry is told to intervene more frequently into the critical zone in an effort to better recover very low levels of contamination.

Unfortunately, available data on environmental monitoring method implies that the variability inherent in these tests, whether done on surfaces or air, may range from 2–10 fold. It is also likely that the sensitivity of these assays is >1cfu/unit of measure. This means two things:

1. There is likely no significant difference between an acceptance criterion of 1 cfu and one of say 4 cfu.
2. It is likely that these tests cannot detect very low levels of contamination and certainly cannot detect the full spectrum of microorganisms including viruses.

Significantly, no current clean room or isolator environmental standard for aseptic processing considers that the environment can really be sterile. All current standards recognize that some low level microbial contamination is likely. In the author's opinion, all standards for contamination levels in aseptic environments should be based upon incidence rates rather than absolute values. Class 5 (EU Class A) environments in modern clean rooms can achieve incidence rates of less than 1% in actual operation. The number of colonies isolated is from a measurement science perspective rather minor, the real measure is the ability to maintain a low incidence of contamination recovery at any level. The present alert and action level system has no real scientific merit and should be abandoned. A logical replacement is a system that looks chiefly at the rate at which any contamination is observed.

It must be said though those since cleanrooms and even isolators are not sterile; it is illogical to think that products made in those environments are provably sterile. It is not possible for industry scientists to prove the negative absolute of sterility under any circumstance of aseptic processing. Therefore, proving state-of-the-art process control must be enough. If we chose to take action against product based upon the discovery of any organism at all, then we are simply playing a game of chance in our environments. Bottom line we cannot prove sterility, therefore we must show that our processes have a validated state-of-control without the constraints of the measurement tools currently available.

## 4. Sharp's Axioms revisited

Another look at Mr. Sharp's Basic Axioms is now possible (the author's comments are italicized):

1. Sterility is the complete absence of living organisms
   *Living can only be measured by the ability to reproduce, organisms that can't reproduce cannot form colonies and cannot cause infections.*
2. Sterility is an absolute. There are no degrees of sterility.
   *Sterility may in fact be an absolute, but all the measures of sterility on our industry are based upon risk and probability. Even in the validation of autoclaves we claim that an acceptable level of performance is one contaminant in a million, which of course falls short of absolute sterility. Even in terminal sterilization we can't prove the absolute absence of*

*contamination. Suggesting that we can do so implies that we have a complete understanding of the nature and resistance of all microorganisms that may inhabit the planet.*

3. A distinction between sterile products and aseptic products is an invalid one. The term sterile may properly be applied to a product. Aseptic is one that is applied to a process.

   *Upon critical examination this statement is incoherent. If there is no distinction to be made between aseptically produced and terminally sterilized products than what possible reason is there to favor one process over the other? The distinction Mr. Sharp attempts to draw between product and process is illogical. How is it possible to expect a process that is aseptic to manufacture products that are all unequivocally sterile, even if we did have a means of truly measuring sterility which of course we do not.*

4. It is not pathogenic organisms alone that can cause harm. Patients can be, and have been seriously damaged by the injection of organisms normally considered to be non-pathogenic.

   *The ability of organisms to produce disease is to some degree situational. Hence, some organisms can be characterized as opportunistically pathogenic. Also, virulence varies in ways that is not always predictable. A further consideration that must be considered in assessing microbiological risk is the ability of organisms to survive in a product. Very low levels of contaminants, even when rather virulent can rarely establish infection, even in a compromised host. Available safety data on injectable products indicates that very high levels of safety are achieved when products are made according to current GMPs.*

5. Great care must be taken to exclude, remove or destroy *all* microorganisms, with the highest level of probability.

   *This statement emphasizes the destruction/exclusion of "all" microorganisms then acknowledges in the next clause that this can only be demonstrated to some level of probability. This statement says we must be able to demonstrate this absence of microorganisms "with the highest level of probability". However, what exactly does that mean? This is the sort of regulatory wording that leads to rather unreasonable expectations, for example, the complete absence of 5um particles because of the belief they may be associated with microbial contamination. Industry needs clear guidance regarding what process control requirements should be established and how they can be verified*

*over time. Setting an expectation of zero is unrealistic, unscientific and uneconomic.*

6. Aseptic processing is not a method of sterilization.
   *This is a statement of the obvious, but what should also be acknowledged is that since aseptic processing is done in non-sterile environments a sterile outcome can never be proven. The measure of effectiveness of asepsis must be safety not sterility. It is unreasonable to expect industry to prove that which cannot be proven.*
7. The concept of "sterility assurance level" has no meaning in the context of aseptic processing.
   *This statement is valid, but one is left to ponder what it means in relation to axiom five above in which Mr. Sharp clearly implies that we must demonstrate the removal of all microorganisms to some undefined "highest level of probability"*
8. Whenever possible, sterile products should be terminally sterilized.
   *This is reasonable and prudent position. However, the number of products that must be produced aseptically will likely continue to increase over time. Also, drug delivery systems that cannot be terminally sterilized may have safety and societal advantages that ought not to be overlooked.*

# 5. Conclusions

The concept of sterility is intrinsically linked to an organism's ability to reproduce. It is the ability to reproduce that enables an organism to grow and establish infection. The understanding of sterility and asepsis that drives our regulations is often confused and the resulting regulatory expectations may not reflect actual product safety realities. It is not possible to prove that aseptically produced products are sterile and it is not possible to know that each unit in each lot is indeed sterile. In fact, it is not logical to think that it is possible to manufacture provably sterile products in an environment that is known to be non-sterile. Regulations should remove legal tensions by being true to the realities of microbiology and measurement science. Industry will be better served by risk and scientific based regulations that recognize sterility cannot be proven in aseptic processing by any of the techniques we have at our disposal including environmental monitoring, media fill testing, or sterility testing. The idea that environmental monitoring can serve as a de facto sterility test is

potentially damaging in that it will result in the rejection of safe product and may put processes at greater risk by requiring greatly increased levels of interventions for the purpose of undertaking monitoring activities. The recent FDA initiatives toward science and risk based regulation should be encouraged as they give industry and regulators the opportunity to develop standards that reflect the realities of measurement and microbiological safety.

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# SESSION ON CONTAMINATION CONTROL IN FOOD PROCESSING AND BIOTECHNOLOGY

# NORDIC CO-OPERATION IN DAIRY HYGIENE – DAIRYNET

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## Abstract

Development of detection and identification methods for assessing microbial and organic soil on surfaces, in air, on packaging and in raw material as well as products are needed to ensure that the results obtained are reliable and repeatable. In an industrial point of view it is important to maintain and extend the interactive contacts between persons dealing with safety and environmental questions in Nordic dairies. The aim of this Nordic dairy hygiene network project, DairyNET – Hygiene control in dairies, is to provide reliable methods for validating and improving cleaning and hygiene in process scale at dairies by applying new knowledge and by using available laboratory and pilot scale equipment in the experimental set-ups. Nordic Industrial Fund, national technology agencies as well as industrial and research partners are funding this network.

The Finnish research started with mapping the air, CIP water and ice water quality in a yoghurt manufacturing dairy. The initial findings showed that the air quality within the controlled environments must be monitored regularly, some of the routines must also be changed to improve the air within the equipment. Cleaning trials in pilot scale is being carried out for optimisation of the parameters in the ultrasound cleaning planned for cleaning milk crates. In the Danish national project the work will mainly focus on sanitising effects and design of hygienic equipment and process flow as parts of hygiene management. The Icelandic project will study the sources and contamination routes for *Listeria* spp. in raw milk samples during different seasons. Samples are also collected from various dairy products and from the production environment. Possible isolates found in the survey will be characterised and tested against sanitizers. In Sweden a checklist for the hygiene in cheese production facilities defining every process step including the function, perquisites, objectives,

methods for measurement and documentation and deviation steps has been processed. The quality of water and air, as well as the effect of aerosols in spreading microbes will be investigated. Studies on existing cleaning routines and innovative solutions in order to prevent contamination during cleaning/ production as well as the efficiency of washing equipment in reducing biofilms will also be studied. Milk from five dairies in Norway using two different disinfection systems will be sampled from one silo-tank through the line to pasteurisation. The occurrence of vegetative and spore-forming bacteria in the milk samples, swabbing samples and on gaskets will be determined. The isolates will be tested for disinfectant resistance and adhesion ability.

# 1. Introduction

The control of hygiene is essential for providing wholesome and safe food products to consumers. The intention of dairies is to minimise the risk of spoiled foodstuffs caused by pathogens or other harmful microbes. The requirements for improved hygiene have risen due to the development of the food industry, including prolonged shelf life, centralised production and long-distance transportation, automated cleaning systems, reduced cleaning time and demands for environmentally friendly cleaning agents. The hygiene of surfaces, instruments and equipment in the dairies are important to the quality of the final product. The surface material and the properties of the surface materials, such as smoothness and cracks, as well as the design of the equipment are very important aspects in preventing the hygiene problems. For instance old conveyer belts can be contamination sources because they collect easily dirt and nutrients. In dairy industry the reasons for microbial problems are often caused by poor equipment design. Also the awareness of the routes for contamination in the dairy is very important. One way of preventing this is hygienic zones. All routes are not known, therefore there should be more investigation in this area. The effect of cleaning is based on chemicals, mechanical forces, time and temperature. Shortening of the washing time and reducing the flow rate decreases the effect of cleaning decidedly. The cleaning programs and habits, detergents and disinfectants used and cleaning utensils must be chosen carefully. Information on microbial resistance against cleaning chemicals as well as chemicals embedded in materials used in product contact and process environments e.g. flooring materials is needed to make correct material choice in equipment and processing facilities. Also attention should be paid on the quality

of process water, steam, pressure air, contaminants in the cleaning system and equipment etc. The good quality of the final product can be maintained and improved with educated and motivated staff, effective and well designed equipment and cleaning programs, right detergents and disinfectants as well as optimal temperatures (Troller, 1993; Anon., 2000; Walker *et al*., 2000).

## 2. Background of the project

The processing of dairy products is continuously changing. This means that there has been, is and will be a constant need for developing new measures in hygiene assessment. The opinion of representatives in the Nordic dairies is that the network is useful as a discussion forum dealing with the theory in the research of practical tasks. It has been built-up in the two subsequent NORDFOOD projects P93156 ”Sanitation in dairies” (1994–1996) and P96049 "Evaluation of cleaning agents and disinfectants for use in dairies: methods and mechanisms" (1997–2000). The results have been published by the project group in the books Sanitation in dairies and Evaluation of sanitation procedures for use in dairies as well as in various book chapters (Wirtanen *et al.,* 1997, 2000, 2002).

## 3. Project structure

In DairyNET-project a project group representing all 5 Nordic countries dairies extended with material producers as well as equipment designers and producers of detergents and diagnostic kits will continue the networking with Nordic research partners involved in cleaning and hygienic design. Figure 1 shows the partners in this project group, which represent dairies (TINE, Valio Ltd., Arla Foods, Milko Mejerier, Norrmejerier, Skåne-mejerier and Norðurmjólk), detergent manufacturers (Mjöll and JohnsonDiversey), material and equipment producers (Lagafors Fabriks AB, Tetra Pak Nordic Processing and Finnsonic Oy), a producer of diagnostic kits (Orion Corp. Orion Diagnostica), universities (BioCentrum-DTU and Royal Institute of Technology) as well as hygiene researchers (VTT Biotechnology, Biotechnological Institute, SIK, Matforsk and IFL). In an industrial point of view the maintenance of interactive contacts between persons dealing with hygiene questions in Nordic dairies is needed alongside the hygiene research. This Nordic project is a co-operation between independent national research or product development projects in Denmark,

Finland, Iceland, Norway and Sweden. Results achieved in the national projects are discussed at the network meetings held 2–3 times a year. The synergy tasks between the national projects are performed by all groups (Figure 2).

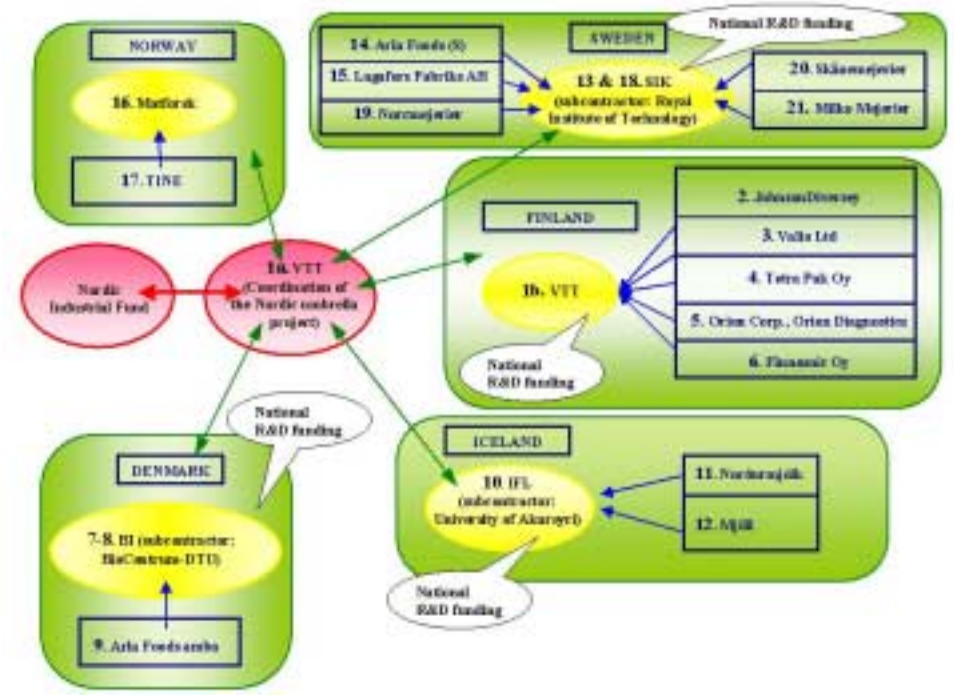


*Figure 1. The partners in the Nordic dairy hygiene network.*

# 4. Project activities

## 4.1 National projects

In the national projects soiling system for validating cleaning in pilot scale is developed. Procedures and methods obtained from other processes is also being transferred into the dairy processing e.g. heat exchangers, ice water systems and supply systems for cleaning. The cleaning will be assessed using both available detection methods and new applications used both in research and in processing.

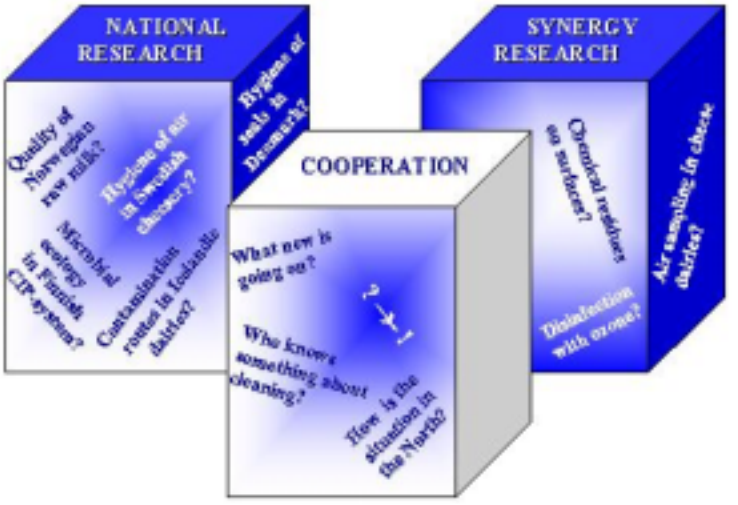


*Figure 2. Building blocks of the tasks in the Nordic DairyNET-project.*

Results from cleaning procedures tested in pilot and full scale in soiling experiments in open and closed pilot scale equipment can be used for planning of optimal cleaning parameter combinations in processing facilities. Further development of detection and identification methods for assessing microbial soil on processing equipment, in air, on packaging material, in raw material and in products are also needed to ensure that the results obtained are reliable. Implementation of new procedures e.g. cleaning systems and detection methods for both equipment and utensils will be carried out in the dairy plants. National research studies reported during this symposium are given in Table 1.

## 4.2 Synergy project

The planned synergy tasks performed in all national groups are: detecting detergent and disinfectant residues, soil detecting with ultraviolet light lamp and detection of airborne fungi using metabolic indicators (Figure 2). The aim of this project is to provide reliable methods for validating and improving cleaning and hygiene in process scale at dairies by applying new knowledge and by using available laboratory and pilot scale equipment in the experimental set-ups. The final results of the first synergy task were discussed at the meeting in April 2003 in Turku. The results are presented in the presentation “Detergent and disinfectant residue testing with photobacteria” at this symposium.

*Table 1. National DairyNET research studies reported at this symposium.*

| National project | Title | Speaker |
|---|---|---|
| Danish | Evaluating hygiene in closed systems – state of the art | A. Friis, DTU, Denmark |
| | The virtual cleaning test – is it possible? | B. Jensen, DTU, Denmark |
| Finnish | Tank cleaning studies using CFD – a case study | S. Salo, VTT Finland |
| | An ultrasonic washing system and its applicability in cleaning of returnable plastic crates | I. Klemetti, Valio Ltd., Finland |
| Icelandic | The source and contamination routes for *Listeria monocytogenes* in the dairy industry | J. Örlygsson, IFL, Iceland |
| Norwegian | Resistance phenomena in dairies due to disinfection | S. Langsrud, Matforsk, Norway |
| Synergy | Detergent and disinfectant residue testing with photobacteria | J. Lappalainen, Aboatox, Finland |

## 4.3 Co-operation and networking

The various forms of communication between the project partners are national and Nordic project meetings, dairy personnel meetings, personal contacts and the confidential internet pages provided by Nordic Industrial Fund. In an industrial point of view it is important to maintain and extend the interactive contacts between persons dealing with safety and environmental questions in Nordic dairies alongside the hygiene research. The Nordic project meetings are co-ordinated by VTT Biotechnology. During the two first years of the DairyNET-project there has been five project meetings. The project partners have by now visited TINE in Oslo, SIK in Gothenburg, IFL in Akureyri, Biotechnological Institute in Kolding and JohnsonDiversey in Turku. National research studies are reported and presented during these project meetings. The dairy representatives involved in this project have met in own meetings in conjunction with the project meetings.

# 5. Project highlights

The partners in the national projects of the Nordic DairyNet-project develop procedures and methods for checking the cleaning efficiency in pilot scale. The aim is to transfer improved procedures and methods into the dairy processes. These subjects as well as summaries of each national project are given below:

- HACCP systems (DK)
- checklist for the hygiene control in cheese dairies (SE)
- air quality in various types of dairies (FIN, SE)
- survey of CIP, ice and process water quality (FIN, SE)
- sanitising effect of different CIP-procedures (DK)
- hygiene in supply systems (IS)
- milk quality through the whole process line (N)
- contamination routes for *Listeria* spp. (IS)
- innovative cleaning systems (FIN, SE)
- ultrasound cleaning of milk transportation packages (FIN)
- cleaning of heat exchanger surfaces (FIN)
- design principles of hygienic seals (DK)
- bacterial resistance to disinfectants (DK, N) and
- microbial adhesion and biofilm formation (N, SE).

## 5.1 Danish project "Sanitation in closed dairy systems"

The research in the Danish national project during 2001–2002 has mainly been performed within the project Centre for Hygienic design and cleaning (HYDEKO). The work has focused on studying the effect of surface structure on biofilm formation in process equipment. At the meeting in Kolding a lecture entitled “Hygienic design and flow modelling of food processing equipment” was given. Based on this practical work concerning hygiene design of tank systems will be performed in co-operation with Finnish researcher.

## 5.2 Finnish project "Hygiene in dairy equipment and processing facilities"

Cleanability studies of a cheese conveyor line using ultrasound cleaning have been continued from the preceding project. The dairy in Lapinlahti has invested

in an ultrasound prototype line. The cleaning parameters for the ultrasound cleaning will be set based on experience from the old system to fulfil the required cleaning level. Ultrasound cleaning has also been tested in the packaging department of a milk dairy as a master thesis work. The findings are also presented in the paper A ultrasonic washing system and its applicability in cleaning of returnable plastic crates. Furthermore, computational fluid dynamics (CFD) has been applied to predict cleanability of various parts in tank systems in order to improve the hygienic design.

## 5.3 Icelandic project "Hygiene in supply systems and contamination routes"

In the dairy sources and contamination routes for *Listeria* spp. were examined. In this sampling all (200) product samples were negative for *Listeria* spp., only some samples from dairy environment tested positive in the screening phase. In screening of raw milk from 143 farms all summer samples were negative but 13 winter samples were contaminated with *Listeria* spp. The isolates are being characterised and tested against sanitizers. The hygiene in the supply system of the milk processing department was tested for microbes as well as for chemical residues. The temperture during the cleaning phase was measured as a function of time.

## 5.4 Norwegian project "The influence of dairy hygiene on raw milk quality"

Five dairies using different cleaning procedures are involved in hygiene survey. The effect of different CIP-methods on the dairy hygiene as well as raw milk quality has started in the Norwegian project. This is performed based on visual and microbial control of the hygiene in these dairies. The occurence of different bacteria (e.g. psykrotrophs and spore formers) is mapped. Recommendations and actions based on these results will be implemented in TINE dairies. The study of the microbial ecology will continue during the project. These strains will be tested for disinfectant resistance and adhesion ability.

### 5.5 Swedish project "Milk plant hygiene in open areas"

Hygiene investigations are being performed at four cheese plants. The microbial level in air, in water and on process surfaces was studied. In the air measurements the flow, temperature and humidity was also studied. Water samples have been taken at these cheese plants from foam systems, high-pressure equipment, water hoses, and water basins. A sampling method for detection of protein residues after cleaning will be developed during the project. Documented hygiene routines at a dairy with low contamination will be spread to other participating dairies The result will be confirmed in a follow up hygiene investigation.

## 6. Acknowledgement

We express our gratitude to the partners in the DairyNET project for the active participation in the project activities. This work is financially supported by Nordic Industrial Fund as well as by national technology funding bodies, which is gratefully acknowledged.

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# AN ULTRASONIC WASHING SYSTEM AND ITS APPLICABILITY IN CLEANING OF RETURNABLE PLASTIC CRATES

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## Abstract

The hygiene of equipment surfaces plays an important role in dairy manufacture. Especially the environment in which products are packed can easily get contaminated. Returnable transport packages or crates can be one source of contamination. The cleaning of these packages is often incomplete and pathogens and spoilage organisms attached to surfaces can easily survive trough the washing process

Hygiene of returnable plastic crates cleaned in washing tunnel was determined in Valio Ltd. Herajoki plant. The cleaning parameters and the microbiological quality of washing liquid were monitored. Using artificially soiled crate pieces were performed the pilot-scale studies with ultrasonic washing system. Design of Experiment (DOE, Taguchi method) was used to optimise the cleaning procedure.

According to the results the bottom parts of the crates were the dirtiest parts with the worst cleaning results. Temperature, duration of washing and washing liquid quality varied much in the washing tunnel. Pilot tests showed that the sodium hydroxide (NaOH) containing EDTA was the most efficient detergent in the ultrasonic washing system. The cleaning time of 100 s and the 5 % concentration of washing liquid gave the best cleaning effects. The ultrasonic cleaning method is applicable for cleaning of returnable plastic crates and it stands comparison with the washing tunnel method.

# 1. Introduction

Returnable transportation packages like plastic trays, wooden/plastic pallets, plastic containers, crates, cages etc. can be a hygiene risk in food industry. They often are difficult to clean because of their construction. Lange *et al.* (1999) found that plastic trays with simple construction and smooth surface were easiest to get clean. Trays with rugged and cracked surfaces were hard to control for cleanliness and returnability after cleaning (Parkes, 1988). There are some limitations in cleaning procedure (e.g. temperature, detergents and pressure) because of materials (Kane *et al.*, 2001a, 2001b). Returnable transportation packages can also be hard soiled or contaminated by pathogens, particularly if they are misused.

Cleaning by ultrasonics has been known and used for a long time commercially in certain specific areas where the level of cleanliness is high, e.g. electronics and optics manufacturing, aviation and maintenance. The first ultrasonic cheese mould washing system was introduced in 1986. Goods to be cleaned are immersed in the liquid and subjected to a high ultrasonic intensity developed by ultrasonic transducers. Small, underpressurized bubbles are generated by cavitation. Those bubbles collide with material surfaces and finally collapse with pressure shocks of up to 1 000 bar. This is the mechanical energy, which is removing the dirt. As long as the goods to be cleaned are submerged, ultrasonics is effective at every point of liquid penetration (Kivelä, 1996). The cleaning result depends on the amplitude and the frequency of the ultrasound as well as the depth and the temperature of the washing solution, the cleaning time and detergent and the target material (Heino, 2000).

The aim of this study was first to make a literature study of traditional and new cleaning methods for returnable transportation packages e.g. plastic crates and trays used in supply chain of fresh milk products. The next step was to make pilot studies of ultrasonic cleaning of artificial soiled and contaminated plastic crates. And the third aim was to find out the hygiene status of different cleaning processes – washing tunnel used at Herajoki dairy plant and ultrasonic cleaning (Arpiainen, 2002).

# 2. Materials and methods

## 2.1 Industrial scale tests

The hygiene quality of plastic crates (materials: HDPE high-density polyethene; blue; external dimensions 430 mm x 340 mm x 280 mm) was monitored at the Herajoki dairy plant. The washing tunnel with two lines (Oy Hackman Ab, Koltek, Finland) has been installed at the beginning of the 70's. Altogether 140 crates were investigated visually and by Hygicult® method (Orion Corporation Orion Diagnostica, Finland) and swabbing before and after washing tunnel. Also new, unused crates were washed to find out if contaminated by the washing liquid in the washing tunnel. 70 returned and unwashed crates were analysed for *Listeria* using traditional method (7473AYF) and Vidas analyser at Valio's microbiological laboratory.

Washing liquid (1 % concentration, pH 13, alkali based combined detergent obtained from Orion Corporation Noiro Farmos Tecnochemicals) used in the washing tunnel was monitored for soiling with the COD method (Chemical Oxygen Demand; ready-to-use cuvette by LCK 514, DrLange, Düsseldorf, Germany) and for microbiological quality with Drycult® (Orion Corporation Orion Diagnostica, Finland) and Petrifilm™ as well as by spread plating. Temperature of washing liquid and total cleaning time of a crate were registered in three different cleaning periods. The cleaning procedure of the washing tunnel consists of pre rinsing, cleaning and after rinsing sections.

## 2.2 Pilot scale tests

In the pilot cleaning tests the wall section of some crates was cut into pieces (10 cm x 12 cm). The pieces were contaminated with NordFood soil (Wirtanen *et al.*, 1997) in combination with either *A. niger* (VTT D-81078) + *E. coli* (VTT E-97836) bacteria or *B. cereus* spores (VTT E-96727) + *L. innocua* (VTT E-98101) bacteria. The contaminated pieces were washed in the ultrasonic cleaner (m40, FinnSonic Ltd., Finland) with ultrasonic frequency of 30 kHz and ultrasonic power of 600 W. Cleaning agents and concentrations and cleaning programmes were variable parameters in the tests (Figure 1).

The survived bacteria combination was enumerated by selective agars. ATP method (HY-LITE®) was also used. Some whole, NordFood soiled crates were also washed in a bigger ultrasonic cleaner (volume 300 litres with two ultrasonic elements, FinnSonic Ltd., Finland).

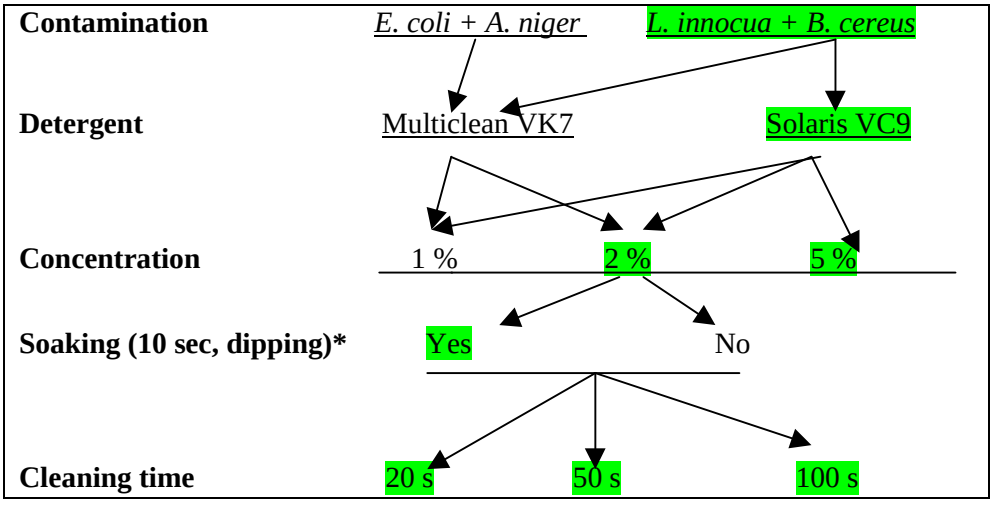


*Figure 1. Cleaning procedures in pilot ultrasonic cleaning tests of crate pieces. * Soaking with either 2 % Multiclean VK7 (< 5 % anionic tensides, pH 12,5) or 5 % Solaris VC9 (15–30 % NaOH, < 5 % EDTA, pH 12,5) depending on the concentration of the detergent (Suomen Unilever Oy DiveserseyLever, Finland). Green coloured parameters were chosen when cleaning whole crates.*

Design of Experiment (DOE, Taguchi method) was used to find out the best cleaning procedure. Whole crates were contaminated, washed and controlled as previous mentioned. The experiment consisted of 11 factors in 2 levels; total number of experiments was 12 with 3 trials per experiment (Table 1). After calculation of experiment results (averages, analysis of variation and S/N ratio) the main effects analysis (S/N response tables) can be done and the best cleaning procedure can be confirmed using confirmation tests.

*Table 1. Description of experiment. Standard Orthogonal Array Model used: L12 ($2^{11}$).*

| Label | Description of factor | Level 1 | Level 2 |
|---|---|---|---|
| A | Soaking, detergent | Detergent (5 %) | SU989 Backdisk (1 %) |
| B | Soaking, time | 10 s | 30 s |
| C | Soaking, temperature | 10°C | 20°C |
| D | Standing time before cleaning | 10 s | 30 s |
| E | Cleaning, temperature | 45°C | 60°C |
| F | Cleaning, detergent | Multiclean | Solaris |
| G | Cleaning, concentration | 2 % | 5 % |
| H | Cleaning, time | 50 s | 100 s |
| I | After rinsing, agent | Water | A8 Sumabrite Zerospots |
| J | After rinsing, temperature | 10°C | 20°C |
| K | After rinsing, time | 10 s | 30 s |

# 3. Results and discussion

The literature study gave only some wool. It seems not to be interesting to evaluate cleaning degree of returnable transportation packages.

The hygiene status of the washing tunnel at Herajoki dairy plant was as follows:

- Temperature of the washing liquid ranged +32 – + 60° C being lowest when milk packaging was most hectic.
- Total cleaning time of a crate ranged 40 sec – over 7 min being 80 s on the average.
- Total bacteria counts of the washing liquid ranged 100–300 cfu/ml and there was no difference between the detection methods.
- New, unused crates were contaminated during washing in the tunnel.
- Returned unwashed crates were visually evaluated into three dirtiness categories: soiled by milk, soiled by dust and ground and visually clean; the crates soiled by dust and ground had the lowest cleaning degree, 30.5 % (on the average 60 %).
- External walls of crates were cleaned better than internal walls; bottoms were the dirtiest parts with the worst cleaning result; yeast and moulds were easily washed out.
- *Listeria monocytogenes* was found in one heavy soiled crate and *Listeria innocua* in three crates.

These results pay attention to the need of continuous control of the automatic refreshing and concentration of the washing liquid. Cleaning parameters should be constant. When investing a new cleaning system for returnable crates these results should be taken into serious consideration. It is a true risk to get *Listeria* with the returnable transportation packages inside a dairy plant. The level of cleanliness depends on the use of returnable transportation packages – if they are in direct contact with the product or if they carry packed product (Colditz & Sowa, 1997). According to the zoning of dairy production the packaging process of milk products demands the strictest hygiene requirements. Returnable transportation packages like plastic trays and crates and cages should be stored and completed in green zone area with less strict hygiene requirements (Heggum *et al.*, 1997). This means minor requirements for their cleanliness but at least they should be visually clean.

In the pilot tests the vegetative bacteria cells were easily detached by ultrasonic cleaning method, but *Bacillus* spores were still left on the washed crate surfaces. ATP measures correlated well with the count of rest bacteria cells. The cleaning effect of Solaris VC9 (NaOH and EDTA based detergent) used as 2 or 5 % concentration with 100 sec cleaning time and without any pre dissolving was better than the cleaning effect of Multiclean VK7 used as 1 or 2 % concentration. According to DOE the washing temperature + 45 °C was better than + 60 °C in the ultrasonic washing system.

# 4. Conclusions

According to the results obtained hygienic classification of washed returnable crates can be determined. To improve the washing results cleaning parameters should be constant. The ultrasonic cleaning method is applicable to cleaning of returnable plastic crates and is a noteworthy method compared to the washing tunnel method.

# 5. Acknowledgement

This work is financially supported by Nordic Industrial Fund as well as by Tekes, the National Technology Agency of Finland, which is gratefully acknowledged.

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# EVALUATING HYGIENE IN CLOSED PROCESS SYSTEMS – STATE OF THE ART

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## Abstract

Computational Fluid Dynamics (CFD) was found to be applicable to evaluate the design of closed process equipment with respect to cleanability. Specifically CFD was used to simulate hydrodynamic characteristics relevant for cleaning. The characteristics were compared to cleaning trials using the certified test method for closed equipment established by the European Hygienic Engineering and Design Group (EHEDG). Simulations show zones in process equipment, which were difficult to clean and thus undesired flow patterns were identified. The results obtained can be used for validation of cleaning as well as for design and redesign of process equipment and will set new standards for hygienic design of equipment. The effect of flow and promotion of desired flow patterns were discussed especially in relation to fully three-dimensional flow, turbulence, unsteady flows, recirculation zones, and other relevant aspects.

## 1. Introduction

Cleaning In Place (CIP) procedures are used throughout the food industry as the only practical way to clean closed process equipment. However, investigations concerning the influence of the hydrodynamics of flow on the cleaning of surfaces in the food industry exposed to real life conditions still lack attention. Furthermore validation techniques are limited to non-invasive methods mainly based on analysis of the rinse water from the cleaning procedure. Such tests do not yield much information about the final hygienic state of the inside of process equipment. In reality we know what we have removed but not what's left in side.

The interest in investigating the influence of fluid flow on hygienic aspects of closed processing equipment is evident when reviewing the literature. Many

study flow parallel to surfaces in order to quantify the effect of hydrodynamics on biofilm formation and mechanisms of microbial adhesion and removal. Such studies employs laminar flow under uni-axial flow conditions. The conclusion is that the wall shear stress is a controlling factor and hence so-called critical wall shear stresses are reported for specific microorganisms on selected surfaces (Duddridge et al., 1982, Powell and Slater, 1982, Fowler, 1980).

The flow patterns observed in industrial applications are seldom similar to uni-axial flows. Normally a higher degree of complexity is found and in many cases flow patterns needs to be considered fully three-dimensional due to turbulence and build-up and break down of flow patterns. Furthermore time effects can play a role yet other flow patterns are unsteady either on a bulk or local level. Examples of poor cleaning are reported due to insufficient fluid exchange and recirculation zones in up-stands, dead-ends, heat exchangers, expansions or contractions (EHEDG, 1992, Anon, 1997, Grasshoff, 1980 and 1992 and Hauser, 1989). These examples can all be considered complex geometries, however a complex geometry is not necessarily causing problems with respect to cleaning. In the housing of a Mix-Proof valve (MPV) recirculation zones are found along with very low wall shear stresses (much below those reported necessary to remove biological material in uni-axial flow), however cleaning is found to be efficient in practical applications (Jensen et al., 2000).

To validate cleaning-in place (CIP) and hygienic design of process equipment test methods are applied. Such a method is developed by the European Hygienic Engineering and Design Group (EHEDG) (EHEDG, 1992) other methods are in-house tests with equipment manufactures for example utilising removal of visible residues from transparent equipment. Such equipment allows for visualisation of both the flow pattern and the removal process on a surface.

The advantage of a simulation over experimental testing is that time can be saved and the number of prototypes can be reduced. The application of flow simulation to assist in design of process equipment and prediction of cleaning efficiency has been suggested previously by Hall, 1998, Hauser et al., 2000 and Jensen et al., 2000. Flow simulation using Computational Fluid Dynamics (CFD) has been used for decades to describe the bulk parameters of flow in process plants. However, describing hydrodynamic parameters close to or at walls is not the traditional intended application. This is due to the fact that the

resolution near the wall requires very much attention. Development of the proper CFD model near the wall is explained in Jensen, 2003.

# 2. Materials and methods

Three different flow cases were studied. A Mix-Proof valve (MPV) were used to show the importance of swirl effects in the flow and the role of wall shear stress in removal of residues from walls. In the second case flow in an up-stand is applied to establish a connection between fluid exchange and EHEDG test results. The last case compares flow patterns in pipe expansions to the nature of re-circulation zones. A mean velocity of 1.5 m/s was used in all simulations assuming fully developed turbulent flow up-stream of the part simulated. The Reynolds numbers in the inlet pipe weres 150,000 for the MPV and up-stand geometry and 70,000 for the expansions. Properties of water at 60°C were used to represent the fluid used in cleaning tests.

The EHEDG test applied is described in detail in EHEDG, 1992. In brief the method consists of the following steps. Soiling with a mixture of Bacillus stearothermophilus var. Calidolactis and sour milk followed by 4 hours of drying with air, rinsing for 1 min with cold water, alkaline CIP for 10 min with 63°C 1% detergent solution, a rinse step for 1 min with cold water. The fluid velocity was 1.5 m/s in the inlet and reference pipe in all steps. In the last step the component was filled with Shapton and Hindes agar and incubated for 20 hours at 58°C. Remaining spores on the surfaces shows as yellowish colouration on the purple agar.

# 3. Hydrodynamics and cleaning efficiency

## 3.1 Mix-Proof valve

The flow field in the MPV were measured in a transparent model valve using Laser Doppler Anemometry yielding quantitative results for velocity components. Furthermore the flow patterns were qualitatively visualised using a laser sheet technique. This proved at the flow in the MPV was modelled in good agreement with the experimental results. When entering the sphere shaped house of the MPV the flow quickly develops from the fully developed turbulent flow

profile in the inlet tube into a fully three-dimensional flow pattern. High velocities are found near the centre of the valve house causing the majority of the fluid to pass through the valve here. As expected a recirculation is found immediately down stream of the stem. Furthermore recirculations were found on either side of the stem in the height of the valve house. The recirculations build up near the entrance and disappear again at the exit of the valve house. Therefore these zones were considered dynamic swirls rather than steady recirculations. Such a flow is indeed fully three-dimensional and by neglecting this valuable information regarding the cleaning effect would be lost.

Several equipment manufacturers produce slightly different types of MPV and these have achieved an approval by EHEDG as being hygienically designed, which coincides with our findings. Removal of dried mustard from the surface of a Plexiglas MPV showed that removal was faster and more efficient than expected based on predictions performed in uni-axial flow (Jensen et al., 2000). The explanation can be found in a combination of the effect of the dynamic swirls and an increased turbulence degree resulting from this flow phenomenon.

The relation between cleaning efficiency and wall shear stress was investigated applying a much milder cleaning compared to that normally used in the EHEDG test. The results show (Figure 1, a-1 and a-2) that just after the entrance and in a strike just below the equator of the MPV house the EHEDG test indicates poor cleaning. The CFD simulations showed (Figure 1, b) that low wall shear stresses were present in the entrance region and in a strike just above the equator in the MPV.

The results are somewhat similar, however, in order to complete the picture the fluid exchange must be included as well. It was seen that slower fluid exchange was present just below the equator. Thus the wall shear stress alone cannot explain the cleaning effect in complex flows but do hold part of the explanation. The result showed that low wall shear stress combined with poor fluid exchange proved a problem, where as the cleaning effect can be good enough if the fluid exchange was better even though the wall shear stress was lower.

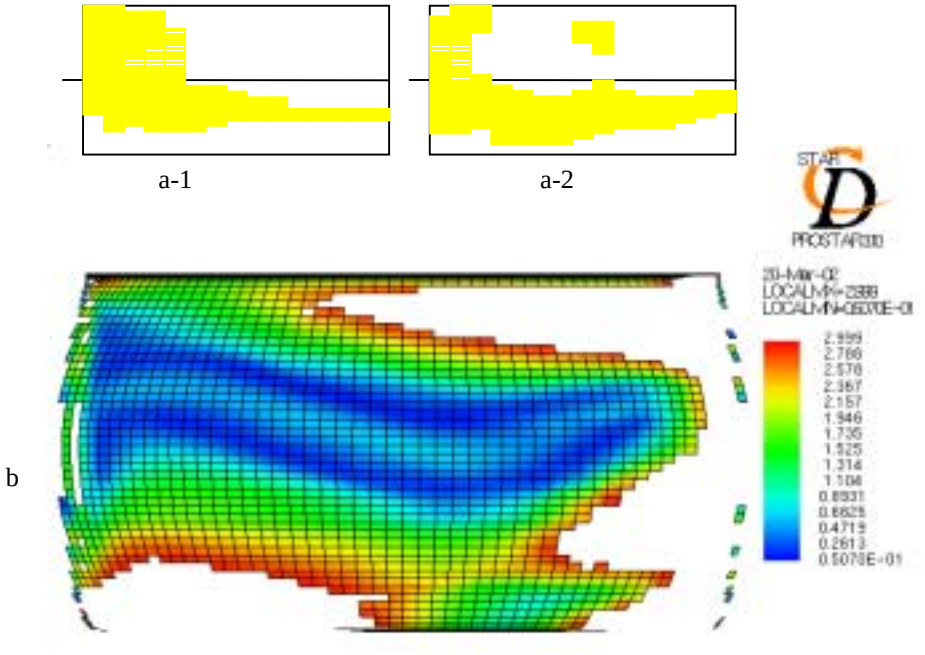


*Figure 1. The pictures (a-1 and a-2) show the result from each two EHEDG test in the upper (a-1) respectively the lower house (a-2) of the MPV (black and grey zones represents discoloration of agar from remaining spores on the surface. The picture (b) shows the simulated wall shear stress on the wall of the upper house of the MPV (darker colours representes low wall shear stresses).*

## 3.2 Up-stand

Cleaning an up-stand on a pipe is known to be a problem. This is shown using EHEDG testing by Richardson, 2000. Furthermore Grasshoff, 1980 shows that a recirculation zone is present just downstream of the up-stand it self. The velocity field from a CFD simulation also identified this recirculation. However, in this case the controlling hydrodynamic parameter was found to be the fluid exchange. The fluid exchange was three times slower in the recirculation than in the main stream and nine times slower in the up-stand it self. The results showed a much poorer cleaning efficiency in the zone with three times slower fluid exchange, which is cooperated by the EHEDG test by Richardson, 2000. The significance of this observation lies in the fact that a relatively simple simulation of bulk flow in some cases can be related to cleaning efficiency.

## 3.3 Pipe expansions

Recirculation zones have been mentioned in the previous two cases as both being able promote and demote cleaning efficiency. Dynamic recirculation zones in the MPV were reported as an advantage where as a permanent recirculation zone in the case of the up-stand was reported a problem. Similar recirculation phenomena are found in pipe expansions.

Two cases a concentric expansion (conventional design) and an eccentric expansion (recommended by the European standardisation committee – CEN in EN 1672-2 as being more hygienic regarding draining of plants) was compared using only CFD simulations. Fully three-dimensional transient simulations of flow in the concentric expansion showed that a recirculation or rather several recirculation zones were found in the expansion zone. However, these recirculation zones were dynamic in size, merged, developed and disappeared again with time (Figure 2-b). The eccentric expansion case showed one permanent recirculation to be found in the expansion (Figure 2-a).

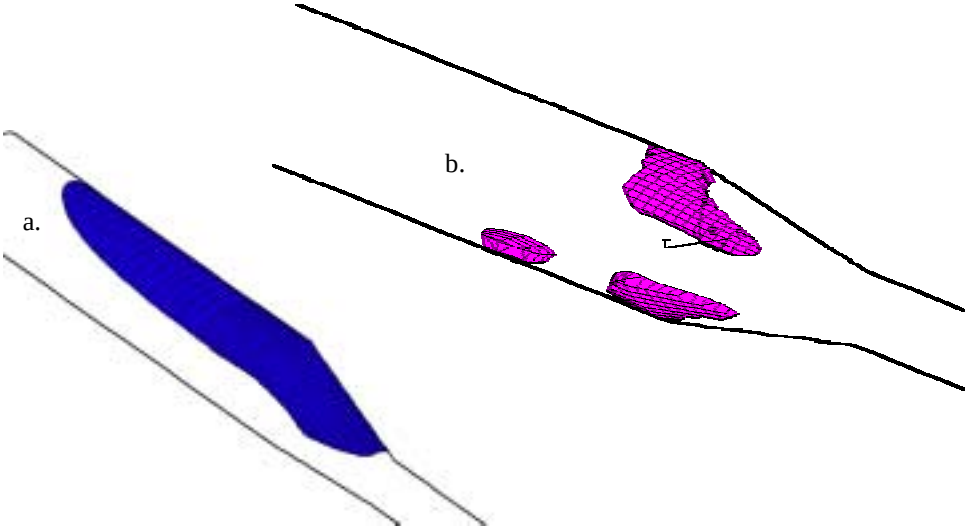


*Figure 2. The permanent recirculation in an eccentric expansion is show in (a) and the dynamic recirculation's in the concentric expansion at an arbitrary time step is show in (b).*

The result from the concentric expansion case would not have been found in a two dimensional model or if only steady state was simulated. This proves that some times also the dynamic effects must be included in order to evaluate the hydrodynamics in certain components. Even though no cleaning test has been performed the concentric expansion was considered superior to the eccentric with respect to cleaning efficiency given that also the fluid exchange was slower in the latter case.

# 4. Conclusions

The results presented showed that CFD could be a qualitative tool for evaluation of cleaning efficiency in closed process systems. It was demonstrated that complex equipment was not necessarily difficult to clean. A set of hydrodynamic parameters was identified as the major controlling factors in cleaning of closed processes. The wall shear stress play a role but cannot explain the whole picture by it self. This is due to the fact that fully three-dimensional turbulent flows

proved to clean better than expected based on uni-axial flow investigations. The nature of recirculations was identified as an additional factor. A dynamic recirculation (concentric expansion) or a swirl zone (MPV) proved much more efficient than a permanent recirculation with respect to cleaning. The results showed that a recirculation zone as in the case of the up-stand resulted in reduced fluid exchange and hence poorer cleaning. The third parameter identified as being limiting for the cleaning efficiency was the fluid exchange.

Concerning the nature of real flows full three-dimensional considerations must often be included since swirl zones and similar phenomena only can be discovered this way. Furthermore transient simulations sometimes can provide additional information since the fluctuations in turbulent flows were reported to have significant influence on the flow pattern.

## 5. Acknowledgment

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# THE VIRTUAL CLEANING TEST – IS IT POSSIBLE?

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## Abstract

Predicting cleanability of food processing equipment is of increasing importance to equipment manufactures. Increased demands from costumers and legislation to guarantee a high standard of hygienic design exist to prevent spoilage, food related diseases and increase the quality of the product. The traditional method to evaluate hygienic design is standardised microbiological cleaning tests for the comparison of different designs of equipment. Reproduction of such a test by numerical fluid simulations (CFD) is presented. A combination of a wall shear stress threshold value and the relative exchange of fluid inside a piece of equipment are shown useable to give a good prediction of the outcome of the standardised test method. Such a tool enables manufacturers to identify and increase the knowledge of hydrodynamic features important to cleaning.

## 1. Introduction

Cleaning in place (CIP) is widely used within the food manufacturing industry for closed processing equipment. Dismantling is avoided, chemicals and water is retained inside the equipment and reuse is possible. Finally, some installations might even be impossible to clean otherwise (Timperley 1997). One major disadvantage of cleaning in place is the difficulties involved in validation of the cleaning procedure, as it requires disassembling; hence, the advantages are diminished. In-line cleanability needs to be proven for a component prior to installation in a process line. The obvious choice is to perform actual cleaning tests in pilot plant scale where the cleanability of a component is compared to the cleanability of a straight pipe (EHEDG 1992). In the ideal case where all components mounted in a process line is proven to be cleaned to the same degree or better than a straight pipe, allows a CIP procedure to be validated by making

microbial testing of the cleaning in a straight pipe only. If the straight pipe is cleaned to a satisfactory degree then the rest of the plant is also assumed cleaned to a satisfactory degree.

# 2. Hydrodynamics in cleaning in place

The alternative presented here is to investigate the different factors affecting cleaning and set-up threshold values for parameters that are "measurable" in process equipment. Cleaning is generally described as an amount of energy that should be applied to soil on a surface in order to remove it. The energy is combined of a chemical, a kinetic and a thermal contribution and then of course the duration of the action of the energy (Schlüβler 1976). The chemical and thermal energy resolves and loosens soil from a surface while the kinetic action removes the soil from the surface. In the case of a straight pipe or a duct both with fully developed turbulent flow and with constant volume flow, the chemical, thermal and kinetic energy can be assumed identical on all surfaces. The only potential change is the oscillating effect from the turbulence in the flow. In such geometries several have suggested and proven the existence of a critical wall shear stress as the threshold value for the kinetic energy (Duddridge et al. 1982, Fowler et al. 1980, Powell et al. 1982, Jensen 2003 etc.). Having knowledge of a threshold value for a combination of cleaning procedure, surface characteristics and soil makes prediction of cleanability possible for a straight pipe or a duct as the wall shear stress acting on these surfaces are well known from analytical fluid dynamics (Shames 1992).

The major difference between the simple flow situations and more complex flow in equipment like valves, dead-ends, pipe bends, pumps, heat exchangers etc. is the nature of the flow patterns and parameters. Hence, the kinetic energy (wall shear stress), the chemical energy (detergent concentration near the soil) and thermal energy (temperature of detergent near the soil) are most likely dissimilar in different areas of a complex component in a process plant (e.g. valves and heat exchangers). The difference in chemical, thermal and kinetic energy is the obvious reason for different degrees of cleanability in certain areas of equipment. In this work, a combination of a threshold value for the wall shear stress and qualitative knowledge of the fluid exchange is shown to enable manufactures of processing equipment to predict the areas that are a potential hygienic hazard. Hereby,

improvement of the hygienic design is possible in the very earliest stages of design processes and further on until an optimum hygienic design is achieved.

# 3. Computational fluid dynamics

To obtain knowledge of wall shear stress (kinetic energy) and the distribution of chemicals and heat through out the flow domain of a component Computational Fluid Dynamics (CFD) is applied. This method involves discretisation of a flow domain into relatively small finite control volumes and the flow inside each of these is calculated by the Navier-Stokes equations (Versteeg et al. 1995). The Navier-Stokes equations express the change in flow rate inside a finite volume under the influence of the forces acting on this small volume. By applying boundary conditions to the flow domain, and transfer information between neighbour control volumes predicts the flow patterns and magnitudes throughout the flow domain. Additional models are applied to take into account turbulence, heat transfer, buoyancy and so forth. Additional information on CFD is given e.g. by (Versteeg et al. 1995). From knowledge of the flow field obtained from solving the Navier-Stokes equations different parameters such as wall shear stress, temperature and concentration of scalars can be derived on a very detailed level. Such information is difficult to come by when using experimental techniques. In addition, some of the parameters derived from the CFD simulations are extremely difficult to measure experimentally, especially if information is needed on a continuous surface or volume and not just in discrete points.

# 4. The virtual cleaning test

The virtual cleaning test itself is based on a threshold wall shear stress derived through experiments by use of the so-called radial flowcell (RFC) that has flow conditions similar to those present in a straight pipe. Furthermore, qualitative information on the fluid exchange is needed. Comparing this information with the wall shear stress derived by CFD for the component during cleaning and the fluid exchange also predicted from CFD simulations makes prediction possible of areas where cleaning in a component is worse than in a straight pipe exposed to fully developed turbulent flow. In case of a component is shown to be more difficult to clean that a straight pipe on an overall level the design should be changed.

However, if all components mounted in a plant is proven easier to clean than a straight pipe then the CIP procedure could be chosen accordingly to cleaning experiments in a straight pipe with different CIP procedures.

# 5. Summary

This work has shown that the virtual cleaning test on a mix-proof valve and an upstand is possible by the use of Computational Fluid Dynamics. A threshold value of the wall shear stress along with the relative fluid exchange is necessary knowledge, but when known, a good agreement between predicted areas difficult to clean is seen compared to the results of an actual cleaning test. In this case, the cleaning test is similar to the one used for certification of equipment for the European food processing plants performed by the European Hygienic Engineering & Design Group (EHEDG). By use of CFD, it is now possible to design equipment that is more cleanable than the existing equipment. Furthermore, a very important piece of information also derived from such investigations is identification of areas where additional attention should paid with respect to cleaning. This could be dismantling of equipment in such areas to allow manual cleaning.

# 6. Acknowledgment

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# TANK CLEANING STUDIES USING CFD – A PRELIMINARY CASE STUDY

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## Abstract

The hygienic state of process surfaces in food production plants crucially affects the quality of food products. Hygiene is important everywhere but fermentation processes where specific microorganisms are used to ferment nutritious raw materials into high quality foods requires special attention. The consequence of contamination of a fermentation can be fatal to the product quality and large batches might be discarded. Therefore hygienic requirements must be included in the design of the process as well as when performing the cleaning procedure. Fluid flow plays an important role in both production and cleaning. Specific hydrodynamic parameters have been proved to control cleaning in closed process systems. The work pertains extension of flow modelling to be applicable for improvement of the hygienic state of food process tanks. The present case study is limited to fermentation tanks for dairy and brewery applications.

## 1. Background

Tanks are used in food production plants for many purposes. These tanks are for many reasons crucial for the operation of plants. The major applications are storage of raw materials and end products, buffers for intermediate products, fermentation, mixing, heat and cooling. When focusing on fermentations the prerequisite is that the tank is clean at the starting point and in some cases it must even be sterile. Of cause the fermentation should run free of contaminations throughout the production as well. Microorganisms in nutrient rich environments are prone to grow in fermentations (Storgårds, 2000). Hence even few uncleaned niches harbouring harmful microorganisms can initiate a fatal contamination. In severe cases poor cleaning can cause biofilm formation

on equipment surfaces, which can cause corrosion and health problems (Geesey and Bryers, 2000; Wirtanen et al., 2000). Therefore tank cleaning has received much attention over the years. Much practical work has been done to improve the design and operation of spray balls for cleaning and today many suppliers can produces customises systems, which has an excellent cleaning efficiency. The challenge is still to optimise the design of cleaning systems with respect to efficiency and economy. This implies choosing the right spray ball for a given tank in such a way that hygienic state is just as required. Minimising cost implies that consumption of water and cleaning agents as well as the cleaning time must be minimised. Such achievements cannot be obtained through the dominantly empirical approaches applied previously.

More fundamental studies are needed concerning the effect of hydrodynamics in tank cleaning processes. Computational Fluid Dynamics (CFD) is shown to be a useful tool for optimisation of hygienic design of closed process equipment (Jensen, 2003). A combination of wall shear stress, fluid exchange and turbulence conditions can be used to predict areas that are not properly cleaned in both simple and complex flow systems (Friis and Jensen, 2002). Other studies show that besides the fluid dynamics also aspects like surface topography and material properties as well as the specific microbiological flora and other components of soil have an influence on the cleaning efficiency (Wirtanen, 1995; Storgårds, 2000). Several studies have shown that only weak relations are found between surface roughness and the cleaning efficiency (Verran et al., 2001). This fact is encouraging for applications of CFD simulations as a tool for optimisation of cleaning since surface roughness is difficult to implement in flow models.

The specific cases studies pertains to dairy and brewery process tanks. The hygienic state of these is important in order to avoid contamination of the end product. In dairies problems concerning contamination of pasteurized milk with psychrotrophic bacteria is likely to be caused by improper cleaning of surfaces (Geesey and Bryers, 2000). Contaminants are also reported to prolong the production of fermentation milk products and to cause undesired competitive cultures to grow or harbour in closed process systems (Wirtanen et al., 2002). In the breweries long production time from wort boiling to final beer, where batch fermentations runs up to several weeks, gives plenty of time and opportunity for unwanted microorganisms to grow. Approximately half of the contamination cases in breweries originate from yeast, wort, fermentation, maturation or tanks

(storage and fermentors) and the consequences are often causing large batches to be discarded or production of poor quality beer (Storgårds, 2000).

It is generally understod that the most succesful ways of prevention microbiological contaminations is to ensure that proper cleaning are performed. Producers of cleaning agents can today deliver effective cleaning programmes applicable in most practical situations. When dealing with closed processes the question is always validation of the cleaning operation. In somes cases visual inspection can be utilised which is the case for large tanks. Different tests are commerically available to evaluate cleaning efficiency by used of flourecent substances (Wirtanen, 1995; Storgårds, 2000). Our hypotosis is that both validation of the cleaning procedure and design of a proper cleaning system can be supported and improved using CFD models. The aim is to evaluate the suitability of CFD simulations for estimation and improvement of tank cleaning and perform cleaning tests in pilot plant in order to establish a correlation to results for CFD simulations. Some preliminary studies are presented in this paper concering flow simulation in tanks with spray balls cleaning test suitable for accurate evaluation of the hygienic state of tanks during and after cleaning.

## 2. Experimental set-up in tank cleaning

The tested equipment was a stainless steel tank. The dimensions were an inner diameter of 400 mm and a height 800 mm yielding a total tank volume of 80 l. A spray ball was mounted in the tank lid. The total length of this spray ball was 145 mm and the rotating tip measured 50 mm. The position of the spray ball it self was 60 mm from the centre of the tank (Figure 1).

Two types of soil was investigated. In the first situation the tank was soiled during production of beer and the soil was beer containing yeast cells. In the second case the tank was soiled with sour milk containing *Bacillus stearothermophilus* var. *calidolactis* (NIZO C953) -spores and subsequently dried at room temperature for three hours.

The CIP-cleaning was performed using a pilot plant cleaning system. The CIP-cleaning procedure consisted of prerinsing with cold water for 1 min, cleaning with 60°C detergent solution for 10 min and final rinsing with cold water for 1

min. Detergent solution was 1% EHEDG Testcleaner. The flow velocity in pipe before spray ball in the lid of tank was adjusted to 1.5 m/s.

*Figure 1. Experimental set-up.*

The hygienic state of the tank was studied using contact agar methods (Hygicult TPC® and Petrifilm AC®) and ATP-detection with a luminometric assay. Samples were taken from the lid of the tank, the spray ball and selceted positions on the walls of the tank (upper, middle and lower parts of the tank wall was sampled). The tests showed that some residuals of microbes were present at the more rough parts of the tank. These being screws in lid, the gasket between tank and lid and some very unhygienic scratches on tank wall. However, the smooth parts of the tank did not have any microbial contamination after cleaning procedure according to detection methods used.

## 3. Computational fluid dynamics

Computational fluid dynamics is used in many applications to model bulk parameters of fluid flows. Recently model developments have made it possible to resolve what happens in specific positions on and near walls, which is of interest when studying cleaning processes (Jensen, 2003). CFD models of tanks

exist for purposes of optimising the operation of processes like mixing, heating and cooling. The extension pertains to combining the models for prediction of hygienic design of valves, pipes etc made by Jensen, (2003) with conventional tank flow models and establishment of more information on the connections between surface characteristics and the cleaning effect of fluid flow. This is believed to result in a tool suitable for evaluation of efficiency of cleaning procedures in tanks.

The commercial CDF-package Fluent 5.4.8 was used in the study. Presently a model of the test tank is under development. Preliminary results from the CFD simulations yield information about wall shear stresses in the tank and the flow rates in different parts of the system.

## 4. Summary and further studies

The results from the preliminary experiments studying a simple case supported each other well in the simple case study performed. This has given us support to the hypothesis that a combination of knowledge in fluid dynamics and microbiology gives an excellent base also for hygienic design of integrated tank and CIP cleaning systems. The main focus has been on the experimental part since sound basis knowledge was required in order to establish a proper CFD model, which can be used for simulation of this type of process system.

In future we will focus on more realistic process applications. The CFD model will be extended to cover different tanks and spray balls and validated using modified microbiological test metods. Development of these test methods is included in the future studies.

## 5. Acknowledgements

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# TOWARDS RISKLESS FOOD?

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## Abstract

European food and feed legislation is currently being revised. White paper on food safety launched by the European Commission in 2000 paved a road to the modernisation and simplification of the common food legislation of the current 15 and of the near future 25 member states.

As a first step the European Parliament and the European Council agreed in February 2002 on the regulation on the principles of the forthcoming European food legislation. In addition to that this piece of legislation erected a new Community institution, the European Food Safety Authority (EFSA). A comprehensive package of hygiene rules is being processed and the main parts of it already agreed on by the Council until to the end of the year 2002.

A twin proposal on genetically modified food and feed and a twin proposal on zoonosis monitoring and control are also agreed on and are now waiting for the second reading in the European Parliament. New legislation on combating of TSE-diseases, on identification of farm animals and on the proper treatment of animal waste is also already approved.

The Commission is expected to give new proposals on the official control of food and feed and on feed hygiene in the beginning of the year 2003. This thorough revision of the Community food safety legislation brings new principles and policies.

This new approach will be discussed in the presentation. The main focus of the presentation is set to the following:

1. *From farm to fork* quality approach and its possible extension to a *from nature to navel and back* quality approach,
2. functional separation of risk assessment and risk management,
3. precautionary principle and its application to the day to day work,
4. guides of good agriculture/hygiene practise as a new innovative tool, and
5. flexibility as a challenge to the official control.

# SAFETY AND HYGIENE MANAGEMENT IN MANUFACTURING PACKAGING MATERIALS

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## Abstract

The importance of hygiene and microbial management in paper and packaging industry has increased considerably as a result of the changes in legislation, tighter international competition and increasing customer requirements.

Microbial risk management in paper and packaging industry is performed mostly through Good Manufacturing Practices and by building up an hygiene and safety management system (own-checking system or HACCP-program) which is recommended to be based on HACCP concept. Good manufacturing practices form the foundation upon which a hygiene and safety management system can be build.

Microbial management is divided to two main strategies: to prevent the entrance of microbes to production premises and to eliminate microbes from production facilities if they have entered the premises. As sterile conditions are impossible the preventive actions should be focused so that the restriction of microbes is in economically and operationally reasonable frame. The control of microbial growth at paper machine environment is traditionally based on the use of biocides. However today the focus is more in the combined use of several control methods. The extreme importance of good housekeeping in successful microbial management has been emphasised.

# 1. Introduction

Microbiological product safety is of utmost importance both for the product manufacturer and for the consumer. This has been witnessed during recent failures in ensuring safe foods for consumers within European community.

Microbiological safety issues are especially important for all industrial fields whose products are intimately in contact with humans and animals. These include the production of feed and foodstuff, packaging, and pharmaceuticals. A number of events in recent years have seriously damaged European consumers trust in the safety of their foods.

Manufacturers of packaging materials for food have become increasingly aware of customer demands relating to concerns of food safety. As an important raw material supplier for food industry manufacturers of packaging materials are expected to bring their standard hygiene in line with expectations of the food industry.

The importance of hygiene and microbial management in paper and packaging industry has also increased considerably as a result of changes in manufacturing process and role of package. The contact time between package with food has extended due to longer selling periods and extended transport time from production plant to market. Furthermore important microbial growth enhancing changes such as increased use of recycled fibre as a raw material and decreased consumption of water (closing of water circulation system) has taken place in manufacturing of paper and board that stress the importance of hygiene and microbial management.

## 2. Microbial risk management

Paper and board are produced in large volumes worldwide: in 2002 the European pulp and paper industry produced 88 million tonnes of paper and board. This is one third of world production. The production of food contact material is about 10 million tonnes. Considering the economic importance of paper and board manufacture, surprisingly little published information is available on the microbes contaminating these processes and the products as well on efficient microbial management.

Some microbes manage to survive throughout the entire packaging material manufacturing process right through to the end product. This is most likely with products having a high grammage, where the thickness of the product protects the microbes from heat during the drying stage. Microbes also withstand heat well in end products containing minerals by adhering to the mineral crystals.

From the point of view of safety and microbiological problems in end products, the most important microbes include spore-forming bacteria (*Bacillus* spp., *Clostridium* sp.), bacteria that are injurious to health, certain moulds, actinomycetes and anaerobic bacteria.

Microbial management is divided to two main strategies: to prevent the entrance of microbes to production premises and to eliminate microbes from production facilities if they have entered the premises. As sterile conditions are impossible the preventive actions should be focused so that the restriction of microbes is in economically and operationally reasonable frame. Focus should be on microbes that cause harm, damage or danger to product, process, worker, environment or customer. The ultimate goal of all these activities carried out in microbial risk management is prevention.

## 2.1 Hygiene and safety management system

Most of the paper and packaging plants have a quality system, which is based on ISO 9000 series standards. Good manufacturing practice (GMP) is a fundamental part of quality control and product safety assurance. GMP identifies the basic requirements and set the basic rules of operation. Preventive microbial risk management in paper and packaging industry is performed mostly through GMP and by building up an hygiene and safety management system (own-checking system or HACCP program) which is recommended to be based on HACCP concept. The prerequisites based on GMP form the foundation upon which a hygiene and safety management system can be built (Figure 1).

Control of hazards that are generic, those that can have an influence at any stage of the process and those that are not associated with specific process points are examples of hazards that are controlled under GMP. On the contrary, the Critical Control Points (ccps) identified in HACCP evaluation are strictly associated with specific process steps and a monitoring system is implemented to ensure that the threshold level set by the HACCP team is met. The seven principles of the HACCP program can be summed up in three elements: hazard analysis, measures for hazard control and verification and documentation of the system, which is the most time- and resource-consuming element in the maintenance of the system. The HACCP system is compatible with the implementation of total quality management system based on the ISO 9000 series of standards. Although

HACCP has been internationally accepted and approved and it has been used as a tool in food safety management, it is known that companies have had problems with its practical application and implementation (Table 1).

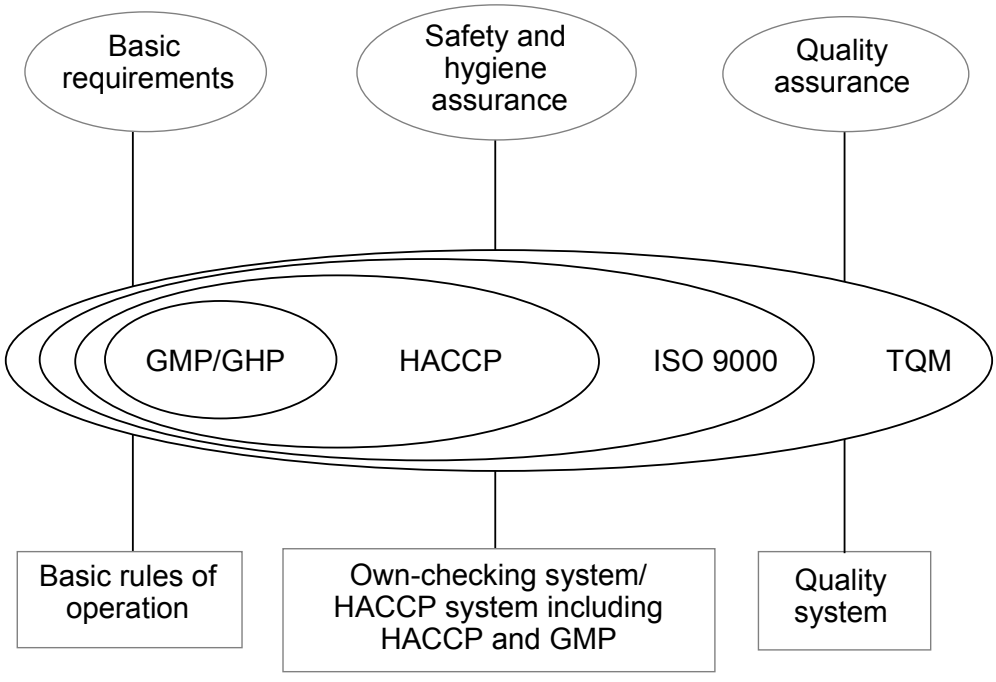


*Figure 1. Dependency of quality and safety management systems.*

The hygiene risk assessment model, HYGRAM®, has been developed to facilitate the risk analysis process in food safety management. HYGRAM® provides a practical model that helps in conducting hazard analysis and in choosing effective critical control points with their critical limits. With the aid of the model it is possible to track the effect of different process stages on the product. The probability and severity are evaluated at each process step. HYGRAM® is also a tool to compare risks of different processes. HYGRAM® has been developed in co-operation between VTT Biotechnology, the Department of Risk Assessment at National Veterinary and Food Research Institute, and Department of Food and Environmental Hygiene at Helsinki University. Presently a modification of HYGRAM® suitable for identification of critical control points and evaluation of GMP activities in paper and packaging industry is under development in VTT. A module evaluating the cost effects and

helping in decision making of monitoring activities will be included in paperHYGRAM model.

*Table 1. Problems in application and implementation of HACCP program (Mortimore 2001).*

| Problem in HACCP-program | Possible solution |
|---|---|
| Getting the scope wrong and confusing safety with quality. If the scope is too wide (e.g. confuse product quality and food safety) then the HACCP study and its implementation will be unmanageable (too many and/or overlapping critical control points). | Keep the control system simple and carry out effective control in only a few most important critical control points. |
| Insufficient commitment to the implementation and application of HACCP program. | Most important is the commitment of directors. |
| Weaknesses in process analysis: Incorrect or incomplete process flow diagrams can mean that steps and processes are missed. Wrong persons participate to the HACCP. | It is important to involve the right people -, and often it is the line workers and supervisors who understand this aspect best of all. The process flow diagram must be verified before doing the hazard analysis. |
| Ineffective monitoring and corrective actions:<br>Can often be found when too many critical control points exist and thus people fail to see the point of doing anything. | Ensure the monitoring frequency is appropriate<br>Keep the documentation simple and easy to use<br>Train the monitors thoroughly<br>Use verification (review of records and audits). |
| Insufficient documentation. | Keep the documentation simple (e.g. computer databases). |
| Continuing motivation and momentum once the HACCP-plan is implemented. | Key workers must be identified and persons in charge of updating the HACCP-plan. |

## 2.2 Control of microbial growth

Paper machines offer microbes a favourable environment to grow and multiply. Microbes or their metabolic products that have an adverse effect on end-product quality comprise those that pose a health hazard or cause odours, taste or colour defects in end products. Microbes can enter the production facilities via raw materials e.g. freshwater, cellulosic raw materials, the broke, solutions or suspensions of additives, fillers, pigments, starches and coatings. Furthermore via packaging materials, transport equipment, open windows, doors and openings, air conditioning, insects and pests and personnel can harmful microbes enter and settle into the production facilities. Contamination risk depends on number of microbes, quality of microbes, conditions in production premises (temperature, moisture, pH), availability of nutrients and efficiency of control methods.

The control of microbial growth at paper machine environment is traditionally based on the use of biocides (Figure 2). However the amount of microbes is not always proportional to the severity of microbial problems. The most important factors in the use of biocides are selection of suitable agents and the optimal feeding strategy. Due to variability and complexity of the microbial flora in paper machines there is no single biocide that would alone be effective in microbial control. Resistance of microbes is a commonly faced problem. Furthermore for environmental reasons new methods are constantly searched. Today the focus is more in the combined use of several control methods. The extreme importance of good housekeeping in successful microbial management has been emphasised. Important housekeeping-related precautions are such as good control of the entire process and plant operation, unnecessary delays for easily spoiling materials are minimised and tanks, containers and machinery are cleaned sufficiently and on regular bases.

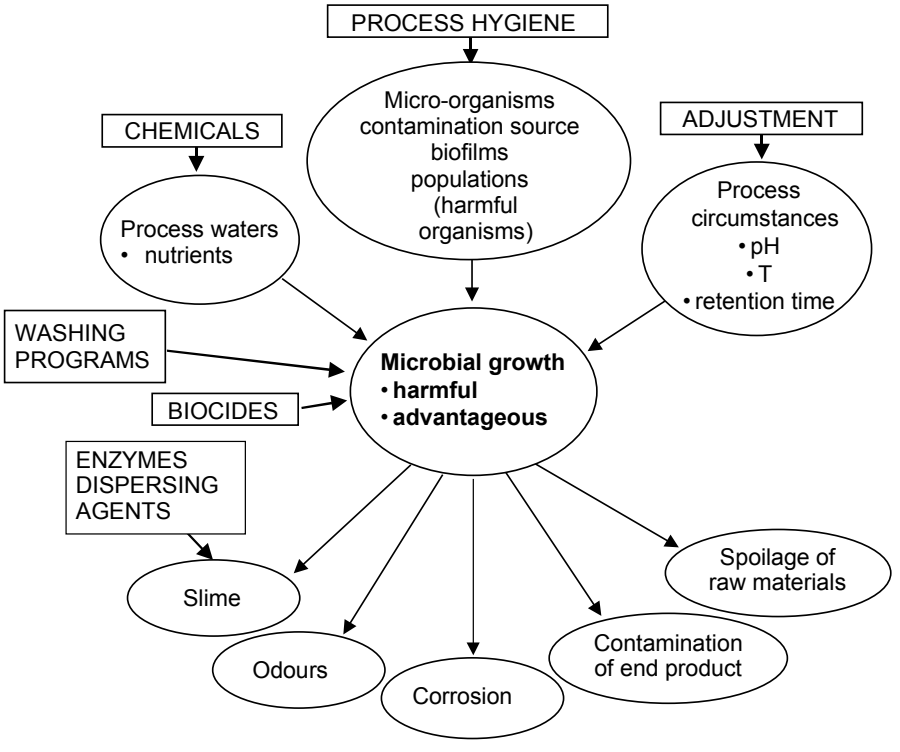


*Figure 2. Control of microbial growth at paper machine environment.*

The effective focusing of risk management activities requires that the characteristics, life cycles and important contamination routes of harmful microbes are known. Focusing the risk management activities on microbes that provide the ecological niche for harmful microbes without being harmful themselves probably the control of microbial growth could be performed more efficiently. Increasingly the focus has sifted from studying the numbers of microbes in paper machine environment to the identification of the harmful contaminants and to the determination of characteristics of these industrial strains.

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# COMPARISON OF THREE AIR SAMPLING METHODS IN FOOD PACKAGING MATERIALS PRODUCTION

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## Abstract

Three air sampling methods: sedimentation, impacting and filtration were compared in a food packaging materials producing factory. Sampling was performed four times during a year in three sampling places. The study showed that the results with all three methods used had similar trend. In general settle plates caught less microbes than other methods. The results for impacting and filtration methods were in many cases similar to each other. These methods were more sensitive than the method based on settle plates. A bigger sampling volume also used in filtration method did not increase the yield.

## 1. Introduction

Concern is growing in the food and packaging industry to determine the importance of the airborne route as a possible source of contamination. There is many different air sampling methods and devices available for air quality monitoring. All methods have their good and weak points and they suit for different purposes and environments. We concentrate on three sampling methods: sedimentation, impacting and filtration, which have been used in industry environments.

Generally, the performance of bioaerosol sampling devices is characterised by their ability to aspirate particles into the inlet, to transmit them through the sampler's interior and to collect them on the collection surface. In the case of viable microbial sampling, the performance of samplers must fulfil the stability of microbial viability as an additional component during sampling (Thompson *et al*., 1994). The samplers must also collect a representative sample of the required

fraction of bioaerosol with a minimum of stress, so that the biological activity of the aerosol is not too much impaired (Griffiths & DeCosemo, 1994).

## 2. Air sampling methods

Sedimentation also referred to as settle plate technique has traditionally been used in the food industry. The plates are easy, inexpensive and collect particles in their original state (Kang & Frank, 1989); however, they have serious drawbacks. The ability of settle plates to collect airborne particles is governed by the gravitational force on the particle, which decreases with a velocity dependent on its mass. Hence, settle plates are biased towards collecting larger particles and are sensitive to air movement (Griffiths & DeCosemo, 1994). The method is not quantitative and in high aerosol concentrations the uncountable numbers of colonies can be a problem (Holah *et al*., 1995).

Impacting is the most common technique for collection of airborne viable particles (Ljungqvist & Reinmüller, 1998). In impactors the inertial forces are used to collect particles. In the collection stage of impactors the air stream is forced to change direction, and particles with too high a level of inertia are impacted on either a solid or liquid surface. (Henningson & Ahlberg, 1994). Sieve samplers are operated by drawing air through a large number of small, evenly spaced holes drilled in a metal plate. The particles are impacted on an agar surface located below the perforated plate (Kang & Frank, 1989). Detection of microbes relies on their ability to grow following sampling. When the concentration of viable particles in an aerosol is high, one sieve hole may allow more than one viable particle to pass through, resulting in the formation of a single colony from two or more viable particles. This inaccuracy can be corrected by reducing sampling time or by using either the microscopic method or a positive-hole method for enumeration (Kang & Frank, 1989). Normally the positive-hole correction tables are included in each commercial sieve sampler.

In filtration several different collecting mechanisms (impacting, interception and diffusion) are active. In general, the filters are inexpensive and simple to operate (Kang & Frank, 1989). The air filtration apparatus consists of cellulose fibre, sodium alginate, fibre glass, gelatine membrane filter or synthetic membrane filters mounted in an appropriate holder and connected to a vacuum source through a flow rate controller (Kang & Frank, 1989). Microbes can be removed

by washing off from the filter to carry out total number rather than viable number enumeration (Griffiths & DeCosemo, 1994). The suspension can also be assessed with appropriate bacteriological techniques. Membrane filters can also be directly placed on an agar surface for incubation. The filter methods are good for enumerating mould or bacterial spores (Kang & Frank, 1989). One of the main disadvantages of using filters in collecting microbes is that they do not protect cells when large volumes of air pass over the filters causing desiccation (Griffiths & DeCosemo, 1994). Shortening of the sampling period for this method may reduce this stress (Kang & Frank, 1989).

The gelatine filter membrane is composed of gelatine foam designed to prevent vegetative microbes from being inactivated by desiccation when air is drawn through the filter (Parks *et al*., 1996). The gelatine membrane is water-soluble so that it can easily be diluted for plating or be dissolved on top of a nutrient agar, resulting in bacterial colonies on the agar surface (Kang & Frank, 1989). Prolonged storage of the filters before assay causes death of sensitive vegetative bacteria (Parks *et al*., 1996).

# 3. Materials and methods

Three air sampling methods: sedimentation, impacting (MAS-100, Merck) and filtration (MD8, Sartorius) were tested. Sampling parameters are presented in Table 1. Sampling was performed four times during a year in a factory producing packaging materials for food industry. Samples were taken from three sampling places, from which one was very warm (+45°C). Other two places were normal industrial environments and temperatures and humidity were 22–24°C depending on the season. The samples for impacting were taken directly on agar plates. Filters used for collecting 200 l of air were placed on agars immediately after sampling and they melted rapidly releasing microbes on agar. Filters used for collecting 1000 l of air were transported to laboratory where they were suspended (within 10 h) into peptone saline waster, diluted and cultivated. All plates were incubated in 25°C for 3 (microbes) or 5 (moulds and yeast) days. Positive-hole correction table was used to calculate impacting plate results. Filter method results from 1000 l samples were calculated to be equivalent to an air volume 200 l.

*Table 1. Methods and parameters (volumes, duration, agar plates and types of microbe) in the air sampling performed.*

| Methods | Sampling volume, duration | Sample into | Organism |
|---|---|---|---|
| Sedimentation | Plates open; 1 h | PC[1] | Microbes |
| Sedimentation | Plates open; 1 h | PD[2] | Moulds and yeast |
| Impacting (MAS 100) | 200 l, 2 min | PC[1] | Microbes |
| Implication (MAS 100) | 200 l, 2 min | PD[2] | Moulds and yeast |
| Filtering (MD8) | 200 l, 2 min | Gelatine filter directly on PC[1] | Microbes |
| Filtering (MD8) | 200 l, 2 min | Gelatine filter directly on PD[2] | Moulds and yeast |
| Filtering (MD8) | 1000 l, 10 min | Gelatine filter, suspended, diluted and cultivated into PC[1] and PD[2] | Microbes, moulds, yeast |

[1] Plate Count agar
[2] Potato Dextrose agar with 0.4 % chloramphenicol, 0.4 % chlorotetrasycline and 0.02 % Triton-X.

# 4. Results

The results with all three methods in all three sampling places and four seasons had similar trend, only few contradictory results occurred (Table 2). In general settle plates caught a little less microbes, especially moulds, than other methods. The results from the impacting and filtration methods were in many cases similar to each other when the sampling volume was 200 l. As the sampling volume increased in filtration method no advantages emerged. On the contrary the amount of bacteria decreased in these samples.

*Table 2. Results for the air-sampling methods at various sampling times during four seasons sampled in food packaging materials production area.*

| Sampling place | time | Sedimentation (CFU/h) | | | MAS-impactor (CFU/200l) | | | MD8 filter on agar (CFU/200l) | | | MD8 filter diluted & cultivated (CFU/200l) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Microbes | Mould | Yeast | Microbes | Mould | Yeast | Microbes | Mould | Yeast | Microbes | Mould | Yeast |
| 1 | May | >1000 | 15 | 0 | 1030 | 5 | 0 | 296 | 4 | 0 | 21 | 23 | 0 |
| | Jul | >1000 | 15 | 84 | >2600 | 43 | 0 | 63 | 20 | 0 | 8 | 20 | 0 |
| | Oct | >1000 | 15 | 122 | 248 | 15 | 0 | 39 | 7 | 0 | 71 | 77 | 0 |
| | Dec | 79 | 0 | 0 | 57 | 3 | 0 | 7 | 2 | 0 | 17 | 12 | 0 |
| 2 | May | 3 | 0 | 0 | 2 | 3 | 0 | 2 | 2 | 0 | 6 | 0 | 0 |
| | Jul | 2 | 1 | 1 | 51 | 4 | 0 | 21 | 6 | 0 | 7 | 4 | 0 |
| | Oct | 3 | 1 | 0 | 4 | 5 | 0 | 13 | 3 | 0 | 11 | 9 | 0 |
| | Dec | 5 | 0 | 0 | 14 | 1 | 0 | 29 | 0 | 0 | 8 | 0 | 0 |
| 3 | May | 13 | 1 | 3 | 9 | 1 | 1 | 5 | 1 | 0 | 4 | 0 | 0 |
| | Jul | 8 | 19 | 0 | 18 | 63 | 0 | 1 | 38 | 5 | 4 | 12 | 0 |
| | Oct | 3 | 5 | 1 | 18 | 13 | 2 | 20 | 9 | 0 | 14 | 8 | 3 |
| | Dec | 4 | 0 | 0 | 112 | 0 | 0 | 116 | 2 | 0 | 5 | 0 | 0 |

Sample place one that was warm was very contaminated with bacteria and moulds. Three out of four settle plates were overgrown, as was one impacting sample. Sample places two and three had a better air quality. Seasonal fluctuation was best noticed in December samples that had in general the least microbes. However some samples had the highest values in December which probably is due to process activities in the sampling place. Mould counts were highest between July and October, when outdoor air also has the biggest mould counts. Yeast was detected sporadically. Their growth in the same agar plate as the sometimes widespread mould growth may have been hidden part of yeast colonies.

# 5. Conclusions

In the food industry, the settle plates and impacting-type samplers are the most commonly used air-sampling methods in routine monitoring. Our test showed that the results with all three methods used had similar trends. Impacting and filtration methods were more sensitive than settle plate method. Settle plate results also describe only a very limited, local area where particles happen to settle whereas air-sampling methods that actively collect air are more reliable to describe the air quality. Our results demonstrated that implication and filtration methods were very similar and both worked well in industry environment. A bigger sampling volume in filtration method did not increase the yield. The filtration method may not be optimal for counting vegetative cells due to the stress it places on cells through dehydration during sampling when prolonged sampling times are used. The use of one air sampling method suits for a routine air quality monitoring. However time to time it is recommended to check the situation with another method because no method is able to reflect the whole variation of air.

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# TOOLS IN ANALYZING THE PROCESS HYGIENE

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## Abstract

In this presentation different tools for process hygiene monitoring are discussed. The common factors of all the methods are the speed of the analyzing process and the ease of use. The methods discussed in this presentation are such new rapid testing methods that are already widely and successfully used in different process hygiene control situations. Traditional methods, like plate counting and dipslide methods, are the most common techniques used to control process hygiene. There are a wide variety of agar types to choose from to meet the monitoring needs. These methods are not, however, included in this presentation. The reason behind this selected focus is the growing need to use the information from the process hygiene analysis as a tool to control and adjust the processes. In most cases this is achieved only if the hygiene information is available quickly, preferably within minutes from the sampling. This can be achieved only by using rapid process hygiene analysis tools.

# 1. Choosing the method for the process hygiene analysis

## 1.1 Surface hygiene

When selecting a method for surface hygiene analysis, one should consider the level of information needed as well as the time available and the amount of money possible to be invested in one single sample. If a suitable hygiene indicator, such as presence of protein or lactose, can be used and the demands about the sensitivity of the measurement are not too high, it may be possible to use the non-instrument test types. With many industry processes, however, the sensitivity needed suggests that a form of ATP testing is to be used.

### 1.1.1 ATP method

If the information is needed quickly in order to control the process and to adjust it, the best choice is usually ATP testing. In most cases the measurement of total ATP, the amount of both microbial and somatic cell load in the sample, is the most effective tool. It is possible; however, to analyze only the microbial cells from a surface hygiene sample, but usually measuring the total ATP gives more sensitivity. Microbes and spores are usually present in any normal process surroundings. The somatic cell load serves as a growth base for the microbes. If, at a certain time, the surface tested is virtually free of microbes but it contains high amount of total ATP, it is only a matter of time before the surface has a high amount of microbes as well.

In addition to speed and sensitivity the other benefit of the ATP method is the possibility to detect also residues of detergents and disinfectants. This possibility is based on the fact that detergents and disinfectants are naturally active and they inhibit the luciferase catalyzed luciferin-ATP reaction by one way or the other. Using an internal ATP standard is an easy way to calculate both the ATP amount in fg and the possible quenching effect of the residual detergents or disinfectants. This is not a widely used method, but it is very simple and cost effective. In order to use this, however, one must use a cuvette based luminometer in order to make the double measurement needed (by means of first measuring the sample and then sample + internal ATP standard).

The ATP standard method also eliminates the errors caused by the residues of detergents or disinfectants. In presence of these, typically chlorine or alcohol based residues, the measurement tends to give too “good” results with many surface hygiene monitoring methods. This is the case with traditional plate count agars, dipslides and the “single shot” ATP-tests.

There are limitations related to every hygiene monitoring tool. Testing of ATP is with most processes limited to the testing of “clean” surfaces, valves and other process equipment. ATP is in other words a testing method for the effectiveness of the cleaning processes. ATP testing is usually not a good method to test the hygiene of process surfaces during the processes, because they usually involve somatic cell load, which can heavily affect the results. In these cases the removal of the somatic cell load prior to the measurement is needed. These techniques are

used with CIP and process water as well as with raw and end product hygiene testing, but they are rarely applied to the testing of surface hygiene.

ATP method is proven to be suitable hygiene analysis tool for a large variety of processes. ATP method is best suited for such processes that involve cell-based products, such as food or pulp. With these products the measurement of both microbial and somatic cell load increases the sensitivity of the method when compared to processes that involve only microbial cell load.

*SnapShot* from Hygiena International Ltd and *Clean-Trace* from Biotrace International Plc can be named as examples of the "single shot" ATP test devices. "Environmental monitoring kit" from Celsis International Ltd can be named as an example of cuvette based ATP test with the possibility to use internal ATP standard in the quantitation.

### 1.1.2 Non-instrument testing for indicator compounds

When analyzing the process hygiene the ease of use and the time between the sampling and the results are significant. Also the cost of test and the cost of the base investments must be taken into consideration. When the financial resources are tight or when the testing must be carried out by the production personnel, non-instrument rapid testing can be the answer. With these tests the level of process hygiene is monitored by detection of indicator compounds, such as glucose, lactose or proteins. The results are usually obtained by color changes within the test devices and the results can be of presence/absence type or semi quantitative. When choosing a non-instrument test, one should always keep in mind the type of contamination or residues that are expected to be found if the cleaning processes are not performed adequately. For example, a protein test is not usable with processes that do not involve protein-based raw materials or products.

Detection of glucose or lactose is a good way to control surface hygiene of processes that contain glucose or lactose. It has been reported to perform well as a hygiene-monitoring tool with many processes, like those within beverage and vegetable industries as well as with the HORECA sector (Hotels, Restaurants and Catering). Test devices that detect glucose or lactose are usable also with many meat and fish processes, because they often involve sugar based raw

materials. Lactose detecting test devices are also suitable for process hygiene monitoring within dairy industry processes as well as with milk production and transport.

Detection of proteins is a useful way to control surface hygiene of processes that involve, for example, meat, fish, egg or milk based products. It has been reported to perform well as a hygiene-monitoring tool with many processes, like those within meat and fish processing industries. Protein testing is also suitable for evaluation of an overall level of cleaning processes and it is quite widely used by many different companies offering cleaning services.

Products like *SpotCheck* (glucose) and *SpotCheck PLUS* (glucose and lactose) from Hygiena International Ltd and *pro-tect* (protein) from Biotrace International Plc can be named as examples of such non-instrument indicator tests. All these test devices are suitable for simple and rapid surface hygiene testing although their overall sensitivity is not at the same level as of ATP method.

## 1.2 CIP and process water hygiene

Many industrial processes that are related to hygienic risks involve the use of tubing, valves, injectors, pumps etc. Surface hygiene sampling techniques can rarely be used with these kinds of components. Sampling from the reachable valves or the open ends of the tubing does not give accurate information about the hygiene level of the entire process. In many cases it does not give any information at all, which can lead to wrong conclusions and unnecessary risks.

Tubing, pumps and valves are usually cleaned by using CIP cleaning or circulation of the detergents in the systems and then flushing them with water. Testing the water from the last flush or flushes by means of ATP testing has proven to be a good and accurate tool for analyzing the hygiene level of such systems. With the use of ATP testing it is possible to gather information about the hygiene level as well as possible residues of detergents and disinfectants, as explained in chapter 1.1.1. The analysis can be made by direct ATP measurement method or filtration method depending on the nature of the water analyzed. The more measurement disturbing factors, like extreme pH, strong colors etc., are involved the easier the results can be achieved by filtration

method. Filtration is also the choice when the microbiological contamination levels are below 1000 cfu / ml. When, on the other hand, the ease of operation is the guiding factor the choice is direct ATP measurement.

### 1.2.1 Direct ATP measurement

Direct ATP measurement is carried out without any enrichment steps. Certain amount of water, usually 100–200 µl, is pipetted into a cuvette. If only the microbial ATP is of interest, the sample is treated with a combination of an agent capable of lysing somatic cells and of ATPase enzyme. After a specified incubation time, from seconds to some minutes depending on the type of the reagents, the agent capable of lysing microbial cells is added together with the luciferase/luciferin agent. If the somatic cell load is low or it is of interest together with the microbial cell load, all cells are lysed at the same time and the total ATP is measured much the same way as when analyzing surface hygiene.

When operating with CIP and process waters, it is often useful to use ATP standard as a quantification tool even if residues of detergents or disinfectants are not of interest. This results in much more accurate information about the hygiene level than measurement without quantification. This is an important factor that has to be considered when choosing the instrumentation. Quantification with ATP standard is usually not possible when using simple, surface hygiene oriented instrumentation, which use “single shot” type of reagents.

### 1.2.2 Filtration ATP method

ATP measurement is, like most enzymatic reactions, easily affected by disturbing factors like extreme pH, strong colors etc. These factors can be eliminated to a certain degree by dilution or by use of ATP standard. Dilution, however, also affects the sensitivity of the method and ATP standard is not always enough to compensate all disturbances. With CIP and other process waters filtration of the sample offers a useful tool to eliminate the disturbance. Depending on the filtration equipment available, as well as on the water to be filtered, the amount of sample can be chosen to suit the sensitivity needs. Usually the amount of the sample is between 500 µl and 5000 ml.

The measurement procedure is much the same as when using a direct ATP measurement technique, but the sample to start with is not the water itself, but the filter used. When filtering with an appropriate filter, pore size usually between 0.45 and 5 µm, only the microbes of interest are left on the filter. All the disturbing factors pass through the filter and cannot affect the measurement any more. Filtration can be used both as a tool to enrich the sample, if the microbiological contamination level of the sample is below the detection level of direct measurement, and as a tool of removing the disturbing factors from the sample. As such, filtration ATP method is a very useful tool of analyzing process hygiene from various processes that involve water or are cleaned by water/detergent solutions.

## 1.3 Raw materials and end product hygiene

In many cases the best way to analyze the effect of hygiene level of a certain process, is to measure the microbiological quality of the end products themselves. The process hygiene is also affected by the microbiological quality of the raw materials, which usually also are monitored. This monitoring is typically performed using plating or antibody techniques. In many cases results can be achieved easier and in a more cost efficient way by the use of ATP methods.

With raw material and end product ATP testing it is vitally important, that the disturbing factors (the matrix effect) are removed to sufficient degree and the measurement can be focused only to microbial ATP. The level of the microbial contamination should also be within the linear sensitivity range (typically between $10^3$ and $10^7$ cfu) of both the luminometer and the reagents in use. To achieve these goals the samples are usually either diluted, when dealing with high contamination levels, or enriched by filtration or incubation, when dealing with low contamination products or sterility testing like for example with UHT milk and other UHT products.

The raw material and end product testing can usually be carried out using traditional and validated microbiological pre-treatment methods. The only difference is that the detection itself is done by ATP measurement, which greatly shortens the time between the sampling and the results. The quicker resulting

makes it possibly to apply the hygiene information to process adjustments much easier than when using traditional techniques.

## 2. Summary

Many factors must be taken into consideration when choosing a tool or method for analyzing process hygiene. The primary factor in many cases is the need for speed – the time needed between the sample taking and the result. If the product or control point is not critical or the information is collected mainly for making long term follow-ups, the tools are often chosen from the traditional methods pool. This is also the case if information about the presence or absence of a specific type of microbe is needed. If, on the other hand, the information about the process hygiene is used to adjust the process itself or some other processes directly linked to it, like cleaning. Effective tools are normally found amongst the new rapid methods discussed in this presentation.

# PROCESS HYGIENE IN FOOD PRODUCTION

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## Abstract

Atria Oy is a Finnish food company that has three production facilities. The establishments are in Nurmo, Kauhajoki and Kuopio:

⇒ in Nurmo: pig slaughtering and cutting, poultry slaughtering and cutting, convenience food production, retail packaged food production, meat product department
⇒ in Kauhajoki we have bovine slaughtering and cutting establishment and
⇒ in Kuopio both pig and bovine slaughtering and cutting lines.

The turnover in year 2001 was 431 with personnel approx. 2200. Our market share here in Finland is around 20% (total in year 2001). Furthermore, we export in many foreign countries. We produce meat products e.g. whole meat, sausages and sliced meat products, retail packaged meat products, poultry, ready-to-eat and convenience products.

The food safety is based on a proper handling of the raw materials, appropriate production facilities, hygienic practices. Good manufacturing practises requires well-motivated and educated production personnel. In Finland there is a new requirement which is to be implemented for persons working with food. Person handling unpacked food products should obtain a hygiene certificate so called hygiene passport. In Atria we have a program that our regular food production personnel have this certificate before year 2004. Furthermore, we have system that ensures that our temporary workers pass this test before working in our establishments.

To maintain acceptable process hygiene in food production appropriate production facilities is needed. The material used in production site would be inert and easy to clean. There are production machines, which are quite simple, but then there is equipment, which contain many complex parts e.g. multihead scales or electrical control systems. Both simple and complex machines should

be handled appropriately to ensure that the foods, which are produced with this machinery, are according to quality requirements set by the company. The machines and other equipment should also be as problem free as possible for production. In addition they should be as simple as possible so that there is no "black corners" for cleaning practises.

The regular maintenance system of different machinery is necessary to ensure that the machines and equipment are working properly the whole time. In addition the maintenance work has also implications to the process hygiene. The proper action of different valves, conveyors etc is crucial to process hygiene. The Air ventilation system should ensure clean air in production facilities. The temperature of the production facilities are checked regularly (preferably with automatic system) and there should be a system which reacts instantly to any abnormalities found in the food production facilities.

In food production facilities there are many requirements which are set by the raw material. Perhaps the greatest interest of concern considering food safety is the correct temperatures in the production. The raw materials and final product should be stored in appropriate temperature. The production should be done in atmosphere is most suitable for each product. Different stages of the production should be separated to avoid cross – contamination. There should be separate area to handle various raw materials: unpacking of carton boxes, weighing spices etc. Many products contain six stages: storage of raw materials, pre-handling of raw materials, production stages (ex. heating), packing, storage of the final product and logistic chain to the customer.

Food production is a clean business. The raw materials should be as clean as possible. The production facilities should be washed and disinfected efficiently to ensure process hygiene. The cleaning practises in food production facilities require high knowledge of washing and disinfection practises. In Atria we have good co-operation with ISS Finland Oy which performs our cleaning practises. The co-operation involves daily discussions between our production personnel and the personnel performing washing and disinfection. The standard of cleaning is additionally verified by both internal and external audits.

The good manufacturing practises performed every day in the production lines are verified by own control systems. Workers, foremen and quality control make a team to ensure the food safety in Atria. Our raw materials and final products

have specifications, which are tested and verified by laboratory tests. The heath status of the workers is regularly checked, the production facilities are checked every day by observation of the workers and foremen. The results of these observations are recorded. The proper hygienic practises are verified daily. The standard of cleaning is furthermore tested regularly by microbiological sampling and for disinfection residues. Furthermore, our quality control makes process studies, in which the different variable process stages are evaluated via physical, chemical and microbiological analysis. These studies are a part of our risk analysis practises, where we describe our processes and evaluate any possible risks involved with each product. In addition we have made predictive microbiological modelling studies of our products to have theoretical information for our quality control which then can be applied in product development. The good manufacturing practises requires own control system to evaluate and follow-up the food safety. It is a tool to find any possible problems in the production, and furthermore it is a tool to further develop our process hygiene.

# FUNCTIONAL FOODS – ARE THEY SAFE?

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## Abstract

Functional foods are a new, interesting group among food staffs. Proper nutrition is becoming more and more important also in prevention and treatment of diseases. Functional foods are commonly called as foods with health effects. In a matter of fact to use food as a remedy is not a new invention. Already in the beginning of Western medicine food was claimed to be the best medicine. Also in the traditional Chinese medicine this has been known and used even much longer time.

The role of nutrition was fading in the medicine in the 21st century due to the invention of synthetic drugs. At the moment the interest in nutrition is increasing again, because of difficulties in financing the growing costs in healthcare in general and also in the development and discovery of new effective drugs. Many drugs have also unpleasant side effects or interactions with other drugs, which have awaken the experts as well as people to search other possibilities to treat and prevent diseases.

Although food is thought normally to be less dangerous than drugs, still lately there have been many occasions where also food poisoning has caused death. It is not only the question of the contamination of harmful microbes, but some new ingredients in foods could have been dangerous in bigger amounts. Even the vitamins (fat soluble) could cause intoxication taken in too high dosages.

In the development of functional foods there are several steps where the possible safety risks should be evaluated. It depends on the origin of the ingredient what kind of risk assessment is needed. Also the health effect which will be claimed should be based in clinical studies. Not only has the claim to be proven but also the novelty of the used ingredient could force for several other studies to show the allergenity, nutritional effects etc.

The whole cycle of food starting from the field and ending up not only to the fork but back to the nature should be clarified. Thus the functional food is a very special group of foods because it will need studies for the safety but also for health effect. Studies in health effect should be completed with the final end product, which means safety studies should have been finalised before the human studies for health effect could be started.

It is very important to make a good action plan for the whole process from the R&D until launching the final product, because the safety studies of the product are necessary to complete in several steps:

- Animal feeding studies
- Microbiological evaluation
- Definition of allergenity
- Analyses of nutritional composition.

Especially if the ingredient belongs to GMO's, also the environmental studies are needed. Animal studies will test e.g. the mutagenity etc. Allergenity is very important to know in order to label packages correctly as well as the information of nutrients. GMO products cause some more studies, because the risk for the nature should be assessed.

The clinical studies in order to show the health effect must be completed by the independent research organization. The studies should be run in placebo-controlled double-blind manner. Also the target group for the product should be defined before the study subjects are recruited. The results are recommended to be published in scientific journals. It is always important to evaluate also the risk of overdose, misleading marketing and long-term effects.

# THE SOURCE AND CONTAMINATION ROUTES FOR *LISTERIA MONOCYTOGENES* IN THE DAIRY INDUSTRY

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## Abstract

*Listeria monocytogenes* is an important food-borne pathogen and its source and contamination routes and detection is crucial in the food industry. Present investigation was directed towards the occurrence and distribution of *L. monocytogenes* in the whole distribution line (farm environment → milk from cow's → milk from containers at the farms → dairy factory environment → products) at one dairy in Iceland. More than 1400 samples were collected over the year in order to get possible season variation. No positive samples of *L. monocytogenes* were found in any product (n = 200) or in the dairy factory environment (n = 250). Samples of milk collected from farms during summer (n = 459) did not contain any *L. monocytogenes*. However, in the milk collected during the winter, thirteen (13) samples were found to be positive (n = 459). These results indicate there is a seasonal variation of the presence of *L. monocytogenes* in raw milk from this area. Three farms (out of 153) were positive for three consecutive weeks, but four farms were positive for one week out of three sampling occasions. For the traceability of *L. monocytogenes*, further samples were collected from all milking cows (n = 20) from one of the infected farms, together with additionally 30 samples from the shed environment and feed (silage). Results show that one cow and eight samples in the shed environment were positive for *L. monocytogenes.* All strains were further characterised by the use of molecular typing technique (Ribotyping) showing that all environmental strains and the strain isolated from the raw milk from this farm belonged to the same ribogroup. Three different ribogroups were observed from all *L. monocytogenes* isolated from seven different sources (farms).

# 1. Introduction

*Listeria monocytogenes* is an invasive food-borne pathogen that can cause serious disease in immunocompromised individuals and pregnant women [1]. It is common in many natural and manmade environments and can grow at refrigerated temperatures [2, 3]. Although *L. monocytogenes* rarely causes diseases in the majority of the population there is a zero-tolerance ruling issued by the U.S. Food and Drug Administration [4] in ready to eat foods (including dairy products) because of high mortality rates. High prevalence of *L. monocytogenes* in food processing environments and in ready to eat food [5, 6] that has undergone pasteurisation, indicate that the process environment represents a significant source of *L. monocytogenes* in the final products. The ability of *L. monocytogenes* to adhere to various food contact surfaces [7-9] and unusual growth and survival properties of the bacterium have supported these findings [3]. Other investigations, however, suggest that raw food material is the most likely source for *L. monocytogenes* [10−11].

More recently there has been an increase in listeriosis outbreak linked to the consumption of dairy products. The types of contaminated dairy products are diverse: pasteurised milk [12], soft cheese [12−13], soft and hard cheese from raw milk [14, 15], ice cream, [16] pasteurised chocolate milk [17] and butter [18]. The occurrence of *L. monocytogenes* in raw milk can vary to a great degree. Most investigations from Western Europe and US have shown the occurrence of *Listeria* to be between cero and five percent [1, 19, 20] but sometimes much higher in other areas as recently reported in Mexico [21]. Although there have been reports concerning the survival of *L. monocytogenes* during HTST pasteurisation [2223] it is generally accepted that pasteurisation temperatures kills the bacteria [24]. However, in dairy factory environments, *Listeria monocytogenes* has been shown to exist and to be persistent [25, 26]. This is especially important in dairies where many products are produced in open areas in a relatively small space due to possible cross contamination risks.

The advent of molecular methodology has revolutionised the possibility to investigate the ecology and pathogenicity of food borne bacteria such as *L. monocytogenes*. These new methods are i.e. multilocus enzyme electrophoresis, pulses-field gel electrophoresis (PFGE), random amplification of polymorphic DNA and ribotyping [5, 6, 27−30]. With the help of this methodology it has been possible to investigate the contamination patterns in foods and in the food

processing environment. Ribotyping is a highly automated and standardised method that has been used for molecular sub-typing and data analysis and was first introduced by DuPont Qualicon TM (Wilmington, DE, USA) in 1995. DuPont ribotyping system characterises and identifies bacterial strains by generation of ribosomal RNA fingerprints from bacteria [31]. The manufacturer has analysed more than 1300 *L. monocytogenes* strains to develop a database [32].

The aim of this study was to examine the occurrence and contamination routes of *Listeria* through the whole production line (farm environment → dairy products) in a small dairy by using ribotyping methods.

# 2. Materials and Methods

## 2.1 Isolation of *Listeria*

### 2.1.1 Isolation of *Listeria* from dairy products

The products investigated for the presence of *Listeria* were following. Pasteurised milk with three different fat content (0.1; 1.5; 3.8), hard cheeses (Gouda; two fat content, 17 and 26%), cottage cheese, soft blue cheese, pasteurised juice (three types), cotton cheese, butter, skyr (made from whey), brown soft cheese, cream and sour milk. The products were taken for five successive weeks both during summer- and wintertime. The total number of product samples was 200. Each sample represented ca. 5 ml or 5 grams of five units of one product which were added together to give exactly 25 ml or 25 grams. To each sample 225 ml of UVM broth was added and incubated for 24 h at 30ºC. From these enrichments 0.1 ml were transferred into 10 ml of Fraser broth and incubated for 48 h at 35ºC. Inoculation loops were used to spread samples from each Fraser broth tube to MOX agar plates, which thereafter, were incubated for 48 h at 35ºC. Suspected colonies (black/silver) were transferred on Tryptic Soy Yeast Extract agar plates and incubated for 24 h at 35ºC before gram stained and catalase activity testing. Finally, after growth for 24 h, at room temperature, in Tryptic Soy Yeast Extract broth, the isolates were checked for mobility with the hanging drop method. To distinguish *L. monocytogenes* from other *Listeria* species a MiniVidas (Model 12 BioMérieux Vitek, Inc. France) was used according to the manufacturer's description.

### 2.1.2 Isolation of *Listeria* from dairy factory environment

The occurrence of *Listeria* in various dairy factory environments was investigated for five successive weeks during both winter- and summertime. Samples were collected from various places in the dairy, i.e. various drainage systems, floors, cartons, packaging material and process surfaces. The number of samples was 250. The samples were taken with sterile cotton swabs. From each sampling site two hydrophobic and two hydrophilic cotton swabs were used. At dry places the cotton swabs were dipped into a phosphate buffer before swabbing. After swabbing, the swabs were placed into sterile 30 ml. plastic bottles and transported to the laboratory. To every sample, 25 ml of sterile UVM broth was added. Thereafter the isolation procedure for *Listeria* was as described above.

### 2.1.3 Isolation of *Listeria* from raw milk

The number of farms delivering milk to the dairy was 143, producing from 10.000 to 600.000 L of milk per year. Samples of raw milk were taken from all farms during three weeks during summer- and wintertime. Because of the high number of farms included in this study, 4–5 samples (farms) were mixed together. Portions of original samples were kept intact at refrigerated temperatures to trace eventual positive samples. One sample of 25 ml was added to 225 ml of UVM broth and further isolations procedure was performed as described above.

### 2.1.4 Isolation of *Listeria* from one farm

At one farm where raw milk was positive for *L. monocytogenes* for three successive sampling occasions it was decided to collect milk from all the milking cows (20) and from the environment of the shed from this farm. Samples were aseptically taken from every udder from all milking cows as well as from the stable environment (silage/drinking water/ floor). Samples were treated as previously described.

## 2.2 Media

Modified Listeria Enrichment Broth (UVM) and Fraser broth was used for enrichment of *Listeria* bacteria. For further characterization Modified Oxford

agar (MOX) was used. For gram staining and catalase test the bacteria were grown over night on Tryptic Soy Yeast Extract agar. For mobility test, Tryptic Soy Yeast Extract broth was used. All media were from Difco.

### 2.3 Ribotyping

Ribotyping was performed using the standard method of automated ribotyping device RiboPrinter System (DuPont QualiconTM) according to the manufacturer's instructions [31]. Cells were grown on Tryptic Soy agar plates (Difco, Detroit, MI, USA) overnight at 30ºC. Colonies were picked, suspended in lysing buffer and heat inactivated at 90ºC (10 min) and placed into the RiboPrinter®. The sequence of steps in the analysis include lysis of cells, digestion of DNA with *Eco*RI, separation of DNA fragments on agarose gel, transfer onto a nylon membrane and hybridization with a chemioluminescent labelled rRNA probe. The RiboPrinter System generates its own ribogroups in the database of the instrument.

## 3. Results

No positive *Listeria* samples were observed from any dairy product or in any of the samples collected from the factory environment (Table 1). However, thirteen (13) samples were found positive for *Listeria* (all *L. monocytogenes*) from the raw milk samples, all collected during winter time. Three farms were found to be positive for all three winter-sampling occasions and four farms were positive for one occasion. To be able to trace the isolates it was decided to choose one positive farm and collect samples from all milking cows and from the shed (environment and silage). One animal was found to be positive for *L. monocytogenes* and suffered from sub clinical mastitis. However, from the stable environment, nine (9) samples of *Listeria* were detected, whereof eight (8) were identified as *L. monocytogenes* and one as *L. seeligeri* (Table 1).

All *Listeria* strains were identified immunologically by MiniVidas and genetically by a RiboPrinter. Based on the Riboprinters system´s software and visual assessment of the data three distinct ribogroups were observed from *L. monocytogenes* strains (Figure 1). Each ribotype pattern generated between five to seven fragments. The sizes of the most typical fragments were two between 2.0 and 2.5 kbp (kilobase pairs used in the system), one at 4.0 kbp, two bands

between 5.0 and 6.0 kbp and one between 9.0 to 9.5 kbp. The distinction between ribogroup I and the other ribogroups is an extra band located at 12.0-13.0 kbp in ribogroup I whereas in ribogroup II, extra bands are located at 3.6 and ca. 35 kbp and in ribogroup III extra bands are located at 3.2 kbp and ca. 35 kbp. Finally no bands are located between 6.0 and 7.0 kbp in ribogroup III as is the case with the other ribogroups.

The strains isolated from raw milk from seven different farms (13 strains) were grouped into three ribogroups (Figure 1; Table 2). In ribogroup I, isolates were represented from farms nr. 1, 3 and 7 (five isolates). In ribogroup II, isolates were represented from farms 2, 4 and 6 (seven isolates) and in ribogroup III, one isolate was represented from farm 5. All strains isolated from the environment and from one milking cow (10 strains) from farm 3 belonged to the same ribogroup as the strains isolated from raw milk from that same farm (ribogroup I). Dendogram pattern generated from the ribotype data also shows the three ribogroups generated (Figure 2).

Most of the strains in ribogroup I (12 of 15) were identical to DUP-1030 database strains. The other three were identical to DUP-1023 strains. All seven strains in ribogroup II were identical to DUP-1039 database strains and the strain from ribogroup III was identical to DUP-1045 database strains (Table 2).

# 4. Discussion

This project is a part of a project within the Nordic countries focusing upon the hygiene in the dairy industry. In this investigation (the Icelandic part) the research was directed to the presence and contamination routes of *Listeria* in the dairy industry, especially the traceability of *Listeria* from producer to consumer.

*L. monocytogenes* can be transmitted by the consumption of raw milk and raw milk products or by the consumption of contaminated dairy products [13, 14, 16, 18]. In the food industry in general it is not clear whether ready-to-eat products are contaminated by *Listeria* from the process environment [5, 6] or from the raw food material [10, 11]. The situation in the dairy industry compared to other food sectors is probably slightly different since, in most cases, the milk is pasteurised. Thus it is reasonable to conclude that dairy contamination products gain the pathogen along the process line.

In this investigation the main aim was towards the occurrence and distribution of *L. monocytogenes* in the whole distribution line, from the farm environment to the final products. However, no positive *Listeria* samples were observed from the 450 samples taken from the factory environment, process equipment and from any of the selected dairy products (Table 1). This was rather surprising since other reports have shown that *L. monocytogenes* is relatively common in the food industry, both in process environment and in products [5, 33, 34]. More than 70% of the raw milk from the dairy investigated in this research is used for cheese production and it is indeed mostly in cheeses *L. monocytogenes* has been reported [12–14, 16].

However, *L. monocytogenes* was detected in 13 samples of raw milk (1.5%; Table 1) which is in the same range as previously reported from Western Europe and US [1, 19, 20]. To trace *L. monocytogenes* strains isolated from the raw milk, samples were collected from raw milk from all the milking cows and from the shed environment at a farm that was positive for all three winter-sampling occasions. Molecular ribotyping is a useful tool for identification and tracking of different strains and defines possible relationships among isolates [35, 36]. In this investigation the ribotyping yielded four different ribogroupes from the 23 isolates. All of the generated ribotypes were identical to *L. monocytogenes* patterns available except for one (*L. seligeeri*) (Table 2). Wiedmann and co-workers classified *L. monocytogenes* into three genetic lineages based on ribotyping and allelic polymorphism in the virulence genes *hly*A, *inl*A and *act*A. Lineages I consisted of isolates from humans, animals and foods, lineage II primarily from foods and animals and lineage III from animals [37]. All *L. monocytogenes* isolated in this investigation belonged to lineage II (Table II) and were probably contaminated through silage as has been reported by others [36, 38, 39]. The four different DUP strains (1023, 1030, 1039 and 1045) also seem to be very common in the food industry as reported from various investigations [33, 34]. The relationship between different strains is shown in Figure 2 with a dendogram. The three ribogroupes are clearly distinct from each other with the exception of three strains belonging to Ribogroup I (LF-5, LF-10 and LHM-3, all DUP 1023 strains) (Table 2: Figure 2). These strains seem to be genetically closer to LHH-5 which belongs to another ribogroup. The reason for this is probably caused by insufficient cell material used for these strains. This can be seen by separate runs for these three strains. The peak at 12–13 kb was significantly less as compared with the 1030 strains (results not shown), thus

allowing the programme software to group them together distant from the DUP 1030 strains although they were grouped into the same ribogroup.

Surprisingly no *L. monocytogenes* strains were isolated from the dairy factory environment but it would be interesting to compare presumable *L. monocytogenes* strains, present in the dairy environment, to the strains isolated in this project. Thus, further efforts will be made to isolate *Listeria* from the dairy factory environment. Additionally it is interesting to note that there seems to be a seasonal variation concerning the occurrence of *L. monocytogenes* from raw milk. The most reasonable explanation is the higher risk for contamination from the shed environment and contaminated silage during wintertime caused by long periods of time the animals are kept relatively tight together. Other reports have indicated higher prevalence of *L. monocytogenes* in the spring [40, 41].

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*Table 1. Total number of samples taken for the isolation of* Listeria monocytogenes *at one farm (shed environment, silage and raw milk), in raw milk from all farms, in the dairy process and in various dairy products as well as numbers of positive* Listeria *spp. and* L. monocytogenes.

| Sampling site | Number of samples | Number of positive *Listeria* samples | Number of positive *L. monocytogenes* |
|---|---|---|---|
| Dairy products | 200 | 0 | 0 |
| Dairy environment | 250 | 0 | 0 |
| Raw milk (cows) | 153* | 13 | 13 |
| Farm I: Raw milk from cows | 30 | 2 | 2** |
| Farm I: Drinking place | 13 | 5 | 5 |
| Farm I: Silage samples | 7 | 3 | 2 |
| Farm I: Floor samples | 5 | 1 | 1 |
| **Total** | **658** | **24** | **23** |

* samples were taken six times (three timed during summer and three times during winter)

** sample taken twice from the same animal

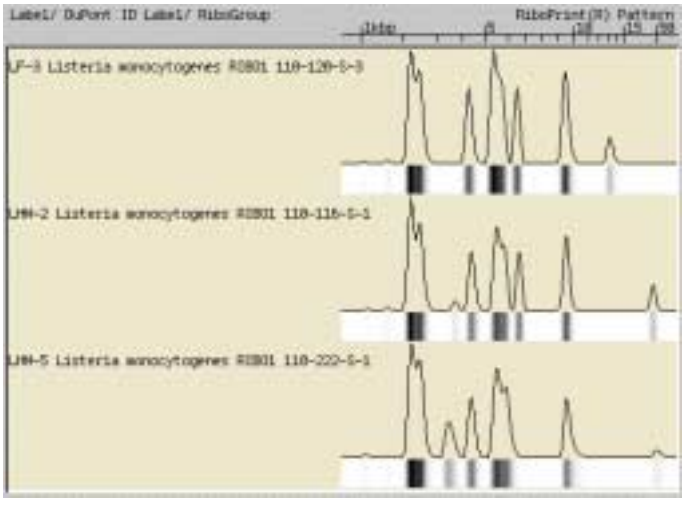


*Figure 1. RiboPrint patterns of three ribotypes of Listeria monocytogenes. The patterns are composites of individual patterns, the number varied from 1 to 15. From top: Ribogroup 1, Ribogroup II and Ribogroup III.*

*Table 2. Listeria monocytogenes ribotypes compared to DuPont database patterns.*

| Strains | Isolated from | Similarity between patterns | Identical to DUP strains | Lineage* |
|---|---|---|---|---|
| LHM-1 | Raw milk (Farm 1) | 0.92 | DUP 1030 | II |
| LHM-2 | Raw milk (Farm 2) | 0.94 | DUP 1039 | II |
| LHM-7 | Raw milk (Farm 2) | 0.90 | DUP 1039 | II |
| LHM-10 | Raw milk (Farm 2) | 0.92 | DUP 1039 | II |
| LHM-3 | Raw milk (Farm 3) | 0.91 | DUP 1023 | II |
| LHM-8 | Raw milk (Farm 3) | 0.93 | DUP 1030 | II |
| LHM-12 | Raw milk (Farm 3) | 0.93 | DUP 1030 | II |
| LHM-4 | Raw milk (Farm 4) | 0.94 | DUP 1039 | II |
| LHM-9 | Raw milk (Farm 4) | 0.94 | DUP 1039 | II |
| LHM-13 | Raw milk (Farm 4) | 0.92 | DUP 1039 | II |
| LHM-5 | Raw milk (Farm 5) | 0.96 | DUP 1045 | II |
| LHM-6 | Raw milk (Farm 6) | 0.94 | DUP 1039 | II |
| LHM-11 | Raw milk (Farm 7) | 0.95 | DUP 1030 | II |
| LF-1 | Farm 3: Environment | 0.95 | DUP 1030 | II |
| LF-2 | Farm 3: Environment | 0.94 | DUP 1030 | II |
| LF-3 | Farm 3: Environment | 0.95 | DUP 1030 | II |
| LF-4 | Farm 3: Environment | 0.94 | DUP 1030 | II |
| LF-5 | Farm 3: Environment | 0.90 | DUP 1023 | II |
| LF-6 | Farm 3: Silage | 0.92 | DUP 1030 | II |
| LF-7 | Farm 3: Silage | 0.89 | DUP 1030 | II |
| LF-8 | Farm 3: Silage | nd | DUP 1065** | nd |
| LF-9 | Farm 3: Raw milk (container) | 0.93 | DUP 1030 | II |
| LF-10 | Farm 3: Environment | 0.90 | DUP 1023 | II |
| LF-11 | Farm 3: Raw milk, cow | 0.95 | DUP 1030 | II |

* From Wiedmann *et al.* (1997).

** *Listeria seligeeri*

nd: not detected

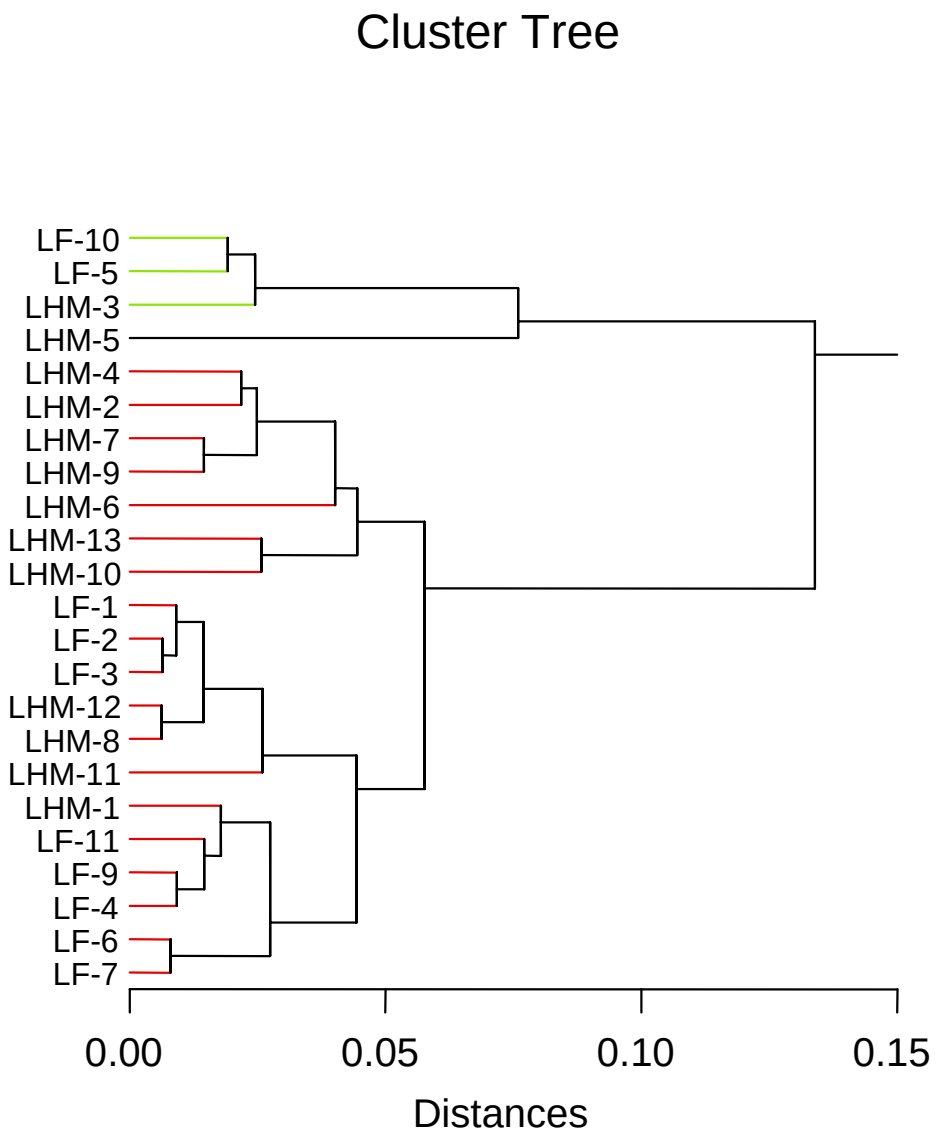


*Figure 2. Dendogram of the generated individual ribotypes. All LF strains and LHM-1, 3, 8, 11 and 12 strains were grouped into ribogroup I. Strains in LHM-2, 4, 6, 7, 9 and 13 are grouped into ribogroup II and strains LHM-5 was grouped into ribogroup III.*

# *LISTERIA MONOCYTOGENES* CONTAMINATION ROUTES IN FOOD PROCESSING INDUSTRY

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## Abstract

*Listeria monocytogenes* is a facultatively anaerobic bacterium, which grows at low temperatures, adheres to various surfaces, tolerates salt and may adapt to disinfectants, thus is very troublesome bacteria for food processing industry. Moreover, *L. monocytogenes* is a causative agent of foodborne outbreaks of human listeriosis and various food products; milk and dairy products, vegetables, salads, fish and meat products, have been associated with the illness. Thus prevention of *L. monocytogenes* contamination of food products, and thereby prevention of listeriosis cases is of major importance. The use of molecular typing techniques has expanded the knowledge of both the epidemiology of listeriosis and the contamination routes of food products. Molecular typing techniques have been used for characterization of isolates recovered in the processing environment, equipment, raw material and the final products in order to analyze sources of contamination. Contamination of products has been demonstrated occur mainly during processing and is due to persistent house-flora strains. The eradication of *Listeria* contamination has shown to be difficult and presumes specific measures, such as structural changes, dismantling of machines and targeted cleaning procedures including use of hot steam, hot air and hot water. Therefore hygienic aspects should be emphasized in the design of processing plant, processing lines and particularly processing equipment in order to prevent persistent contamination of the machines and thereby the spread of *L. monocytogenes* to products.

# 1. Characteristics of *Listeria monocytogenes*

*Listeria monocytogenes* is a common bacterium in nature. It is a causative agent of listeriosis, which occurs both in humans and in animals. Invasive human listeriosis is a rare, but a severe disease associated with high case fatality rate. *L. monocytogenes* was considered as a food-borne pathogen as late as in 1980's, even though it was recognized as human pathogen as early as 1926. Several food products have been linked with epidemics and sporadic cases of listeriosis including meat, dairy, vegetable and fish products. *L. monocytogenes* is a facultative anaerobic bacterium, thus able to grow in foods packaged under vacuum or modified atmosphere (Buchanan *et al.* 1989). *L. monocytogenes* can grow at wide pH range, pH 4.3–9.6. The minimum $a_w$ is as low as 0.90, but it can survive for extended periods even at lower $a_w$ values (Lou and Yousef 1999). *L. monocytogenes* has shown to grow at up to 12% NaCl (w/v) (Lou and Yousef 1999), thus limitation of the growth in foods by salt is unrealistic. Even though the optimal growth temperature of *L. monocytogenes* is between 30 and 37 °C, it is able to grow at refrigeration temperatures and has shown to survive even freezing (Seeliger and Jones 1986, Junttila *et al.* 1988). *L. monocytogenes* is susceptible for heat and is destroyed by pasteurization at 71.6°C in 15 s (Lovett *et al.* 1990). *L. monocytogenes* is sensitive to disinfectants commonly used by food processing industry (van de Weyer *et al.* 1993). Organic material has, however, shown to reduce the activity of disinfectants (Best *et al.* 1990, Aarnisalo *et al.* 2000) and bacteria growing on biofilms are shown to be more resistant than planktonic cells (Ronner and Wong 1993).

# 2. Contamination analyses

The understanding of *Listeria* contamination routes in food processing industry is of a major importance in order to prevent *L. monocytogenes* contamination of food products. Intensive sampling of various sites including raw material, product, processing equipment and environment, and food handlers among others is the basis of contamination analyses. The sampling of product at different stages of processing with environmental sampling at same time has proved to be an effective approach for tracing the bacterial contamination in food processing industry (Mäkelä and Korkeala 1987, Björkroth and Korkeala 1997). Characterisation of isolates of interest by molecular subtyping techniques has shown to be great advantages in tracing both pathogen and spoilage bacterial

contamination (Destro *et al.* 1996, Nesbakken *et al.* 1996, Björkroth and Korkeala 1997). The comparison of isolates recovered e.g. in different stage of process, in processing environment and equipment, in air, and in final product, makes possible to determine specific sources and sites of product contamination. Molecular approach has been used in contamination studies of spoilage organisms and various pathogens. A number of methods, e.g. multilocus enzyme electrophoresis, plasmid profiling, analysis of fatty acid profiles, ribotyping, RAPD, AFLP and PFGE typing, have successfully been applied (Dykes *et al.* 1993, Destro *et al.* 1996, Nesbakken *et al.* 1996, Björkroth and Korkeala 1997, Lin *et al.* 1998, Autio *et al.* 1999, 2000, Fredriksson-Ahomaa *et al.* 2000, Autio *et al.* 2002, Aarnisalo *et al.* 2003).

# 3. Typical features of *Listeria monocytogenes* contamination

Unlike many other organisms *L. monocytogenes* contamination has shown to be associated with the strains existing in processing facilities and it has been suggested that animal strains do not easily contaminate meat and are replaced by other better adapted strains (Boerlin and Piffaretti 1991, Nesbakken *et al.* 1996). The specific feature of *Listeria* contamination is prolonged processing plant contamination as it has been established that some *L. monocytogenes* strains have caused persistent plant contamination over several months or years (Unnerstad *et al.* 1996, Miettinen 1999, Lundén *et al.* 2002). The specific sites of contamination are probably numerous, but processing equipment have been suggested to be important sources as the contamination often occur after heat treatment (Rørvik *et al.* 1995, Ojeniyi *et al.* 1996, Miettinen 1999, Rørvik 2000, Aguado *et al.* 2001, Norton *et al.* 2001).

Contamination of products mostly occurs during processing. The sites associated with contamination are processing equipment, such as mechanical saws, brining, slicing, dicing, freezing, and packaging machines and conveyors. Thereby machines are considered as essential niches of the bacteria and they are important sites of product contamination (Rørvik *et al.* 1995, Ojeniyi *et al.* 1996, Autio *et al.* 1999, Miettinen 1999a, Rørvik 2000, Aguado *et al.* 2001, Norton *et al.* 2001). The equipment are very complex and often have narrow opening and dead areas, and the routine dismantling in order to conduct efficient cleaning and disinfection procedures is cumbersome.

Airborne contamination has been associated with bacterial contamination (Björkroth and Korkeala 1997, Rahkio and Korkeala 1997). However, in *L. monocytogenes* contamination air-mediated contamination may not be of major importance, as in several studies no Listeria has been found from air samples taken in food processing industry (Jacquet *et al.* 1993, Salvat *et al.* 1995, Autio *et al.* 1999). However, Spurlock and Zottola (1991) experimentally showed that high populations of *L. monocytogenes* survives in aerosol suspensions for hours, thus practices encouraging extensive aerosol formation should be avoided in a food processing environment.

Even though contamination of product has shown to be associated with processing, the initial origin of processing plant contamination is obscure. It is well known that *L. monocytogenes* is present in raw materials both of animal and plant origin, and in nature (Seeliger 1961, Fenlon *et al.* 1996, Fenlon 1999, Autio *et al.* 2000), thus it may be introduced into processing environment by several ways such as via incoming raw materials, other materials, incoming traffic and people.

# 4. Eradication and prevention of *L. monocytogenes* contamination

The eradication of *Listeria* contamination has shown to be difficult and presumes specific measures, such as structural changes, dismantling of machines and targeted cleaning procedures including use of hot steam, hot air and hot water (Autio *et al.* 1999, Miettinen *et al.* 1999, Lundén *et al.* 2002). Therefore hygienic aspects should be emphasized in design of processing plant, processing lines and particularly processing equipment in order to prevent persistent contamination of the machines and thereby the spread of *Listeria* to products. In addition to specific niches the lay-out and maintenance of facilities as well as working and cleaning routines may effect on the persistence of contamination, and it has been shown that well-maintained facilities had preventive effect on *Listeria* contamination in processing plants (Rørvik *et al.* 1997).

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# PERSISTENT *LISTERIA MONOCYTOGENES* PLANT CONTAMINATION

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## Abstract

*Listeria monocytogenes* causes a serious illness, listeriosis, in 20 to 50 people per year in Finland. *L. monocytogenes* causes sepsis, meningitis, meningoencephalitis, abortions, or febrile gastroenteritis. The pathogen is food borne and several epidemics have been linked to different food items. The preventive measures have focused on elimination of the bacteria from food processing facilities. However, the elimination of *L. monocytogenes* from food processing plants has proven to be very difficult. *L. monocytogenes* has been shown to persist i.e. cause prolonged contamination in meat, dairy and fish processing plants. The contamination has been observed to persist in plants in several years despite regular cleaning and disinfection procedures. Especially certain *L. monocytogenes* strains persist in the plants while other strains are of a sporadic nature. The elimination of the persistent strains is important as they dominate in the food plants and cause a continuous contamination pressure on the products. Reasons leading to persistent contamination are not clear but some factors influencing the survival of the persistent *L. monocytogenes* strains, such as enhanced adherence to food contact surfaces and differences in disinfectant resistance, have been recognized. The processing machines have also been shown to harbour persistent *L. monocytogenes* contamination due to complex structure and poor hygienic properties.

## 1. Introduction

*Listeria monocytogenes* is a food borne pathogen that causes a serious disease, listeriosis. The first scientifically proved food related listeriosis epidemic occurred in 1981 in Nova-Scotia in Canada where coleslaw was reported as vehicle of *L. monocytogenes* (Schlech *et al.* 1983). Since that *L. monocytogenes*

has been linked to several food borne epidemics with a range of different food products acting as vehicles. In Finland there has been two listeriosis epidemics, one due to cold-smoked rainbow trout in 1997 (Miettinen *et al.* 1999b) and another one due to contaminated butter (Lyytikäinen *et al.* 2000), resulting in 5 and 25 cases, respectively.

The prevention of contamination of food products with *L. monocytogenes* in food processing plants has proven to be difficult. *L. monocytogenes* contamination has been observed to persist for extended periods of time in different food processing plants (Rørvik *et al.* 1995, Nesbakken *et al.* 1996, Unnerstad *et al.* 1996, Autio *et al.* 1999, Giovannacci *et al.* 1999, Miettinen *et al.* 1999a, Senczek *et al.* 2000, Chasseignaux, *et al.* 2001, Norton *et al.* 2001, Suihko *et al.* 2002, Hoffman *et al.* 2003) despite of routine cleaning disinfection procedures. It has also been observed that some strains are capable of causing persistent plant contamination while other strains are of sporadic nature (Rørvik *et al.* 1995, Nesbakken *et al.* 1996, Unnerstad *et al.* 1996, Autio *et al.* 1999, Giovannacci *et al.* 1999, Mettinen *et al.* 1999, Senczek *et al.* 2000, Chasseignaux, *et al.* 2001, Norton et al. 2001, Suihko et al. 2002, Hoffman et al. 2003). These observations on persistent contaminations and persistent strains versus non-persistent strains have raised the question whether the persistent strains have properties that enables survival in food processing plants for extended periods of time. The persistent contamination causes a serious threat towards the product safety. Increased knowledge of persistent strains and non-persistent strains is valuable in planning preventive measures in the food processing plants.

## 2. Properties of persistent and non-persistent strains

*Listeria monocytogenes* has been shown to adhere to food contact surfaces in several studies. It can adhere and form biofilm on commonly used food contact materials such as stainless steel, rubber, and plastic surfaces (Mafu *et al.* 1990, Wirtanen and Mattila-Sandholm 1993, Blackman and Frank 1996, Sinde and Carballo 2000, Beresford *et al.* 2001). Adherence and biofilm formation is important in the survival in food processing plants as the adhered and biofilm cells show markedly increased resistance towards stress factors. Adherence to surfaces has been shown to increase the resistance of *L. monocytogenes* towards

e.g. disinfectants and heat (Frank and Koffi 1990, Ronner and Wong 1993, Oh and Marshall 1995, Aarnisalo et al 2000). Both of these factors are in an important role in the cleaning and disinfecting procedures.

Persistent *L. monocytogenes* strains have been observed to show increased adherence to stainless steel surfaces (Norwood and Gilmour 1999, Lundén *et al.* 2000). Norwood and Gilmour (1999) observed that persistent strains belonging to the serotype 1/2c showed the most efficient adherence to stainless steel after 24 h contact time. Lundén et al (2000) showed that persistent strains showed enhanced adherence to stainless steel surfaces after a 2 h contact time. It was concluded that the enhanced adherence of the persistent strains after short contact time might influence the survival and initiate the persistent plant contamination.

Disinfectant resistance has been shown to differ between *L. monocytogenes* strains. Aase *et al.* (2000) observed that disinfectant resistance to benzalkonium chloride, a quaternary ammonium compound (QAC), differed between 200 *L. monocytogenes* strains the minimum inhibitory concentration (MIC) ranging between 2 µg/ml and 7 µg/ml. Three persistent strains out of 4 were shown to possess a high initial resistance towards the benzalkonium chloride. The high initial resistance was suggested to influence the survival in the food processing plants. Lundén *et al.* (2003) observed also differences in the disinfectant resistance between persistent and non-persistent strains. *L. monocytogenes* strains have also been observed to adapt to disinfectants. *L. monocytogenes* has been shown to adapt to QACs, tertiary alkylamine and sodium hypochlorite (Aase *et al.* 2000, Lundén *et al.* 2003). Differences in the adaptive responses of persistent and non-persistent strains have not been shown. However, the adaptation of *L. monocytogenes* strains to disinfectants may influence the survival in food processing plants.

Persistent *L. monocytogenes* serogroup 1 strains have been observed to show more often cadmium resistance than non-persistent serogroup 1 strains (Harvey and Gilmour 2001). The persistent serogroup 1 strains have also been observed to produce more often monocin E than the non-persistent serogroup 1 strains. Monocin E is active against *L. monocyogenes* serogroup 4 strains and *L. ivanovii*. It was suggested that the production of monocin E give the producing strains an advantage against other bacteria in the same ecological niche (Harvey and Gilmour 2001).

# 3. Processing machines

Food processing machines have been shown to be in an important role in the contamination of processed foods. There are several studies showing that processing machines have sustained *L. monocytogenes* contamination and transferred the contamination to the products. Miettinen *et al.* (1999a) showed that *L. monocytogenes* contaminated a dairy plant over 7 years. The contamination was localized in the packaging machine and the conveyor belt of the packaging machine. The contamination was eradicated when the packaging machine and the conveyor were cleaned and sanitized with improved methods. Autio *et al.* (1999) showed that the contamination in a fish processing plant was mainly due to the contamination of the brining, slicing and packaging machines. Lundén *et al.* (2002) observed that the relocation of a dicing machine from one plant to another transferred *L. monocytogenes* contamination. The dicing machine was transferred from plant A, which was found contaminated with a certain *L. monocytogenes* genotype (type 1), to plant B. *L. monocytogenes* type 1 contamination was soon after the transfer observed in plant B in the dicing machine and the dicing line. The contamination persisted in plant B. The dicing machine was further sold to plant C where the *L. monocytogenes* type 1 contamination was soon observed in the dicing line and the dicing line. The contamination persisted in the dicing line in plant C over one year. The contamination was finally eradicated when the machine was taken apart every second week and cleaned and disinfected with alkaline-acid-alkaline washes. The adherence capabilities of the persistent *L. monocytogenes* type 1 strain were investigated and it was observed that the strain shoved enhanced adherence to stainless steel after short contact time. This study elucidates that processing machines with poor hygienic qualities and *L. monocytogenes* strains with enhanced adherence may lead to persistent contaminations of processing lines (Lundén *et al.* 2002).

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# HYGIENE ASPECTS IN CHOOSING FOOD PROCESSING EQUIPMENT

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## Abstract

When choosing food processing equipment, factors considered are the operational reliability, costs, economical efficiency of use, reliability of manufacturer/importer, availability of maintenance, availability and price of spare parts, and cleanability. Hygienic design of food processing equipment is an essential part of maintaining high food safety and quality. It will also reduce the time and effort required for cleaning the equipment and so in long-term reduce the costs of cleaning.

In Finland and in Europe the most important legislation for hygienic equipment design of food processing equipment is in the machine directive 89/37/EEC (revised 1998, 98/37/EC), which contains safety requirements and basic principles of hygienic design.

After 1995 all machines sold in EU have to have a symbol “CE” showing that they fulfil the criteria of the directive. To help the equipment manufacturers to fulfil the criteria, standards and guidelines are available from different organisations e.g.:

- CEN (The European Committee for Standardization)
- EHEDG (European Hygienic Engineering and Design Group) in Europe
- ISO (International Organization for Standardization)
- IDF (International Dairy Foundation
- 3-A (Sanitary Standards Committee)
- AISI (American Iron and Steel Institute)
- the National Canners Association
- American Society of Mechanical Engineers (ASME)
- Baking Industry Sanitary Standars Committee,) in USA and

- other national standards such as BS (British Standard), DS (Dansk Standard) and DIN (Deutsches Institut für Normung).

When choosing the equipment for food processing, the food manufacturer should consider, to which purpose the equipment is being used. Will the equipment be used for only one purpose or can it be used for many purposes?

An important thing to consider is also what type of product is going to be processed with the equipment. Furthermore, we need to know the hygiene requirements for the product product. Is it going to be further processed or is a ready-to-eat product? What type of soil is left on surfaces of equipment after use. Food manufacturer should consider how often the machine is going to be used, how often the equipment will be cleaned and dismantled and where it is going to be placed. The equipment should be cleanable in routine cleaning processes.

The factors which effect on the cleanability of food processing equipment are:
- the constructions of the equipment
- materials of construction
- cleaning procedures used (cleaning agents, disinfectants, time, temperature, pressure, mechanical force, frequency)
- personnel using, cleaning and maintaining the equipment
- placement of the equipment
- cleanliness of air and surroundings of the equipment
- lubricants used for the machine and
- type of product processed with the equipment.

The constructions of the equipment should be cleanable and as simple as possible. All surfaces especially in contact with food should be easily reached in routine cleaning procedures. Dead legs must be avoided. Screwheads and bolts on product contact side of the equipment are not recommended. Metal-metal connections should be avoided. When joints are needed, they should preferably be welded and the welding should be smooth. The correct function of seals should be checked regularly. If possible, panels containing electronics should not be situated in places, where water is needed for cleaning. The equipment should also be situated so, that the cleaning procedures are as easy as possible and so that there are no potential contamination sources just next to the equipment.

For product safety, the most important surfaces are the surfaces in contact with product. The surface materials should be cleanable, non-absorbent, durable and they should not contain scratches or crevices which may harbour soil and microbes. A surface roughness value ($R_a$) of $\leq 0.8$ is recommended. When choosing the materials, the conditions, where the equipment is going to be used, are important. E.g. if the equipment will be washed with water of high temperature, the materials must tolerate this. No toxic or otherwise harmful substances are allowed to get into the product from the surfaces.

Cleaning procedures used should be selected according to the process and materials of the equipment. If hygienic design of the equipment is good, less cleaning agents and stress on equipment surfaces can be used.

Personnel using, cleaning and maintaining the equipment must be trained in hygiene aspects. E.g. the maintenance personnel unavoidably touch a multitude of surfaces in contact with unpacked products. For example, they dismantle machinery for cleaning procedures and reassemble it after cleaning as well as maintain machinery operation during production. The responsibility who cleans the surface after the maintenance work must be clear to all workers.

Lubricants used for the machinery can cause microbiological problems. Samples for microbiological analysis of the lubricants used in the equipment should be taken to determine the frequency how often lubricants should be washed off the surfaces completely and changed to new.

A 3-year project "Hygienic equipment in the food industry" co-ordinated by VTT Biotechnology was launched in 2001 together with University of Helsinki Faculty of Veterinary Medicine, National Technology Agency of Finland, Foundation of Food Research and with six companies: two equipment manufactures, two cleaning agent companies, a cleaning company and a maintenance company.

The aim of the project is to produce information of the hygiene problems of equipment in the food industry: Which are the most problematic equipment and why are they problematic. In this project improvement in the hygienic level of the equipment used in the food processes are produced in co-operation of participants.

The activities of the EHEDG organisation are also introduced to Finnish companies in the project. For collection of information, an enquiry was sent to Finnish food companies concerning the equipment hygiene. In the presentation, some results of this enquiry, are presented.

# HYGIENE REQUIREMENTS IN THE FERMENTATION PROCESS

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## Abstract

Although modern control systems have made sterilisation of fermenters and fermentation media in a small scale very easy, understanding the system design may well help in finding the cause when problems arise. In larger fermentation systems understanding engineering solutions and the reasons behind them will be very valuable, if contamination problems occur. Valve systems configurations with steam traps play an important role in sterilisation of fermentation systems. Avoiding dead legs, remembering "six tubediameter" rule and the nature of hydrophobic membranes will help in successful fermentations.

In this presentation system design is considered more than calculating times and temperatures to achieve a certain Z-value or calculating heat transfer across a fouling tubular heat exchanger used in continuous sterilisation. Calculations of this kind can be found for example in Raju and Cooney (1993). Some comments are also presented in comparing batch and continuous sterilisation of fermentation media.

# 1. Introduction

In biotechnological production different levels of asepticity are used: from beer fermentations simple boiling of the "medium", wort, for 1 to 1½ hours to excluding the last bacterium/spore in animal cell fermentations. A fermentation process is very sensitive to failures in asepticity, because in fermentation process, usually, good growth conditions prevail. If the contaminant has a better growth rate, it will overgrow the actual process organism (Figure 1).

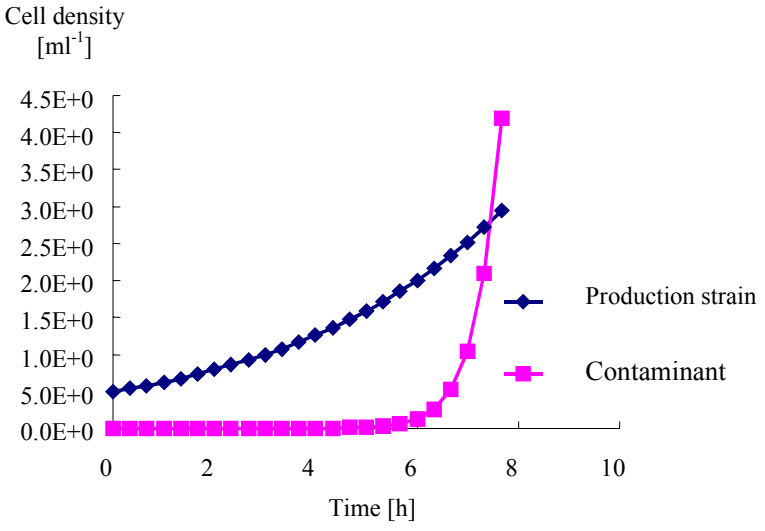


*Figure 1. Cell densities with time when the ratio of contaminant: production strain is 1:1 000 000 and the doubling times are 20 min and 180 min.*

The methods used to fulfil the needed asepticity level of the fermentation process – and the product – can be divided into thermal and non-thermal methods. The latter include filtration, UV-light, γ-radiation, the use of sterilising chemicals and some other methods and are not dealt in this presentation to a large extent. The thermal methods include autoclaving and direct use of steam and this last method is the main topic of this presentation.

The use of steam is the most common method to sterilise equipment and materials used in fermentation processes. The recommended temperatures for steam sterilisation are presented in Table 1 and the usually chosen combination is 121°C for 15 minutes. The "standard" steam pressure used is 1.5 bar (gauge), which gives a good margin of safety (Oakley, 1994).

## 2. Vessel sterilisation

Basically, the sterilisation of a vessel is easy. In practise there are many possibilities to fail: cold spots, temperature probe wrongly placed, condensate not removed etc. Manufacturers offering their own designs and automated protocols have seemingly removed all the need for the operator to understand the design, the number and the use of the valves and steam traps. But to ensure the

required level of asepticity every time the operator and the people purchasing the equipment should be well familiar with different designs involved with vessel sterilisation.

*Table 1. Recommended temperatures and times for steam sterilisation (Medical Research Council, Kelsey 1959, Perkins, 1959).*

| Temperature [$^{o}$C] | Time [min] | Time [min] |
|---|---|---|
| 116 | 30 | |
| 118 | 18 | |
| 121 | 12 | 15 |
| 125 | 8 | |
| 126 | | 10 |
| 132 | 2 | |
| 134 | | 3 |
| | Kelsey | Perkins |

In laboratory scale fermentations and small industrial scale fermentations (say up to 1m$^3$) sterilisation seems to be a very straight forward procedure: medium placed in the fermenter, the button is pushed and all goes automatically: 121$^{o}$C for 20 minutes is maintained. Let the fermenter cool down and start working. Understanding is needed, but only when things start to go wrong. In a larger scale fermentations the sterilisation of the vessel is usually separated from the medium sterilisation. The reason for this is the long heating and cooling times of large, filled industrial fermenters (up to 1.5 million litres).

In the Figure 2 a well-designed vessel sterilisation assembly is presented (Oakley, 1994). From the figure 2 it is clear that, the number of valves involved in sterilisation of a whole vessel (vessel, sparger line, air inlet filter, air outlet filter, media inlet line, transfer line and CIP line) is large. Any false closure or opening or even leakage can cause a contamination of the fermentation process. Automation, of course, eases the operational procedures, but if the design and/or the placement of the valves is incorrect, the reliable sterilisation will remain a dream. To understand the whys of the design it may be better to take the whole design in parts and look the items separately.

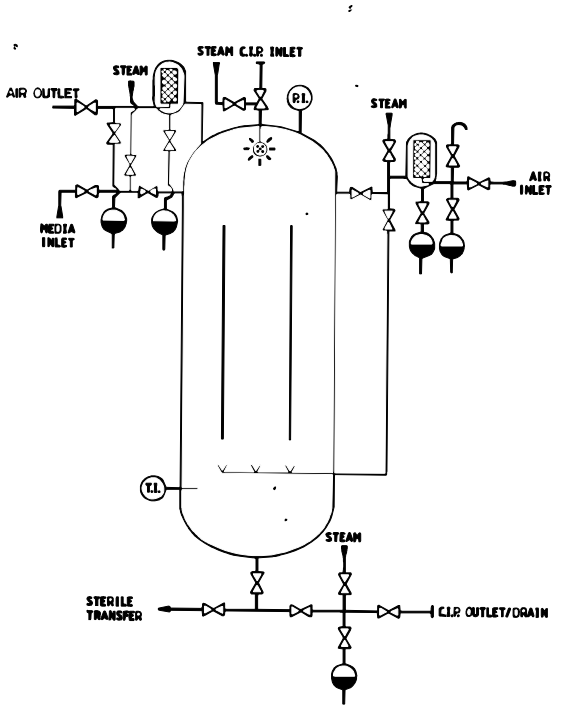


*Figure 2. Valve configuration for sterilisation of a whole vessel (Oakley, 1994).*

## 2.1 Sparger line and dip pipe

The valve configuration for sparger line sterilisation is presented in Figure 3 (Oakley, 1994). At least in larger fermenters the sparger should have drain holes additionally to the aeration holes. When steam sterilising the vessel the sparger line is steamed first, until the pressure in the vessel reaches about 0.5 bar (gauge). In the initial phase only the valve B is open (A is closed). After reaching the pressure the both valves A and B (Figure 3) are open. A dip line should be steamed from both sides (inside and outside) at the same time. The line above the closing valve in the dip line must be sterilised, too.

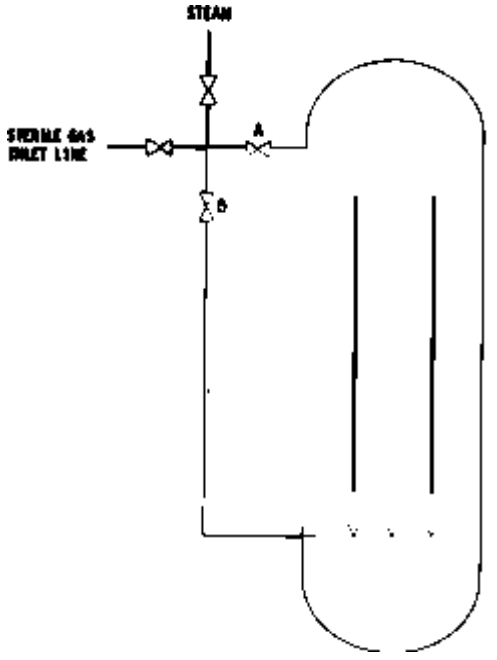


*Figure 3. Valve configuration for sterilisation of sparger line (Oakley, 1994).*

## 2.2 Vessel outlet

The steam will form condensate during the sterilisation and this condensate must be removed from the equipment, because it protects the contaminants from the steam. The outlet of the vessel must be at the very lowest point of the vessel in order to drain properly. As emptying the product and CIP solutions from the vessel is important the vessel outlet serves the three purposes. This leads to multiple lines and need for careful valve positioning underneath the vessel. A good design is shown in Figure 4, right (Oakley, 1994).

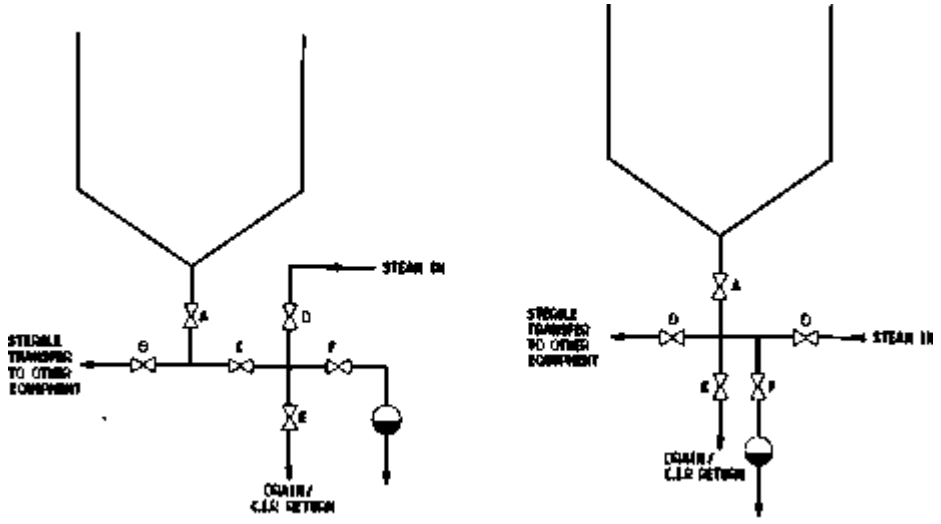


*Figure 4. Left: A good valve configuration for vessel outlet. Right: A poor valve configuration for vessel outlet (Oakley, 1994).*

During the sterilisation of the vessel, valves A, C and F are open and B, D, E are shut. Emptying is done via valves A and B and during cleaning the valves A, C and E are open and B, D and F shut. This valve configuration allows the vessel to be sterilised and filled from top with sterile medium. All the time at least two valves separated the sterile vessel contents and unsterile outside world. The part of piping between valves A, B and C is steamed, too, so the bacteria have to cross two valve seats in order to get into the vessel. In Figure 4 a poor configuration of outlet valves is presented. In this configuration the drain CIP valve forms a dead leg and a contaminant may be left under he valve seat and therefore the transfer line is under a risk of contamination.

## 2.3 Agitator shaft seal

The sealing for agitator shaft in the fermenter should be a double mechanical seal and the steam and valves for this are provided by the manufacturer. The valve arrangements for the double mechanical seal are left outside of this presentation.

## 2.4 Air filters

Air and vent filters provide a barrier that prevents contaminants from entering the sterile vessel. The sterile side of the filter faces the vessel, obviously, but some filters work both ways and then there exists a possibility to assemble the housing so that the vent and bleed valves of the housing are on the sterile side. Which is not a good thing for sterility. The modern air filters are hydrophobic membranes which means that sterilisation of them requires care: rupture and wetting must be avoided. To avoid wetting the steam must be dry. For this reason draining of condensate in the incoming and in the outgoing line must be arranged.

A correct arrangement for air filter sterilisation is presented in Figure 5 and three incorrect ones in Figure 6. The configuration A and C in Figure 6 can wet (blind) with the condensate, because steam is likely to push the condensate up the air line into the middle of the air filter. Once the water is inside the filter it has no way out because of the hydrophopicity of the membrane. The arrangement in B in Figure 6 steaming the vessel through the air filter poses a great stress on the filter and may rupture it.

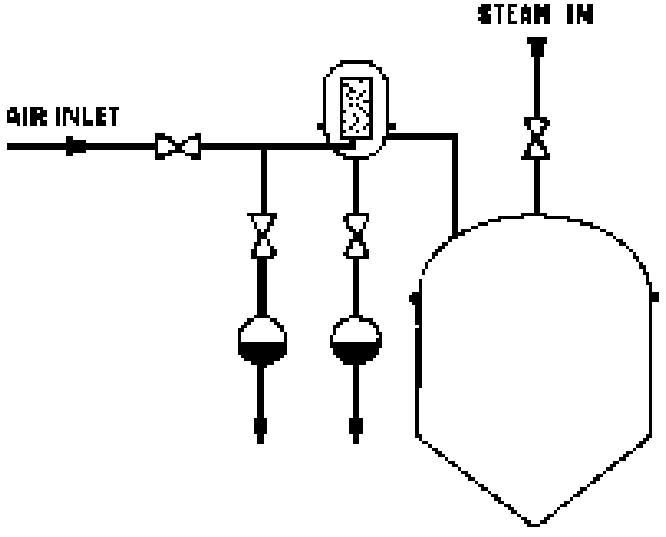


*Figure 5. A good valve arrangement for an air inlet filter (Oakley, 1994).*

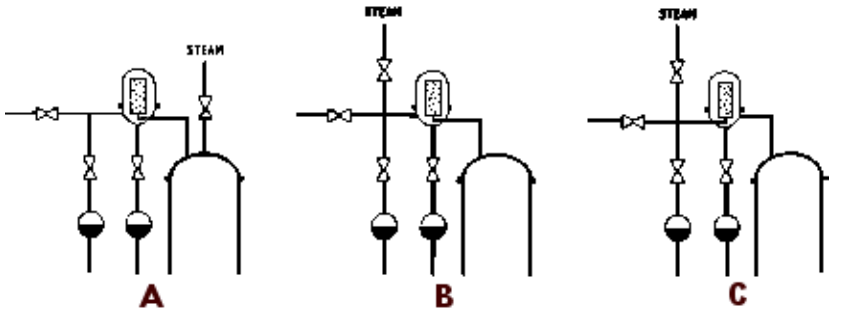


*Figure 6. Three poor valve arrangements for an air inlet filter (Oakley, 1994).*

## 2.5 Piping

The first thing in designing pipelines is that they must slope: a minimum of 1 cm per 1 m is recommended. This applies also between pipe supports, not just for the whole line. The lines for sterile transfers should be designed so that they can separately sterilised. This will increase the amount of valves and causes again potential dead legs. For materials and joints see e.g., Holah and Timperley (1999), but welding is recommended. TriClamp™ and other hygienic flanges can be used, too.

A dead leg can form a cold spot and this is illustrated in Figure 7. When steam enters a cold pipe, it forms condensate, which will further cool down, while travelling the pipe. When this condensate is pushed past the steam trap it travels down the pipe and is stopped by the next valve. The condensate fills the pipe between the valve and the connection to the trap. The rest of the condensate and steam will go the trap/drain. Now, if the length of the dead leg is more than six times the diameter of the pipe, it is possible that the temperature at the valve is not high enough to kill the spores/microbes. And the sterilisation has failed. This "six pipediameter" rule i.e. that the length of a pipe is less than six times its diameter, is important, not only in transfer lines, but in all systems.

An example of sterile transfer of an inoculum is illustrated in Figure 8. The two vessels are connected with a flexible pipe (or other type of pipe) at A and B. Direct steam injection from valves J and G is used to sterilise the contents of the fermenter via valves D, E and F. During this stage valve C is closed and I and H are slightly open to remove the condensate and bleed the steam. At the completion of sterilisation valves J, G, H and I are closed, while valves F, E and

D are left open. The medium is cooled under sterile air (connections not shown). After cooling the valve C is opened and inoculum culture is transferred into the fermenter. Finally, valve C is closed and the transfer line is sterilised with steam.

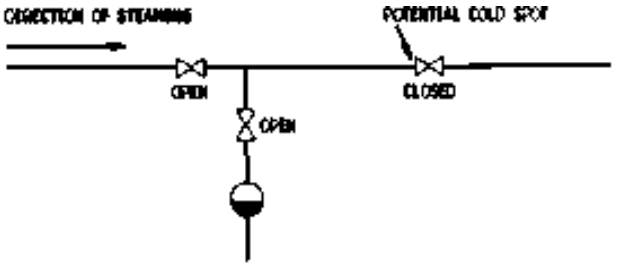


*Figure 7. A cold spot with a dead leg (Oakley, 1994).*

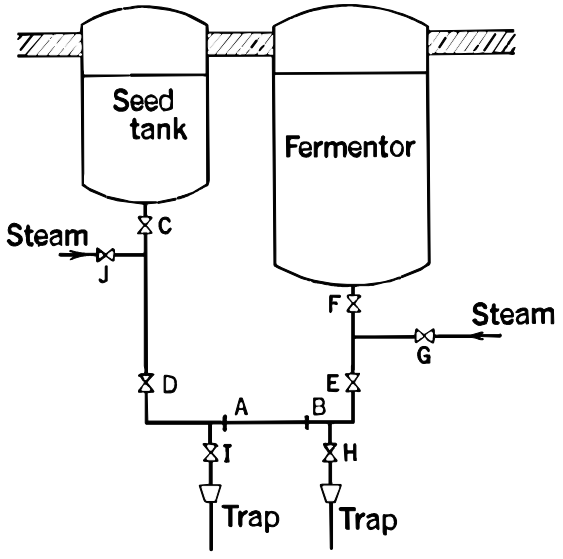


*Figure 8. A transfer line design (Aiba* et al.*, 1994).*

## 3. Sterilisation by filtration

Some components of a fermentation medium may not stand heat, e.g. vitamins, and they are sterilised by filtration. These filters are easier to steam sterilise – if they stand it! – because they are not hydrophobic and water can pass the

membrane. Today very efficient sterile filtration systems can be bought from a number of suppliers. In the pharmaceutical production sterile filtration is much used and of high standard, therefore this presentation leaves it to that.

# 4. Medium sterilisation

Two goals of the medium sterilisation process are a sterile medium with minimal damage to nutrients. The contradiction of these goals comes into play with increasing scale. This is schematically shown in Figure 9 (Raju and Cooney, 1993). $V_1$ and $V_2$ are to volumes of nutrients to be sterilised $V_2 > V_1$. Although the concentrations of contaminants (assumed or otherwise) are the same, the actual numbers differ. Therefore, the larger volume needs more time to reduce the number of remaining contaminants to the pre-set level. This leads to an increase in the sterilisation time ($t_2$–$t_1$), but as the concentration of nutrient decreases at a constant rate, the remaining concentration of the nutrient is lower in the larger scale. The above example also alleviates the benefits of continuous sterilisation of fermentation media.

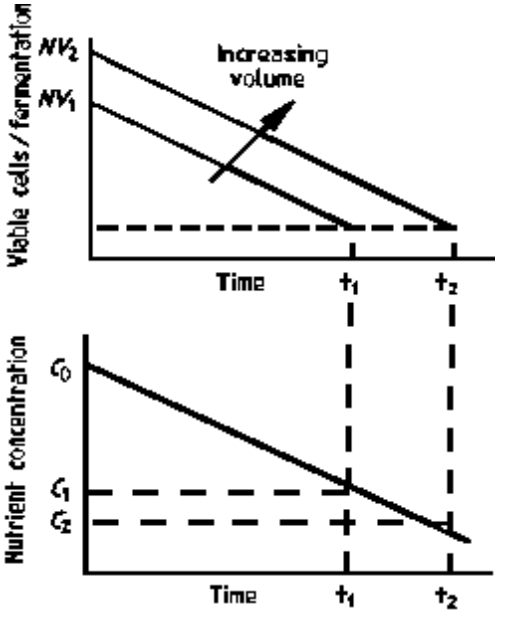


*Figure 9. Sterilisation at two different scales (Raju and Cooney, 1993). N = number of contaminants, V = volume of medium $V_1 < V_2$, t = time and C = consentration of a nutrient. Subscripts 1 and 2 refer to cases.*

The continuos medium sterilisation can be carried out by direct steam injection as in Figure 10. This process provides a rapid temperature increase of the medium and very rapid temperature decrease. A drawback is slight dilution of the nutrients, which may sometimes be critical.

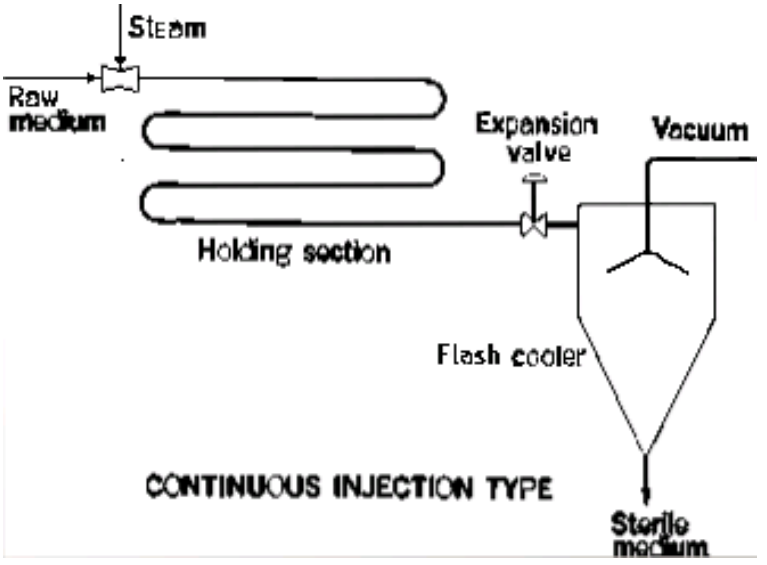


*Figure 10. Continuous sterilisation by direct steam injection (Aiba* et al.*, 1973).*

The other continuous sterilisation process uses heat exchangers (Figure 11). The process uses the hot medium to warm up the cold medium and steam is used only in the last (part of) the heat exchanger. The exchanger can be plate or tube type. Spiral heat exchangers were used in the Pekilo process in Mänttä, Finland to sterilise a side stream of a pulp process to produce a feed protein. The process was continuous and used three parallel fermenters 420 $m^3$ each and the feed tank was 1700 $m^3$. The warming up and cooling down are slower with heat exchanger process than with direct steam injection and the hot surfaces are prone to fouling, but the dilution of the nutrients is avoided.

# 5. Conclutions

To avoid problems and to solve those that have occurred knowledge about design of equipment, especially valve systems used in sterilisation processes is useful. The aim of this presentation was to point a few useful things for the larger scale fermentation systems.

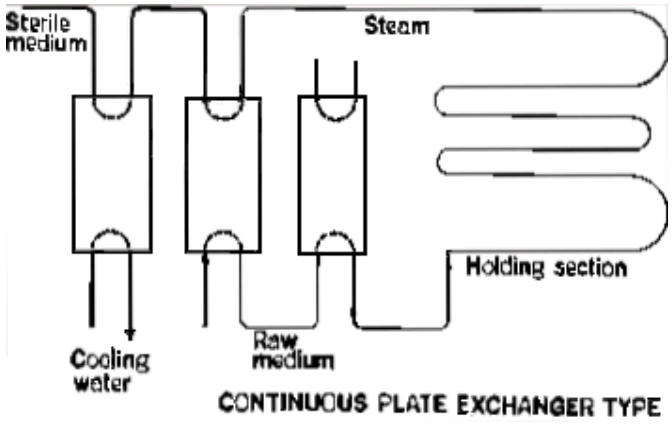


*Figure 11. Continuous sterilisation by direct steam injection (Aiba* et al.*, 1973).*

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# RESISTANCE PHENOMENA IN DAIRIES DUE TO DISINFECTION

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## Abstract

The focus on hygiene in the been questioned if misuse of disinfectants has imposed a selective pressure and contributed to the emergence of disinfectant resistant microorganisms in these environments. We have investigated the occurrence and resistance of microorganisms food industry has resulted in an increased use of chemical disinfection. It has surviving fogging disinfection and in disinfecting footbaths in dairies. Bacteria were isolated from about 75% of the footbaths tested and none of the disinfectants used totally prevented bacterial survival. *Serratia marcescens* isolated from footbaths containing Tego were resistant to the recommended in-use concentration. Six microorganisms isolated from two Norwegian dairy production plants after cleaning and fogging disinfection with alkyl amine/peracetic acid has also been characterised. The isolates were identified as three *Methylobacterium* sp., one *Rhodococcus erythropolis*, one *Sphingomonas* sp. and one *Rhodotorula mucilaginosa*. Four isolates as well as the two control strains *Pseudomonas aeruginosa* and *Staphylococcus aureus* were subjected to bactericidal suspension tests using the recommended user-concentrations of six different commercially available disinfectants. The *Sphingomonas* sp. and the *S. aureus* showed little resistance, while the other isolates showed resistance to several disinfectants. Biofilm growth experiments on stainless steel indicated that the *Sphingomonas* sp. has a much higher ratio of early attachment than the other isolates, indicating the possibility that biofilm growth serves as a survival mechanism for this less resistant isolate.

# 1. Background

The aim of disinfection is to reduce the number of microorganisms present on food-contact surfaces thereby avoiding contamination of raw materials and products with pathogens and spoilage organisms. When the disinfection process fails, this can in most cases be explained by the use of to low disinfectant concentration, temperature, exposure time or failure in the cleaning process leaving soil on surfaces to be disinfected. However, in some cases bacteria survive after an apparently effective cleaning and disinfection program. One explanation for this could be that the susceptibility of the bacteria present in the process facilities is lower than expected. The recommended in-use concentration of disinfectants is often based on laboratory suspension tests and laboratory strains. Therefore, the range of bacteria tested in the documentation of the disinfectant applied may not reflect the flora present on the food production equipment.

For the users of disinfectants in the food industry and in other applications, it is most relevant to define resistance as survival in practical use. However, since the efficacy of disinfectants is strongly related to the testing method used, resistance should be used as a relative term in a scientific context. If a microorganism (or species) survives or grows in a higher concentration of disinfectant than another microorganism (or species) it is said to have higher resistance. Within a species, strains that survive (or are not inhibited by) a concentration of disinfectant that kills (or inhibits) the majority of the strains of that species will be termed resistant. The level of resistance varies considerably between different species, and the natural level of resistance of a species is termed the intrinsic resistance. An example of a microorganism with relatively high intrinsic resistance is the spore-form of *Bacillus* spp. Spores will survive most chemical disinfection processes used in the food industry. Another type of intrinsic resistance is the phenotypic, physiological adaptation of microorganism resulting from the growth conditions. Bacteria growing as biofilms on surfaces may survive 10-1000-fold higher concentrations of disinfectants than bacteria in suspension. Resistance may also be acquired by mutation(s) or acquisition of genetic material (plasmids, transposons). An example of microorganisms with acquired resistance is Staphylococcus sp. harbouring plasmids with genes encoding efflux of quaternary ammonium compounds.

## 2. Resistance to disinfectants among food-related bacteria

Quaternary ammonium compounds (QACs) are commonly used disinfectants in the Norwegian food industry. Screening of food-associated *Pseudomonas* spp., coliforms, *Listeria monocytogenes*, *Staphylococcus* spp. and lactic acid bacteria showed huge differences in the level of resistance and the frequency of resistant strains within each species. It was also demonstrated that bacteria isolated from food processing equipment after cleaning and disinfection are more likely to be resistant than bacteria isolated from raw materials and food products. Gram-positive bacteria were in general more susceptible to QACs than Gram-negative bacteria. The isolates with highest resistance to QAC among all strains tested were isolated after disinfection with a QAC and identified as *P. fluorescens* and *S. marcescens*. (Langsrud *et al*. 2003b).

Resistance to QACs and other tenside-based disinfectants is well documented in the scientific literature, but less attention has been paid to oxidative antibacterial agents, such as hypochlorite- and peroxygen-based disinfectants. Enhanced resistance has been described for bacteria surviving disinfection with hypochlorite (Higginbottom *et al.* 1964).

## 3. Resistance to disinfectants in dairy isolates

### 3.1 Resistance of bacteria isolated from disinfecting footbaths

Disinfecting footbaths are used for elimination of microorganisms on footwear to prevent cross-contamination between areas with different hygienic level. A questionnaire about the use of footbaths was distributed to 30 Norwegian dairies. The most commonly used disinfectant was hypochlorite followed by amphoteric tensides. Microbial analysis of samples from the footbaths showed that bacteria could be isolated from all types of disinfectants used and bacteria were isolated from about 75% of the footbaths tested (Wirtanen *et al.* 2002). *S. marcescens* isolated from footbaths containing the amphoteric tenside TEGO were resistant to the recommended in-use concentration. A laboratory strain of *Serratia*

*marcescens* was susceptible. Also, one of the strains tested multiplied in user-concentration of TEGO. The isolates were cross-resistant to a QAC, but could be eliminated by hypochlorite or peracetic acid. The conclusion from the investigation was that disinfecting footbaths should not be used without regular cleaning and hygienic monitoring. (Langsrud *et al.* 2003a)

## 3.2 Resistance of bacteria isolated after fogging disinfection

Fogging disinfection is the use of finely dispersed droplets of a disinfectant within the production facilities. It has been demonstrated that fogging disinfection can reduce the microbial counts in the air and on surfaces (Burfoot *et al.* 1999). In this study, the effect of fogging disinfection was tested using contact agar plates before and after fogging disinfection in 5 dairies. A total of 10-19 control points were sampled in each dairy. The efficacy of the cleaning and the extent to which the fog filled the room varied greatly among the dairies and this was reflected in the microbial counts (Wirtanen *et al.* 2002). Six microorganisms isolated after apparently effective cleaning and disinfection in two dairies using rotational fogging with a peracetic acid based disinfectant and an alkyl amino acetate-based disinfectant were identified and further characterised. The microorganisms were identified as *Methylobacterium* sp. (3 strains), *R. erythropolis*, *Sphingomonas* sp. and *R. mucilaginosa*. Four isolates as well as the two laboratory strains of *P. aeruginosa* and *Staphylococcus aureus* were subjected to bactericidal suspension tests using the recommended user-concentrations of six different commercially available disinfectants. The *Sphingomonas* sp. and the *S. aureus* showed little resistance, while the other isolates showed resistance to several disinfectants. Biofilm growth experiments on stainless steel indicates that the *Sphingomonas* sp. has a much higher ratio of early attachment than the other isolates, indicating the possibility that biofilm growth serves as a survival mechanism for this less resistant isolate.

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# DETERGENT AND DISINFECTANT RESIDUE TESTING WITH PHOTOBACTERIA

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## Abstract

Fundamental to the HACCP philosophy is the identification of specific hazards and measures for their prevention. The method involves the identification of critical control points and procedures for monitoring them. The definition of a hazard includes unacceptable contamination of a biological, chemical or physical nature. However, the total cleanliness of the process facilities prior to initiating productions is based mainly on measuring the bacterial load with traditional microbiological tests. It is possible that the test result is not an indicator of the cleanliness, it only shows that there are no viable organisms on the surface. It is possible that there are clemical residues of the cleaning agents left on the surface. This is not allowed but usually no tests are run to avoid this.

The luminescent bacteria light inhibition method can be used to measure low amounts of residues both in liquids and on surfaces. In this study we present data on the method and results obtained in the food industry in Scandinavian countires during the 'DairyNet – Hygiene Control in Dairies' project.

## 1. Introduction

The luminescence inhibition method with *Vibrio fischeri* photobacteria is a useful tool to estimate the toxicity of different chemicals and effluents by measuring the reduction of light production due to interactions between bacteria and toxic compounds (Dutka *et al.* 1991). The method is rapid, because the incubation time is from 5 to 30 min. The test is standardized for water and effluent samples (ISO 11348-3, 1998). The test method can be used to measure residual cleaning agents and disinfectants on production surfaces (Lappalainen *et*

*al.* 2000). According to the findings there could be a need for this kind of test. However, there is very limited number of published data available about the situation in the food industry regarding the residues, because the chemical methots availabe are difficult and expensive to perform. Therefore a study was performed during the years 2001–2002 in Sweden, Finland, Norway, Denmark and Iceland as a part of the 'DairyNet – Hygiene Control in Dairies' project to obtain more data about the performance and usefulness of the test.

Because most of the people involved in this study had no previous experience about toxicity tests and photobacteria tests especially, the study was performed in three different phases. Phase one was the introduction to the method, reagent and instrumentation. The users were given detailed instructions and reagents to perform the test and after receiving acceptable results they moved to phase two. In phase two 10 unknown samples were shipped to the laboratories for an intercalibration study. In phase three samples from the production facilities were tested.

# 2. Materials and methods

## 2.1 Principle of the BioTox test for the detection of detergents and disinfectants

The test is performed by combining specified volumes of the test sample or diluted sample with the luminescent bacteria suspension in a cuvette. The parameter measured is the decrease in luminescence after a contact time of 5 min. The values measured are compared to a control sample and the intensity chances in the control sample are taken into account by using a correction factor when the results of the samples are calculated.

The test was carried out using *V. fischeri* bacteria from the BioTox kit (Aboatox, Turku, Finland). A standard ISO protocol was followed with the exception that the pH of the samples was not adjusted and the salinity was not adjusted for swab samples. The pH should be between 6–8 and salinity should be 2%. The light-producing *V. fischeri* bacteria were reconstituted from the freeze-dried vials with the rehydration solution and stabilized for 1 h at 4° C. Instead of adjusting the salinity of the liquid samples the salinity of the test bacteria solution was adjusted with 1 ml of 20% NaCl solution from the kit. The NaCl addition gives a final NaCl concentration of 1.7% during the measurement. For the swab samples

no salinity adjustment was done, because the sample volume is about 100 µl and the resulting NaCl concentration during the measurement same as with liquid samples. Dilution serie of disinfectants and cleaning agents was made in tap water.

The test was performed as follows in all phases. A 500 µl aliquot of *V. fischeri* suspension was transferred into the measuring cuvette and the light output was measured with a luminometer. This represents the light production of unstressed bacteria and was performed for the control tube and sample tubes. After this first measurement dilutions of detergents (500 µl) were mixed together with the bacteria, and the tubes were incubated for 5 min. The second reading was taken after the incubation, and the light production was measured from the control and samples. The effect of agents causing inhibition in light production was calculated and compared with the light output of unstressed bacteria. The results are expressed as inhibition percentage (INH%), which was calculated with BioTox software according to formulas [1] and [2]:

$$CF = IC_5/IC_0 \quad [1]$$

$$INH\% = 100-[IT_5/(KF*IT_0)]*100 \quad [2]$$

where
CF = correction factor
$IC_5$ = light production of the control after incubation (5 min)
$IC_0$ = light production of the bacterial suspension (control tube)
$IT_5$ = light production of the sample after incubation (5 min)
$IT_0$ = light production of the bacterial suspension (sample tube).

The effective concentration of the detergent tested that resulted in an inhibition of 50% of light production (EC-50 value) was measured and calculated according to standard for with the phase 1 results. For the factory samples only the inhibition percentage was calculated.

## 2.2 Measurement of selected samples (1st phase)

Laboratories were qiven instructions to test a dilution series from three different types of detergent or desinfectant samples normally used in the dairy. Results were calculated and assistance given if needed. This was done in order to see that the instrumentation was suitable for the testing in each laboratory and that the reagent handling was acceptable. A dose response curve was the target result for each chemical.

## 2.3 Measurement of supplied samples (2nd phase)

Ten coded samples were supplied in 4,5 ml tubes (chemicals and concentrations in table 1) to five different laboratories to six different users.The concentrations were chosen so that the results should show all types of results from clear inhibition to no inhibition. Also swabs were supplied except one laboratory. One of the samples was water sample (= zero sample and diluent for the samples). If a swab sample was measured it was first inserted to the sample liquid and then the stick was cut with scissors and left to the bacteria cuvette during the incubation and measurement. The samples were run in duplicate. Chemicals that were suggested to be rapidly disintegrated in diluted concentration were shipped in stronger concentrations and laboratories were instructed to do more dilutions from these. FIN 2 was the reference laboratory. They prepared the samples and tested the samples first.

## 2.4 Measurement of the samples from the dairy (3rd phase)

The participating laboratories were taking samples from a dairy and they tested the samples with the Biotox kit. Both swab samples and liquid samples were tested.

## 2.5 Results of the detergent residue test compared to total plate count results

Total plate count results were obtained from the same locations where the residue samples were taken. This was done in order to find out if there is a correlation between the residues and viable organisms.

## 2.6 Instrumentation

The test is based on the inhibiton of light and therefore most luminometers can be used for the test. The instruments used in this study we Unilite (Henkel Ecolab), Luminator (Henkel Ecolab), Lumax (Lumac) Charm LUMinator T (Charm Sciences), Luminometer 1253 (Bio-Orbit) and PD10 (Kikkoman).

# 3. Results and conclusions

## 3.1 Results from the 1st phase and 2nd phase tests

All participating laboratories were able to obtain a typical dose response curve from different types of chemicals with the photobacteria test in the 1st phase. They were able to perform the test reliably and repeatably. Therefore 2nd phase samples were shipped to the laboratories. Most of the problems encountered in the 1st phase were caused by the unsuitabillity of the laboratory's luminometer for the test (three out of five laboratories). The photobacteria are producing lots of light with the used volume and therefore some of the instruments we in overload or were not operating decently. Detailed information about the problems and possible corrective actions are not available and therefore no comments about different instruments are given. If a laboratory found out that they were unable to use their own instrument another instrument was used in the location (PD10).

The results from the supplied 2nd phase test samples are presented in Table 1. The chemicals used were I = isopropanol based, H = hypochlorite based and P = tensides.All laboratories received similar results (in the same order of magnitude) from chemical I and chemical P dilution series. With H there was much more variation in the results. One reason for this may be that the

laboratory with the smallest inhibition tested their sample several weeks later than the other laboratories. It is possible that chlorine in the sample is not any more reactive. The correction factor is reasonably low (from 0.562 to 0.981) in every test series and therefore the results are reliable.

With swab samples the variation between different users is much higher than with liquid samples. The correction factor with the water samples is much higher (0.124–0.660) and therefore the results are comparable only intraseries, not interseries. A small correction factor means in practise that a clean water sample together with the swab inhibit the light production. The sample volume is about 100 µl when swab samples are used and therefore the sensitivity of the test is different compared to the 500 µl liquid samples. A five times difference can have very high increase in the inhibition especially because the light inhibition of the bacteria is linear only in the double logarithmic presentation. It was noted during the testing that different users dipped the swab to the tube in a different way and the sample volume is not the same with everybody.

The negative values in the table represent induction of the light production compared to the control instead of inhibition. This is a very common phenomena with different chemicals within a certain concentration range.

## 3.2 Results from the dairy samples

Most of the samples taken in dairies were swab samples because it is easier to obtain a swab sample than a liquid sample. There were totally 501 samples from different locations in different countries. The inhibition values are presented in Figure 1a and 1b. In Figure 1a the samples are divided in different groups according to the result and in figure 1b the results are sorted according to the inhibition. The results are spread over the whole possible area under 100% inhibion (Figure 1b). If we compare the results obtained in the 2$^{nd}$ phase with theoretical diluted chemicals it is interesting to see that the situation is the same in the factories: there are samples with very clear inhibition (inhibition percentage over 50), samples with moderate inhibition (inhibition percentage between 20 to 50%) and also samples that induce the light production (inhibition value negative). About 40% of the samples showed a clear inhibition. In other words this means that there are residues before starting the production if no preventive rinsing is performed.

*Table 1. Results from the 2nd phase samples. H = hypochlorite based disinfectant, I = isopropanol based disinfectant, P = surface active detergent. The concentrations are in percentages. D = diluted by the operator. FIN 2 was the reference laboratory which prepared the sampes. Therefore their results are concidered as 'correct'.*

| Sample/ c | | Inhibition/ % | | | | | |
|---|---|---|---|---|---|---|---|
| **Liquid** | Tube | SWE | FIN 1 | NOR | DEN | ICE | FIN 2 |
| cf | 10 | 0,562 | 0,769 | 0,610 | 0,685 | 0,981 | 0,612 |
| H 0,1 | 1 | 99,4 | 100,0 | 100,0 | - | 100,0 | 100,0 |
| H 0,02 | 8 | 100,0 | 99,5 | 2,5 | 1,9 | 12,2 | 100,0 |
| H 0,005 | 9 | 19,0 | 6,4 | 7,6 | 10,4 | 14,9 | 97,3 |
| H 0,0017(D) | 9 | 29,8 | 13,3 | -3,4 | 16,6 | 23,8 | -25,0 |
| I 10 | 4 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 |
| I 5 | 6 | 100,0 | 99,7 | 100,0 | 100,0 | 100,0 | 100,0 |
| I 1 | 2 | 96,5 | 92,5 | 99,8 | 99,6 | 94,2 | 99,6 |
| I 0,3 (D) | 2 | 77,3 | 73,6 | 92,5 | 97,1 | 63,5 | 64,9 |
| I 0,17 (D) | 2 | 33,0 | 42,8 | 47,8 | 65,0 | 34,4 | 12,9 |
| P 0,15 | 5 | 99,9 | 99,2 | 100,0 | 100,0 | 99,6 | 99,9 |
| P 0,07 | 7 | 99,8 | 99,2 | 99,8 | 100,0 | 99,1 | 99,8 |
| P 0,015 | 3 | 61,4 | 57,4 | 82,2 | 71,5 | 33,4 | 50,2 |
| | | | | | | | |
| **Swab** | Tube | SWE | FIN 1 | NOR | DEN | ICE | FIN 2 |
| cf | 10 | 0,340 | 0,124 | 0,531 | | 0,660 | 0,353 |
| H 0,1 | 1 | 67,6 | 87,0 | 75,5 | na | 85,3 | 99,9 |
| H 0,02 | 8 | 3,9 | 49,8 | -8,5 | na | -8,9 | 43,0 |
| H 0,005 | 9 | -30,9 | 93,7 | 0,3 | na | -25,4 | -7,8 |
| I 10 | 4 | 56,6 | 99,9 | 94,5 | na | 20,9 | 99,9 |
| I 5 | 6 | 45,5 | 99,1 | 64,0 | na | 71,0 | 99,4 |
| I 1 | 2 | -15,1 | 58,2 | 18,2 | na | 25,2 | 47,3 |
| P 0,15 | 5 | 31,1 | 99,9 | 72,2 | na | 77,5 | 89,9 |
| P 0,07 | 7 | 32,8 | 63,0 | 47,4 | na | 55,0 | 80,6 |
| P 0,015 | 3 | 4,1 | 87,9 | 20,6 | na | 22,8 | 24,4 |

The best results regarding the residues were obtained with CIP systems. If the cleaing process is optimised, there are no residues (and also no viable organisms) after the cleaning. The highest inhibition values were usually obtained from places which are difficult to wash and no automated washing is possible to perform.

One of the dairies was followed for several times. When the residues were found during the first tests more attention was paid to the rinsing. The inhibition values dropped significantly in the last series.

a b

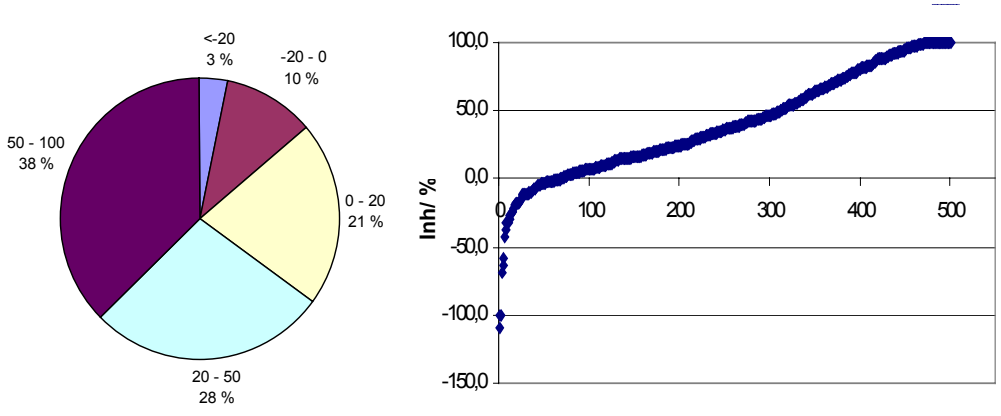


*Figure 1. Residue test results from dairies. 501 samples were tested. The results are divided in 5 groups according to the inhibition result (1a). The distribution of all results (1b) shows clearly that there are residues left on the surfaces in about 40% of the samples (inhibition greater than 50%).*

## 3.3 Residue results compared to TPC results

The preliminary results of the residue tests and total plate count results show that there is no correlation between the TPC results and residue test results. The microbial cleanliness is describing the whole cleaning process whereas the residue test measures only the residues after the last cleaning step. If only one production line is followed with the method during the washing cycles it is shows that the microbial load drops at the same time when the chemicals reach the location and the photobacteria are killed totally in five minutes.

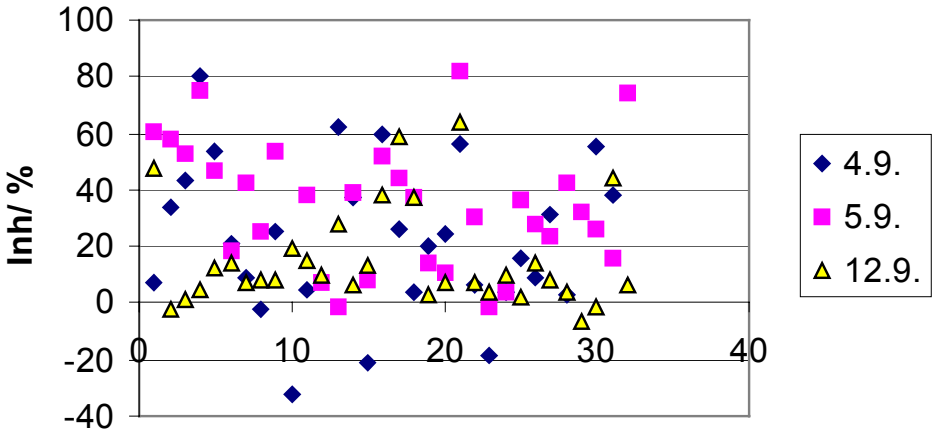


*Figure 2. One dairy was tested several times. The inhibition values are significantly smaller during the last series of samples (September 12) compared to the previous ones (September 4 and September 5) due to enhanced rinsing.*

The photobacteria method used in this study was rapid to perform because the incubation time is only 5 minutes. The distribution of the results show that residues do exist on the production surfaces prior to starting the production. The method offers a useful alternative for residue testing.

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# VALIDATION OF HYDROGEN PEROXIDE GENERATION METHOD FOR SANITATION OF PHARMACEUTICAL CLEANROOMS

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## Abstract

A variety of methods and antimicrobiological agents are used for decontamination and sterilization. For example, for periodic critical environment decontamination, conventional wipe down techniques with antimicrobiological products are widely used. A variety of products are applied but range in antimicrobial efficacy and material compatibility. In general, these methods are very time consuming and labor intensive. As an alternative, traditional fumigation with formaldehyde has been used for larger areas, but it is uncontrolled, variable, slow acting and, more importantly, has significant safety and health concerns. Further, although they can be validated for smaller areas, they are more difficult to ensure coverage and reproducibility over larger areas. Alternative, automated decontamination methods are becoming widely used due to ease use, higher levels of sterility assurance, cost savings and validatability. The most widely used and accepted is Vaporized Hydrogen Peroxide (VHP®) systems used for decontaminating a wide variety of isolated areas including rooms and separative enclosures.

# AUTOMATIC MILKING SYSTEM IN FINLAND

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## Abstract

Study of automatic milking system (AMS) in Finland started in summer 2000 and first AMS-unit started at the end of year 2000. The aims of study are to find out quality of milk, systems effect on udder health of cows and cows behaviour. Also interview study was done on 12 farms in August and September of 2002. Farmers with AMS experienced many advantages as less physical labour, more flexibility at work, better quality of work, better safety and better animal health compared to old milking systems. According to farmers AMS disadvantages are expensive and vulnerable system, user must be reachable 24 h a day, capacity too low and system uses too much water in washings.

# MILK QUALITY ON THE FINNISH AUTOMATIC MILKING SYSTEMS

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## Abstract

The chemical and microbiological quality of milk was monitored on Finnish farms with automatic milking systems (AMS). AMS-milk was compared to conventional parlour-milk. The somatic cell counts (SCC) and total bacteria counts (TBC) were determined to evaluate the quality of milk. The counts are also a criterion of milk price. Both SCC and TBC were elevated after the introduction of AMS. However, the mean values stayed below the E-class limits (SCC 2.5 $10^5$ cells/ml; TBC 5.0 $10^4$ cfu/ml). The fat, protein and lactose content were on the same level in both groups. TBC and coliformic bacteria were determined from the equipment before and after the washing. These samples proved that the cleaning process was effective.

## 1. Introduction

The physiological status, health, stage of lactation, (living) environment and feeding of the animal affects the quality and chemical composition of milk. Elevating SCC are usually a sign of mastitis. The TBC tells about the animal health, but also about the milking hygiene and the cooling of milk. When the conventional milking systems is used, the farmer is responsible for the cleaning of equipment and for detection of mastitis.

However, in automatic milking system the presence of man during the milking is not required. This means that the requirements of the AMS are high. The machine should prepare the cow for the milking, for example, clean the udders and teats and minimise the transferring of mastitis bacteria. The system should also reliably find those cows, which produce milk with a high SCC. Both SCC

and TBC have been negatively influenced when the AMS has been taken in use (Everitt *et al*., 2002; Rasmussen *et al*., 2002).

The cleaning system in AMS consists of three parts: the system cleaning, 2–4 times per day, the unit flush, after a cow with abnormal milk or after some idle time and the third is the cluster flush, to avoid cross contamination with pathogens between cows. The system cleaning can be a boiling water cleaning with alkaline and acid detergents or a circulation wash (Schuling *et al*., 2001).

The milk quality is a criterion of milk price in Finland. The upper limit of superior class (E) milk is 50 000 cfu/ml (2 months geometric mean) for TBC and 250 000 cells /ml (3 months geometric mean) for SCC. Since 1998 over 90 % of the Finnish milk has been in the E-class (Anon., 2003).

The first automatic milking systems (AMS) in Finland started to operate in November 2000. This study, which is a part of a larger Finnish research project, focused on milk quality, udder health and the behaviour of cows in AMS started at the same time. The aim of this work is to study the effect of robotic milking on the quality of milk.

## 2. Materials and methods

Three farms participated in this study: two private farms and the Helsinki University´s research farm. The reference group included the research farm (conventional milking parlour) and also selected private farms (26) having conventional milking system.

Bulk tank milk samples were collected two times a week during the first few months and later on once a week. From all samples the SCC, TBC, fat, protein, lactose and freezing point was determined. Total bacteria counts were determined with Bactoscan 8000 and the somatic cell counts with Fossomatic cell counter. MilcoScan 605 was used to determine for the fat, protein and lactose content in milk. The freezing point was analysed using a standard method (IDF 108B:1991/ETY 91/180).

The effectiveness of the cleaning process was evaluated using swabbing technique. The samples were plated on Plate count agar (Total bacteria count)

and incubated for 48 h at 30 °C (IDF 100B:1991). Coliformic bacteria were determined on VRB-agar by incubating for 24 h at 30 °C (IDF 73 A:1985).

## 3. Results and discussion

According to the present study the main composition of milk was not affected by the milking system. The fat, protein and lactose contents were at similar levels in both AMS-milk and conventionally milked milk. The freezing point was increased from –0.530 °C to –0.524 °C during the first six months. However, after this period it returned to the normal level. It is suggested that some of the higher values of freezing point may be caused by technical problems during the introduction period. Both the SCC and TBC elevated when the farms took AMS in use. The levels stayed below the E-class limits.

The samples taken to evaluate the cleaning process showed that the cleaning system works efficiently. The total bacteria counts before cleaning varied from 1.5 to 2.4 log cfu/100 $cm^2$ and after the cleaning procedure from 0.8 to 2 log cfu/100 $cm^2$. The amounts of coliformic bacteria were less than 10 per ml.

## 4. Conclusions

The present study showed that the AMS affected the hygienic quality of milk compared to conventional milking systems. The TBC and SSC were elevated in AMS but changes were not so extensive that they would influence the farmers income. Moreover the results of this study showed that in this case the cleaning process was effective.

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# MILKING HYGIENE IN AUTOMATIC MILKING

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## Abstract

Automatic milking unit prepares and milks the cow independently of human interference around the clock. Quality of milk of an individual cow is no longer detected visually, but the system informs the manager when there seems to be deviations in the parameters that the system measures. Cleaning of the udder and teats before milking and detection of abnormal milk are the main challenges of this new technology. Hygiene of the stalls, cows and the milking parlour are extremely important in barns with automatic milking system.

## 1. Introduction

The automatic milking system (AMS) is independent of human interference in the milking process. Major differences compared with conventional milking are variable milking intervals, increased milking frequency, different udder preparation, lack of visual inspection of foremilk, and quarter based milking. Milking hygiene is important in all milking systems, and has a direct effect on the quality of the end-product, i.e.; milk. Good milking hygiene includes effective udder preparation for milking, fluent milking process, reliable detection and rejection of abnormal milk and pipeline rinsing after milking. Automatic milking should not have an adverse effect on udder health, because good quality milk can be obtained only from healthy cows.

## 2. Udder preparation

The purpose of udder preparation is to initiate milk ejection reflex and clean the teats and udder before milking. Udder preparation methods vary depending on the type of the AMS. Automatic milking machines can use rotating brushes or separate cleaning cup that washes the teats. To date there is no method for

distinguishing between clean and dirty or injured teats. Unless programmed otherwise (for example cleaning of the teats of a dirty cow twice), the cleaning process is always the same. The outcome of cleaning cannot be assessed afterwards. AMS can also have problems in finding the teats. The cleaning devise may miss a teat and leave it dirty, and still AMS milks the teat. The new Finnish legislation presumes that AMS informs the manager about these missing cleanings.

According to a Norwegian study, 10 to 20 percent of cleanings were classified unsuccessful (one ore more teats missing) (Hvaale *et al*., 2002). The cleaning efficiency of AMS has been good when compared with manual cleaning when the teats have been dipped in bacterial spore suspension (Melin *et al*. 2002). According to practical experience no system can remove dirt from heavily soiled teats. In conventional milking the efficacy of cleaning depends on the milker.

## 3. Detection and rejection of abnormal milk

Foremilk should be visually investigated before milking according to the EU Hygiene Directive 89/362, in order to detect and reject abnormal milk from the bulk tank. In the AMS the detection of abnormal milk or milk from an inflamed udder is based on the electrical conductivity or other measurements from milk. To date there is not yet a complete agreement of the definition of abnormal milk.

Electrical conductivity is not a very reliable method for detection of abnormal milk or milk from inflamed quarter (Trilk, 2002, Knappstein *et al*. 2002). Milk colour can be detected by optical analysis as described by Espada *et al.* (2002). This could improve the detection of blood in milk, colostrum and sometimes changed milk from an inflamed udder. The detection method of abnormal milk can also be based on the amount of milk. Measuring milk yield is not a reliable method for finding inflamed quarters, if the cow has subclinical mastitis (Hovinen & Pyörälä, 2002). As many as 50 percent of the positive detections of abnormal milk can be false (Trilk, 2002; Knappstein *et al*., 2002; and Hovinen & Pyörälä, 2002). By combining different parametres like milk yield with electrical conductivity, the accuracy of the detection of subclinical mastitis can be increased (Maatje *et al*., 1997).

Problems in reliable detection of abnormal milk leads to insufficient rejection of abnormal milk from the bulk milk tank. According to new Finnish legislation this should happen as soon as abnormal milk is detected, before it enters the tank. Unsuccessful detection of mastitic cows may also lead to the spreading of mastitis between cows, especially if the equipment rinsing the cups between the cows is not working efficiently enough. On the other hand, false positive detection leads to rejection of normal milk.

# 4. Conclusions

In order to maintain good milking hygiene special attention should be paid on hygiene and udder health of the cows. Stalls and milking parlour as well as the cows should be kept clean. Udders should be kept hairless and cleaned manually when needed. Conventional methods for detecting abnormal milk or milk from an inflamed udder (for example milk cell count analysis) should be used regularly to inspect milk and the udder in addition to the methods used by the AMS until these are more reliable. By taking these actions it is possible to produce high quality milk from automatically milked cows.

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# COW BEHAVIOUR IN AUTOMATIC MILKING SYSTEM

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## Abstract

Automatic Milking System (AMS) provides the stock person with several new tools to monitor the cows' behaviour, e.g. data on activity and eating. The behaviour of a cow, its individual characteristics as well as social behaviour is emphasised in AMS. Hence, a stock person should be aware of the social structure of the herd in order to minimise social stress among cows and provide enough resources (enough space and time to eat and rest). Cow traffic arrangements are decisive in the behaviour point of view because it is not only the moving of the cow in the barn but also her eating, lying, and milking which is controlled by the traffic arrangements. Forced cow traffic, where the only option for the cow to go from the lying area to the feeding area is by passing the milking unit, can be used at the start to train the cows to use the milking unit. However, it is not recommended for sustained use because it may restrict the cows' behaviour. Feeding during milking is necessary in AMS, because the milking itself is not attractive enough for the cows to pay enough visits to the milking unit.

## 1. Introduction

The number of cows per farm is increasing in Finland. Thus, the amount of work needs to be reduced on the farm. Automatic milking system (AMS) is a potential labour-saving solution. In AMS the cow and the stockperson are the users of a new technology. Therefore, cow behaviour must not be forgotten when this new technology is introduced.

## 2. AMS and cow freedoms

According to the Five Freedoms (Brambell Commission) the welfare of an animal is fulfilled if the animal has freedom to express normal behaviour and freedom from:

- hunger and thirst
- discomfort
- pain, injury or disease
- fear and distress.

The Five Freedoms standards should come true in AMS, and welfare of cows should be at least at the same level as it is in conventional free stall system. Therefore, cows should have a possibility to eat ad lib roughage together and good-quality water should be available at all times. In addition, every cow should have at least one stall where she can lie down. This gives all the cows the possibility to rest together. In the context of AMS it is often mentioned that the cows have freedom to make their individual choices. However, the cows are highly gregarious animals and thus, synchronised behaviour belongs to their natural behavioural pattern (Castrén 1997; Boissou *et al*., 2001).

## 3. Possibility to move

The possibility of a cow to move in a barn with AMS is controlled by cow traffic arrangements. Traffic arrangements are decisive in the behavioral point of view, because it is not only the moving of the cow in the barn but also her eating, lying and milking, which is controlled by the traffic arrangements. The aim is to get the cows voluntarily to the milking unit at even intervals. The feeding and the lying areas and the milking unit can be separated by different gates, which either prohibit or allow the cows to move between these areas.

In forced cow traffic the only possibility for the cow to go from the lying area to the feeding area is by passing through the milking unit. In free cow traffic the cows have the possibility to move freely between these areas. Forced cow traffic can be used at the start to train the cows to use the milking unit. However, it is not recommended for prolonged use because it restricts the cows' behaviour. In free cow traffic the milking unit may not be attractive enough for the cows to go to be milked often enough (Ketelaar-de Lauwere *et al*., 1998). Possible

compromise between free and forced cow traffic is an arrangement where cows can freely move between roughage feeding and lying areas, but in order to reach the concentrates they have to pass through the milking unit (Ketelaar-de Lauwere, 1999).

## 4. Possibility to eat and rest

Roughage and water should be ad lib available for the cows and they should have enough time to eat (Lindström, 2000). With forced cow traffic there can be rush near the milking unit when several cows want to go feeding or milking at the same time. Rush can appear at the most favourable milking or eating times, during the maintenance or repair of the milking machine, and when too many cows behind the milking machine overload its' capacity. The high-ranking (dominant) cows will go first to the milking and the low ranking (subordinate) ones will wait and go after the higher ranking cows (Ketelaar-de Lauwere *et al*., 1996). While waiting for access to the milking unit the subordinate cows mainly stand idle. This time is out of their eating and lying time. However, it is equally important for the subordinate cows as it is for the high-ranking cows to have enough time to eat.

Due to their synchronised behaviour the cows should be able to eat roughage together. Therefore, enough space should be available for eating. In AMS the cows have to ''work in shift''. Thus, the synchronised behaviour of the cows is, in some extent, broken down and it is not possible for all of them to eat together. It is therefore, assumed that less eating space is needed in AMS compared to conventional loose housing system (Morita *et al*., 2000). However, there still remains a need to thoroughly examine the need of the feeding space in AMS. The resting time for the subordinate cows may be reduced in the forced cow traffic if the cows have to wait for the access to the milking unit. In conventional loose housing and also in AMS every cow should have at least one lying stall (Morita *et al*., 2000). Again, cows like to rest together due to their synchronised behaviour and on the other hand not all the cows can rest side by side due to the differences in their social hierarchy. Behavioural observations from Suitia research farm of the University of Helsinki showed that automatically milked cows lay less and stood more (resting: 48 vs. 56% and standing: 25 vs. 15% of observations) compared to cows in conventional loose housing.

## 5. Possibility to be milked

Concentrates should always be offered to the cow during milking. In a study by Prescott et al. (1998), milking itself was not attractive enough for the cows to pay enough visits to the milking unit. There were significantly more visits to the milking unit when concentrates were offered compared to the situation when concentrates were not offered. This phenomenon may be even clearer when the cow is low yielding (Prescott *et al*., 1998).

The efficient use of the milking unit demands that the machine is used constantly round the clock. Anyhow, there are more and less popular milking times during the day. The cows with lower dominance values may have to use the unpopular milking times whereas cows with the higher dominance values can go milking during the more popular times (Ketelaar-de Lauwere *et al*., 1996).

## 6. Possibility to go on pasture

AMS cows should have a possibility to go on pasture during the summer. Grazing with the AMS is easier if the pastures are in the vicinity of the AMS barn. Ketelaar-de Lauwere *et al*. (2000) observed that the cows did come into the barn from the pasture if the distance between the pasture and the barn was less than 350 meters. Wredle and Spörndly (2001) observed that if the distance between the barn and the pasture was 50 meters instead of 260 meters the cows returned more frequently from the pasture into the barn. Restricted grazing compared to the unrestricted grazing gives higher milking frequencies (Ketelaar-de Lauwere *et al*., 1999).

The willingness of the cows to be outside in the pasture or inside the barn was dependent on the outside temperature and the sward height. For lower sward heights cows were more inside the barn compared to higher sward heights and at higher outside temperatures cows were more inside the barn compared to lower outside temperatures (Ketelaar-de Lauwere *et al*., 2000). The cows clearly preferred lying in the pasture than inside the barn (Ketelaar-de Lauwere *et al.*, 1999). The cows also wanted to be outside although there was extra feed offered inside the barn (Wredle and Spörndly, 2001). The cows had the tendency to come from the pasture into the barn as a group, due to their gregarious behaviour, and this caused rush to the milking unit (Ketelaar-de Lauwere *et al*.,

1999). Using water as a way to persuade cows to come from the pasture into the barn (if water is only offered inside the barn and not on the pasture) is not ethical animal husbandry as water should always be ad lib available for the animals.

## 7. The impact of a stock person

AMS provides the stockperson with several new tools to monitor the cows' behaviour, e.g. data on activity and eating. Notwithstanding, the stockperson is responsible for the welfare of his animals. He makes sure that all the cows are properly fed, checks the health of the cows and detects their heats. In addition, the cleanness of the barn and animals is even more important in AMS compared to the conventional system. The stockperson should also maintain positive contacts with the cows to avoid animals' fear of humans and in this way assure easiness of handling. The behaviour of a cow, its individual characteristics as well as social behaviour is emphasized in AMS. Hence, a stockperson should be aware of the social structure of the herd in order to minimize social stress among cows and provide enough resources, space and time to eat and rest, for all his cows.

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# PRODUCTION OF IMMUNE MILK

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## 1. Background

Novatreat Ltd. (”the Company”) is a Finnish biotechnology company, founded in 1997, which specialises in developing and producing novel immune milk products for the treatment of antibiotic-associated hospital infections. Company’s roots date back to 1987 when the key founders began their research on colostrum in Valio, the biggest dairy co-operative in Finland.

The Company’s main innovations are related to collecting, processing, modifying and using bovine colostrum. Colostrum is produced by cows after delivery to provide the newborn calf with larger quantities of antibodies and growth factors than found in normal milk. These ingredients in the colostrum protect the calf from infectious agents as it’s own immune system is yet not functioning optimally.

There is increasing evidence that the use of colostrum by humans has many potentially beneficial effects. Novatreat’s approach includes modifying the natural colostrum by immunising the cows with specific immunogens, resulting in colostrum, which has specific effects against certain targeted pathogens, for example *Clostridium difficile*. Further, Novatreat’s proprietary processes allow the collection of the whey of the colostrums and concentration of this resulting in very high yield of the beneficial proteins.

The primary target end-users for Novatreat’s products are patients suffering from hospital infections, for example antibiotic-associated diarrhoea caused by *Clostridium difficile*. The prophylactic and therapeutic efficacy of the products is achieved through high concentrations of specific antibodies present in the colostrum whey concentrate from immunised cows.

# 2. Production overview

Novatreat's new production line is based on a proprietary design. It is the result of intensive development work to obtain the maximum yield of antibodies and retain the important compounds in colostrum. Novatreat will produce aseptically packed liquid products with a long shelf life, as well as later also powdered products of extremely high microbial quality.

The manufacturing of Novatreat's products has four distinctive steps:

1. Vaccine productions
2. Vaccination of the cows
3. Collection and handling of the colostrum from contract farms
4. Processing of the colostrum to the final product.

All these steps will be performed according to the prevailing US and EU regulatory requirements. Novatreat has already followed the main principles of GMP in development of the production line, Quality Assurance system and the new facilities. Novatreat is currently in the process of finalising the documentation for the manufacturing process, quality control, analytics and writing the corresponding Standard Operating Procedures.

# 3. Vaccine production

A key element of Novatreat's proprietary product concept is the immunisation by which colostrum rich in antibodies specific to desired pathogens are obtained. Important part of production is to have a vaccine that will effectively immunise the cow to get high amount of specific antibodies. Also these antibodies must react efficiently with the desired pathogen.

Once Novatreat has developed a vaccine with desirable activity, the production of the vaccine will be out-sourced to a licensed European veterinary manufacturer. The vaccines must be produced under licence for vaccine production to guarantee the health of the cows, as their milk and meat will be later used as normal food.

## 4. Vaccination of the cows

Effective immunisation protocol has been tested. It has proved to be technically feasible and there have not been safety problems. Novatreat has already vaccinated over 100 cows for the ongoing clinical trials. This was the first time cows had been vaccinated on private farms in connection with immune milk production in Finland. The logistics of large-scale immunisations was developed and basic data also for veterinary officials in safety issues was collected. The logistics of immunisation will be further developed and project to lower the number of required injections will be carried out.

## 5. Collection and handling of the colostrum from contract farms

Farmers collect, label and freeze the colostrum after calving. This system and its documentation have been tested both with normal and immunised colostrum.

Due to the good training level of the farmers and excellent hygienic level in farms this part of production is already working well. Currently the collection of normal colostrum is organised for large-scale production in cooperation with one dairy company. There is intensive development of logistics for collecting the frozen colostrum from the farm and transporting it to the factory going on. The same logistics system will be used also in the collection of the immunised colostrum.

## 6. Processing of the colostrum to the final product

The new process plant which is located at Turku BioValley has offices, laboratory and process space of 2350 square metres. This new production facility will have capacity in the first phase for 1000-litre batches, providing annual production of 500 000 daily doses of final product. The first phase capacity will is operational at the moment. In the second phase production will be carried out in 2000-litre batches that provide an annual maximum production of 2 000 000 daily doses of final product. The phase II systems will be installed in

2004–2005. The phase II production capacity should fulfil the production requirements at least until 2008. The colostrum process system consists of three major parts:

1. First part is handling of the raw colostrum with specially designed milk processing equipment to melt, skim and pasteurise the colostrum. The special design of this part is Novatreat know-how.
2. The second part is combination of tangential micro- and ultrafiltration. It makes possible to produce sterile liquid product that contains more that 95 % of the active antibodies from filtered colostrum. Filtering technology is the best production scale process method for processing high protein concentration liquids. Also the technology allows sterile filtering of the end products resulting in ready to use long shelf life and safe end product.
3. The third step is aseptic packing of the end product where special combination of sterile cartridge filtering and aseptic packing with Tetra-Brik packing machine is used. This unique system packs the product in active form into normal consumer packages that are easy to use. The final product can also be spray dried to powder type product. Novatreat has also tested the spray drying technology and the new facility has place ready for powder type product line.

This processing unit and the technology can be used to make all the planned immune milk products. In addition to this later also component type of products that can be added to other products can be made with this technology.

# SESSION ON CONTAMINATION CONTROL IN PHARMACEUTICAL PROCESSES

# FINALLY A NEW TEST METHOD FOR AIR FILTERS – EN779:2002

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Camfil Farr, Trosa, Sweden

## Abstract

During the past decade people have become much more aware about the environment and environmental concerns. Knowledge about the exterior and interior environment has increased significantly. At the same time, manufacturing and process industries have become more and more advanced. These trends, as well as requirements to conserve energy, are clearly reflected in today's choices of air filters for HVAC systems. Filters must meet stricter performance requirements and there has been a significant need for developing and establishing new test methods. When it comes to air filters for HVAC systems, the member states of the European Union agreed to revise the present filter testing method considerably. This has resulted in the development of a new European norm – EN 779:2002 – to determine a filter's fractional particle efficiency. Another new feature in this method is that the discharged or neutralized efficiency would also be tested and reported to give an indication of a filter's performance in real conditions with outdoor air.

## 1. Why introduce a new test method?

During the past quarter century air filters for HVAC systems have been tested in Europe in accordance with Eurovent 4/5, a number of national standards, and after 1993, in accordance with the common European test method EN 779. All are based on the U.S. norm and test method ASHRAE 52/68, dating from 1968. Laboratory tests based on this method are no longer sufficient for evaluating filter performance, in view of present-day requirements for good indoor air quality (IAQ) and environmental conservation [1].

To solve IAQ problems and regain confidence in the function of air filters, it is important that test methods reflect an air filter's performance "on-the-job", in actual operating conditions. CEN, the European Committee for Standardization – therefore proposed a revision to the EN 779 testing method. In August 2002, CEN's member states approved the new norm *EN 779:2002 Particulate air filters for general ventilation – Determination of the filtration performance*.

In the new revised norm, tests are conducted to determine a filter's fractional particle efficiency. The method provides greater knowledge about a filter's performance characteristics, making it possible to evaluate filter performance properties in relation to IAQ requirements and process demands and find better agreement between lab test results and filter performance in real operating conditions. However, to simulate the dust load, synthetic test dust is still being used from the 1968 ASHRAE 52/68 method, despite its inability to test and evaluate a filter's dust-holding, expected life, or efficiency in real operation.

## 2. EN 779:2002 test method

The procedures described in this standard have been developed from those in EN 779:1993 and Eurovent 4/9:1997. The basic design of the test rig in EN 779:1993 is retained with the exception of the equipment used to test the "dust-spot" atmospheric aerosol opacity. Instead, a challenge aerosol of DEHS (or equivalent) is dispersed evenly across the duct upstream of the filter that is being tested. Representative upstream and downstream samples are analyzed by an optical particle counter to provide particle size efficiency data for the filter.

Two different types of dust are used to compare and classify filters: one fine dust, DEHS, to measure efficiency as a function of particle size in the 0.2 µm - 3 µm range, and one coarse (ASHRAE dust) to obtain information about the dust-holding capacity and arrestance for synthetic dust. Filters with an efficiency higher than 98% on 0.4 µm particles are tested in accordance with EN 1822 (HEPA and ULPA filters).

Some types of filter media are dependent on electrostatic effects and can reach high efficiencies and a low pressure drop measurement in lab tests when they are tested with synthetic dust. However, in real operating conditions, particles in outdoor air neutralize (discharge) the electrostatic effect, altering the filter's

performance properties drastically. It is important that end-users of air filters are aware of this risk. The revised EN 779 test method therefore includes methodology for determining if efficiency depends on electrostatic effects (Figure 1), and if it does, quantitative information is provided about these effects to determine the filter's minimum life efficiency (MLE).

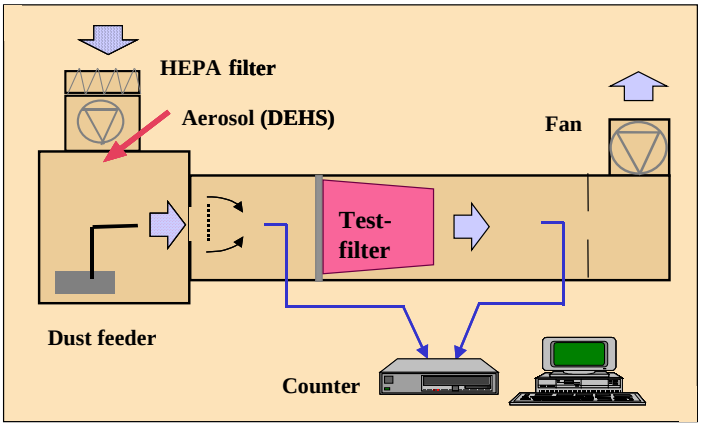


*Figure 1. EN 779:2002 test trig: test aerosol is added to the particle-free supply air. A particle counter is used to measure the filter's efficiency the new filter and then after the gradual introduction of synthetic dust.*

## 2.1 Test aerosol

The test aerosol consists of untreated or undiluted DEHS. Any other aerosol proven to have an equivalent performance may also be used. Test aerosol of DEHS (DiEthylHexylSebacate), produced by a Laskin nozzle, is widely used in performance testing of HEPA and ULPA filters, and experience has already been gained by users of the Eurovent 4/9 method. This challenge aerosol was also chosen for the following reasons:

- liquid aerosols are easy to generate in the concentrations, size range and degree of consistency required;
- the DEHS could be used both as a neutral test aerosol without charge, or it can be charged to the Boltzmann equilibrium charge level;
- The determination of the particle size of spherical liquid particles, using optical particle counters, is more accurate than would be the case with solid particles of non spherical salt and test dusts.

It is important that the aerosol is generated in a way that renders it neutral so that particles contain an equal balance of positive and negative charges (Bolzmann electrostatic charge distribution), which is accomplished with a beta or gamma radiation generator, or by a corona discharge ionizer. The aerosol is brought to the Boltzmann charge distribution to represent the charge distribution of aged ambient atmospheric aerosol.

### 2.2 Synthetic ASHRAE test dust

The filter is loaded with ASHRAE dust consisting of Arizona road dust (72%), cotton linters (5%) and carbon black (23%). Fifty percent of the particles in the Arizona road dust are to be larger than 10 µm in size by weight. In practice, the particles agglomerate and become larger when they are generated in a dust feeder. The atmospheric dust contains few large particles and differs sharply from the lab dust. Since ASHRAE dust has been used for more than 30 years, many filters have been developed to meet laboratory requirements instead of performance in actual applications.

## 3. Reporting in accordance with EN 779

In addition to the air flow and pressure drop, the test is also required to report a summary of measured efficiencies for a new filter, and then its efficiency after the addition of synthetic dust.

The filter's efficiency, as a function of particle size, is determined when the filter is new, after 30 g of dust and after dust loading up to final pressure drops of 250 Pa, 350 Pa and 450 Pa. The average efficiency on different particle sizes is calculated at the various pressure drops (Figure 2). Filters with an average efficiency lower than 40% on 0.4 µm particles are classified as coarse filters, or G-filters, and the results of the particle efficiency test do not have to be reported. Filters with an average efficiency of 40% or higher on 0.4 µm particles are classified as fine filters, or F-filters, and all test results are reported.

The dust load, dust holding capacity and average arrestance for synthetic dust is also reported at final pressure drops of 150 Pa and 250 Pa for G-filters, and at final pressure drops of 250 Pa, 350 Pa and 450 Pa for F-filters.

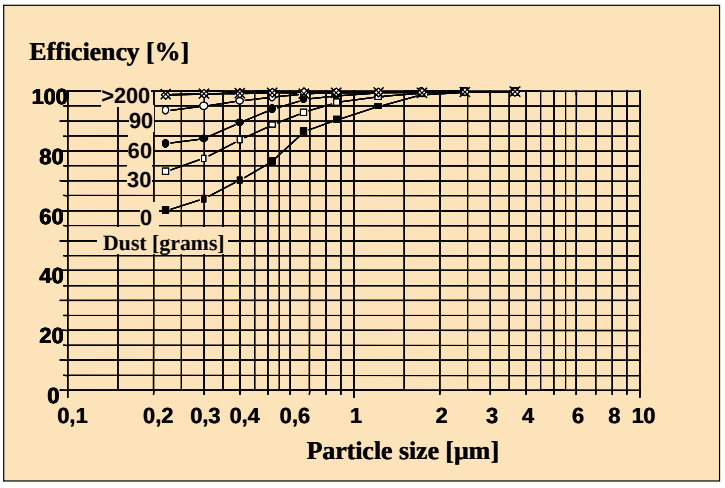


*Figure 2. Example of efficiency as a function of particle size after different dust measurements during tests conducted in accordance with EN 779:2002.*

## 3.1 Summarizing test results with EN 779:2002

The summarizing report includes initial test results (new filter), as well as curves indicating pressure drop, particle efficiency (0.4 µm) and arrestance for synthetic dust as a function of the quantity of dust fed at the test air flow rate (Figure 3). In addition to data about the actual filter, the average efficiency and arrestance at different final pressure drops are also to be reported.

Filters are tested and classified in their “natural state”, without any pretreatment or preparation. However, the efficiency for neutralized (discharged) filters, filter media or parts of the filter are also reported in the summary as supplementary information. Even if the classification is not affected, the efficiency for discharged material provides information that can indicate how much of a filter’s efficiency originates from the electrostatic effect and the minimum life efficiency (MLE) that can be expected for the filter in real operating conditions.

Testing organisation: Filter Testers Ltd. Report nr: 2xx3

**EN 779:2002. AIR FILTER TEST RESULTS**

GENERAL

| | | |
|---|---|---|
| Test no.: 12345 | Date of test: yyyy - mm - dd/dd | Supervisor: Mr. S. Tandard |
| Test requested by: WorldBestFilters Inc. | | Device receiving date: |
| Device delivered by: WorldBestFilters Inc. | | yyyy.mm.dd |

DEVICE TESTED

| | | |
|---|---|---|
| Model: WBF Leader 200 | Manufacturer: WorldBest Filters Inc. | Construction: Compact filter 4 V-shaped pockets |
| Type of media: Glass & plastic fiber WBF MixG&F | Net effective filtering area: 19 $m^2$ | Filter dimensions (width x height x depth) 592 mm x 592 mm x 292 mm |

TEST DATA

| | | | | |
|---|---|---|---|---|
| Test air flow rate: 0.944 $m^3/s$ | Test air temperature: 20°C to 24°C | Test air relative humidity: 26 % to 61 % | Test aerosol: DEHS | Loading dust: ASHRAE |

RESULTS

| | | | | |
|---|---|---|---|---|
| Initial pressure drop: 99 Pa | Initial arrestance: 98 % | Initial efficiency (0,4 µm): 70 % | Dust holding capacity: 254 g / 369 g / 461 g | Untreated / discharged efficiency of media (0,4 µm, Annex A): 71 % / 70 % |
| Final pressure drop: 250 Pa / 350 Pa / 450 Pa | Average arrestance: 99 % | Average efficiency (0,4 µm): 93 % / 95 % / 96 % | Filter class (450 Pa): F9 | Remarks: |
| Note: The performance results are only valid for the tested ite m cannot by themselves be quantitatively applied to predict filter performa nce in service. | | | | |

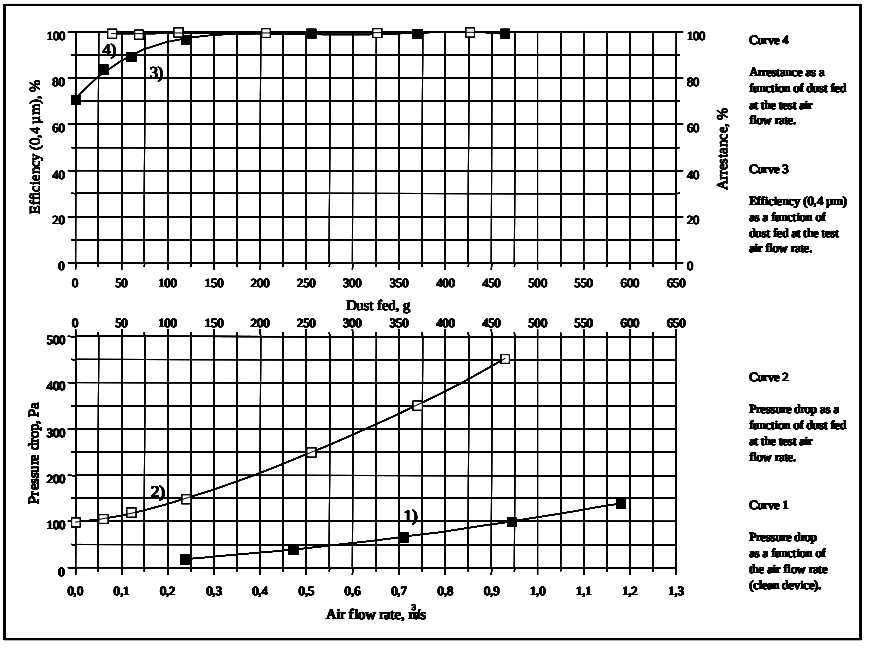


*Figure 3. Example of a summary of test results reported in accordance with EN 779:2002. One new feature is that the discharged efficiency of electrostatically charged filter media must be reported (circled area of report in the figure).*

## 3.2 Discharged efficiency

The efficiency of a neutralized or discharged filter or filter media is tested in accordance with “Annex A, Electrostatic discharging procedure”. The efficiency of untreated (new) material, together with the efficiency for discharged material, is to be reported in the summary, but does not affect the filter’s classification. This information is provided only to indicate how much of the efficiency originates from the electrostatic effect and how much it can deteriorate in real operating conditions. Figure 4 shows an example of how an F7 filter with an initial efficiency of 88% declines to 16% when it becomes discharged and the electrostatic effect disappears. Thus, the efficiency is strongly dependent on the electrostatic properties of the filter media.

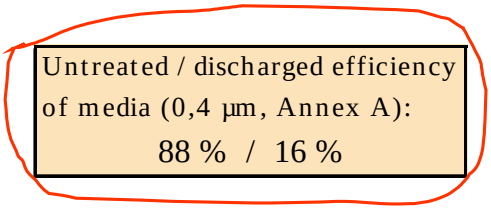


*Figure 4. Example of how the discharged efficiency should be reported in the summary of test results for a filter – in this case for an F7 filter with electrostatically charged material. The efficiency of untreated (new) material is to be reported together with the efficiency for neutralized (discharged) material. Despite the high initial efficiency, and the fact that the filter has been tested and classified as an F7 filter, it can be expected that the minimum life efficiency (MLE) will decrease to 16% in real operating conditions.*

# 4. Classification

The original classification system has been maintained with F (fine filters) and G (coarse filters), while the class will depend on the average efficiency on 0.4 µm particles. The classification with 0.4 µm particles is based on tests indicating that the average efficiency for these particles produces results that are in agreement with the average dust spot efficiency in accordance with the original system. The current classification system could therefore be kept this way. An F7 filter according to the former system will in all probability still be classified as an F7 filter.

Filters with an average efficiency (on 0.4 µm particles) lower than 40% are to be classified as G-filters and the classification follows the former system. Filters with an average efficiency of 40% or higher are classified as F-filters and the classification is based on the average efficiency on 0.4 µm particles, instead of on the average dust spot efficiency used in the previous system. There is one difference, however. In the former classification system, an F-filter was required to have a minimum initial dust spot efficiency of 20%. In the new norm, an F-filter is only required to have an average efficiency of 40% or higher.

Classification still has to be based on testing filters at a nominal air flow rate of 0,944 $m^3/s$ (3 400 $m^3/h$) and a final pressure drop of 250 Pa for G-filters and 450 Pa for F-filters.

*Table 1. Classification of air filters in accordance with EN 779:2002. The standard uses an average efficiency on 0,4 µm particles for classifying F-filters. G-filters are classified, as before, on the basis of a filter's average arrestance for synthetic dust. The filters are to be tested at an air flow rate of 0.944 $m^3/s$ (3400 $m^3/h$).*

| **Filter Type** | **EN 779 Class** | **Average arrestance ($A_m$) synthetic dust (%)** | **Average Efficiency ($E_m$) 0,4 µm particles (%)** | **Final Pressure (Pa)** |
|---|---|---|---|---|
| | G1 | $50 \leq A_m < 65$ | - | 250 |
| Coarse | G2 | $65 \leq A_m < 80$ | - | 250 |
| filter | G3 | $80 \leq A_m < 90$ | - | 250 |
| | G4 | $90 \leq A_m$ | - | 250 |
| | F5 | - | $40 \leq E_m < 60$ | 450 |
| Fine- | F6 | - | $60 \leq E_m < 80$ | 450 |
| filter | F7 | - | $80 \leq E_m < 90$ | 450 |
| | F8 | - | $90 \leq E_m < 95$ | 450 |
| | F9 | - | $95 \leq E_m$ | 450 |

As in previous test methods, there are problems when synthetic dust is used. Classification, for example, is based on lab tests with synthetic dust and does not produce documentation for calculating a filter's service life or its performance properties in a real installation application [2]. In addition, the dust holding capacity and average efficiency vary with the final pressure drop.

## 4.1 Final pressure drop

To save energy, filters are dimensioned for a final pressure drop that is much lower than the one used for classification. From a hygienic standpoint, filters must also be replaced at certain time intervals and not on the basis of their final pressure drop. Thus, in reality, filters do not measure up to their filter class. For optimum operating economy, an F7 filter usually has a final pressure drop of 150 Pa, and not 450 Pa, which the classification is based on. The classification therefore overestimates the filter's performance properties in real operating conditions.

## 4.2 Minimum life efficiency (MLE)

It is important to be aware of a filter's performance properties in different operating environments. As a filter accumulates dust, the pressure drop increases and the collected dust improves the filter's efficiency. The opposite effect can be seen with electrostatically charged material. During operation, impurities neutralize the material and the filter's capacity to separate is reduced considerably. There are cases when efficiency drops from 90% to 20% after a few weeks of operation.

The standard or classification does not establish minimum efficiency requirements for discharged material. However, if this efficiency is more than 10 percentage units lower than the efficiency of a new filter, the filter, in reality, is most likely one class lower than the classification in accordance with lab test results. In Sweden, this problem was brought to the attention of the industry several years ago, and the Swedish National Testing and Research Institute has subsequently introduced certification regulations for "P-labeling" of filters [3], which have established the minimum requirements for efficiency in real conditions. The system has been appreciated and is of great value to end-users when they select filters.

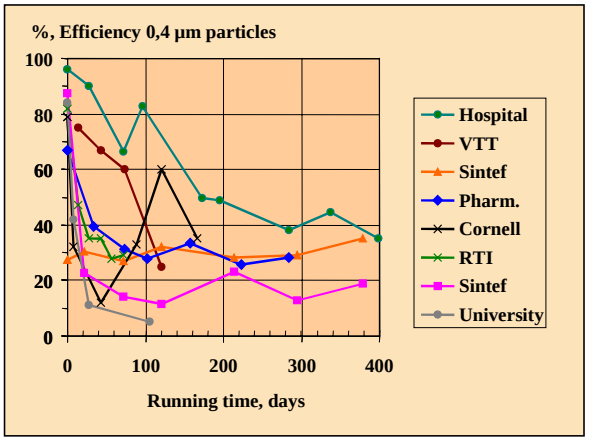


*Figure 5. Example showing some independent tests with electrostatically charged filters. Tests on different filters, in different locations, show that efficiency can drop dramatically in real life.*

## 4.3 Dust holding capacity

The filter's accumulation of synthetic dust is often used as a measurement of the filter's dust-holding capacity or service life in real operating conditions. Due to the use of coarse dust, the dust will accumulate in the filter media during lab tests in a completely different way than it would in real operating conditions. Figure 6 shows an example of filters with electrostatically charged filters that have been tested in the lab and in real operating circumstances. According to measurements, the filter can accumulate approximately 500 g of synthetic lab dust at a pressure drop of 450 Pa, but only 50 g of atmospheric pollutants at the same press drop. Thus, the lab test indicates that the filter has a dust-holding capacity that is about ten times higher than in reality. Agreement between the lab and reality depends entirely on the design of the filter material and its structure. Filters with glass-fiber material often show the opposite properties – that is, a dust-holding capacity for atmospheric dust that is higher than the capacity measured in the lab.

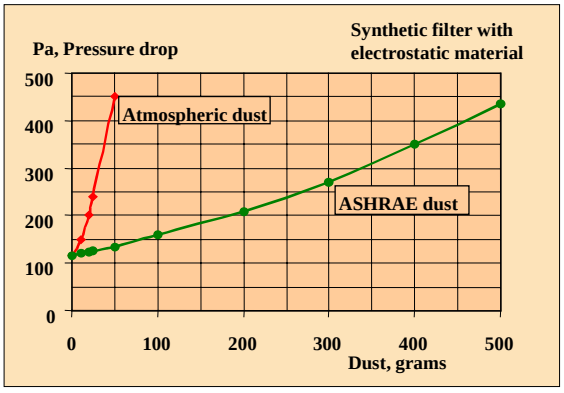


*Figure 6. Example of lab test results compared with performance in real operating conditions for a synthetic filter. The filter accumulates about 10 times more synthetic dust than atmospheric dust [4]. Calculations of a filter's life based on ASHRAE synthetic dust can therefore be completely misleading.*

## 5. Annex A – Discharging

Annex A, which requires reporting the efficiency of a filter material or filter without electrostatic effects, was a subject of hot debate when the new test method was being developed. The background is that filter media with electrostatically charged fibers have a very high efficiency, as long as the electrostatic effects are present. Coarser fibers can be used, which improves the pressure drop and dust-holding capacity of the filter. The coarse synthetic ASHRAE dust improves efficiency and compensates for the discharging of the electrostatic properties. Thus, it is possible to achieve a high classification based on lab measurements. In real conditions with atmospheric dust, the filter media becomes discharged and does not benefit from the compensation effect from the synthetic lab dust.

The efficiency of electrostatically charged filter media can therefore deteriorate dramatically in real operating conditions, compared with lab tests. Pretending that the problem does not exist, instead of addressing it, is to mislead the end-user or customer. This is why the standard requires the publication of the discharged efficiency. Even if the discharged efficiency does not affect the classification of the filter, it will provide the filter user with a guideline for the MLE (minimum life efficiency). The discharged method, based on the Nordtest

method [5], has been in use for several years. The method's requirements have had a major impact on the development of new filter medias and have helped increase understanding of real life filtration.

This procedure in Annex A is used to determine whether the filter efficiency is dependent on the electrostatic removal mechanism and to provide quantitative information about the importance of the electrostatic removal. This is accomplished by measuring the removal efficiency of an untreated filter or filter media and the corresponding efficiency after the effect of the electrostatic removal mechanism has been eliminated. The test is based on the elimination of the electrostatic removal mechanism. Any treatment to give a completely discharged material may be used (isopropanol, diesel fume, detergents or surfactants in water).

## 5.1 Diesel fume test

Isopropanol alcohol (IPA) treatment of material is described in the norm. It is an easy test for small samples but causes practical problems for full size filters. IPA can also affect some filter materials. The best discharging procedure is to use diesel fumes. The entire filter can be easily tested and the filter can be discharged in a few hours. A filter is usually exposed to diesel fumes in real operating conditions and a few hours of lab testing simulates weeks or months of operation in real life. With this test method, results can be obtained 100 to 1000 times faster than in real conditions. In addition, diesel fumes do not destroy the filter media and do not affect the pressure drop.

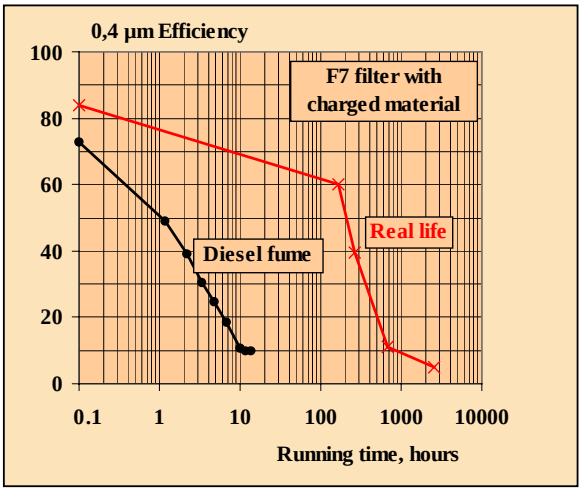


*Figure 7. Example of how efficiency changes over time, in real operating conditions and when a filter is loaded with diesel fumes. In both cases, the same type of filter with electrostatically charged material was tested. One filter was installed in a ventilation plant near a motorway, while the other filter was loaded with fumes from a diesel engine.*

# 6. Summary

In EN 779:2002, efficiency and classification are based on normal filters without pretreatment of the filter or filter media. Classification is based on the average efficiency on 0,4 µm particles for fine (F) filters, and in the case of coarse (G) filters, on the average arrestance for synthetic dust. The efficiency of discharged filters or filter materials will also be reported and gives a good indication of a filter's performance properties in real operating conditions with normal outdoor air.

The new norm gives the filtration industry modern methodology that is better suited to deal with today's IAQ requirements and process demands. However, despite all the advantages of EN 779:2002, there are still problems with classification based on a high final pressure drop and the misleading results for efficiency and dust-holding capacity, compared with real-life performance. It is important to establish a European certification system of air filters that is focused on the performance of filters in real life conditions, and that filters are classified on the basis of their minimum life efficiency (MLE).

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# AIR CLEANING DEVICE FOR DESTRUCTION OF MICROBES BASED ON ELECTROPORATION EFFECT

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## Abstract

Based on many years of experience and special investigations, the Scientific – manufacturing firm "Potok Inter" (Russia) has developed a unique air-treatment technology, as a result of which microbes and viruses are completely destroyed, and a large volume of sterilised filtered is ensured. The technology is realised in POTOK 150M-01 unit, whose diagram is given below. After an initial "Pre-filter" the polluted air is directed using a fan towards the "Biocellular structural disrupter". The integrity of microbes is disrupted using electrostatic field effects. The cells are then pushed through the "Biocells structural disintegrator" where microbes are totally inactivated by intrastructural energy liberation. Finally aerosolized debris contained in the air (incl. disintegrated cellular residues) are precipitated on the surface of the "fine electrostatic filter" due to electrostatic attraction. Sterilized residues remain inside the system.

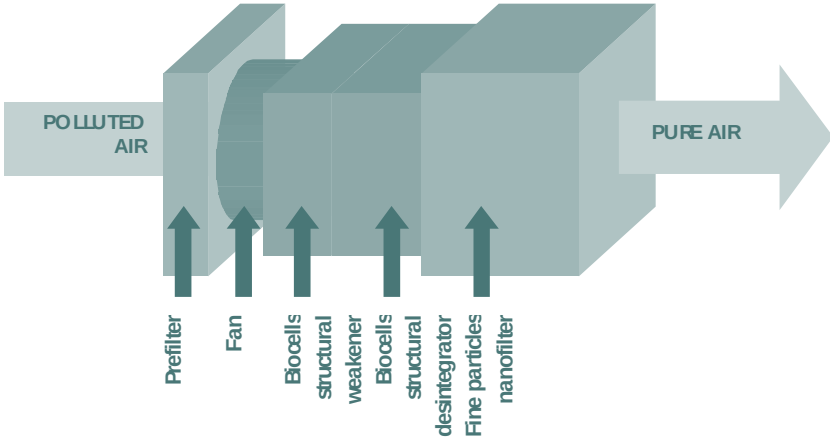
## 1. POTOK 150M-01

The use of "POTOK 150M-01" air sterilizers in medical institutions provides a reliable source of sterilised air with a modest capital investment. The high levels of sterilisation can be attained reliably, and not influenced by time if the "POTOK 150M-01" air sterilizers are arranged on the premises correctly taking into account the prefiltration of air. More than 600 operations were carried out at Turner Clinic of Military Traumatology and Orthopedic Surgery during the clinical tests of the unit at the Military Medical Academy (Saint-Petersburg). 500 of these operations were complex ones e.g. replacement of the hip joints, trauma surgery, surgical treatment of diseases of the vertebral column and arthroscopic surgery. The level of infectious complications was 0.32%).

During the three-year period using the "POTOK 150 M-O1" air sterilizers in the orthopaedic operating room of the Vishnevsky 3rd Central Military Clinical Hospital, about 1000 complex operations were done per year, including total replacement of the hip and knee joints. After the operating suites of the trauma and orthopaedic departments were equipped with the "POTOK 150 M-01" system, the level of infectious complications dropped more than 12-fold, and currently is approximately 0.25%. No other existing technology offers a broad spectrum control of air born contaminants, which also is proven to be cost effective.

## 1.1 Main technical data

| | |
|---|---|
| Efficiency of sterilization | $10^{-3}$ and better |
| Efficiency of filtration particle size 0.01…10 μm | up to 99% |
| Airflow capacity | 150$m^3$/h |
| Power consumption | 40 W |
| Weight | 9 kg |
| Dimensions | 420*322*360 mm |




## 1.2 Functional possibilities of POTOK technology

The air sterilizing device "POTOK 150M-01" is certified in Russia and Europe. High efficiency of air sterilization and reliability of this device were confirmed by the results of tests and use in leading institutions and clinics of the Russian

Federation and Europe, as well as by the experience of using the device aboard the orbital space station "MIR". Currently there is a POTOK 150MK in use aboard the International Space Station. The functional possibilities for this technology (see also Figure 1) are that:

- it creates sterile, dust-free and hypoallergenic air-medium in closed premises,
- it deodorizes and removes toxins from air,
- it does not change the ionic and gas composition of air,
- it does not create interference for electronic equipment and
- it is ecologically safe and has no contraindications for use in the presence of humans.

The high efficiency particulate air (HEPA) filtration do not inactivate viable microorganisms, provides an environment for growth of the retained organisms and does not retain virus sized particles. The ultraviolet and germicidal irradiation (UVGI) is highly dependent on the ventilation rate. It is not effective in high humidity environment and may damage products. It also shows low efficacy against fungal spores and exhibits inconsistent effectiveness depending on microbial susceptibility. The light source requires regular cleaning and the effectiveness is lowered over time. Ozone generators are plagued with safety concerns and little to no efficiency under closely monitored conditions. Ionizers do not provide protection against airborne contamination and suffer from a “black wall” effect and they show toxicological problems.

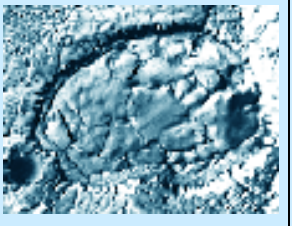
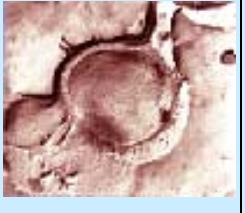
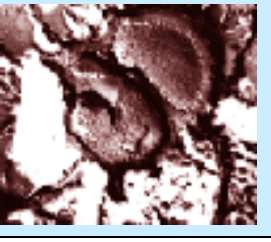
| | Before | Effects of POTOK 150M-01 | After |
|---|---|---|---|
| Saccharomyces cerevisiae |  | **Full structural disintegration**<br>The membrane organelles are almost totally disintegrated. The cytoplasmic material has been expulsed out of the cellular membrane Plasmalemma is totally losing its structure and intracellular organisation. |  |
| Pseudomonas fluorescens |  | **Multi focal breakage and mincing of the membrane**<br>The shape is changing and becoming rounded the cellular membrane is covered by multiple microscopic gaps. The cytoplasm is significantly vacuolated due to loss of cytoplasmic material through the local gaps in membrane (ribosomes and nucleids). |  |
| Micrococcus lateus |  | **Major large membrane and cytoplasm disruption**<br>Loss of integrity of cell walls and leaking cytoplasm material is visible on right photo |  |

*Figure 1. Effects of POTOK 150M-01.*

In Figure 2 we show a recent study done by the Russian Academy of Science at the Institute of Epidemiology and Microbiology "Gamaleye" that proves the efficacy of the POTOK 150m-01 under extremely severe conditions.

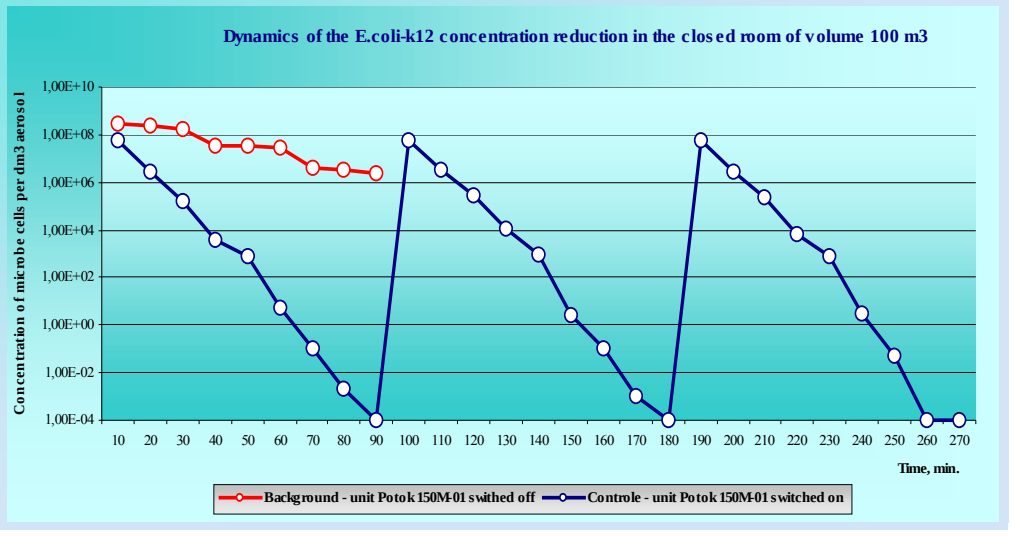



*Figure 2. In a closed room with a volume of air of 100 $m^3$, three times repeated viable aerosolized* E. coli *cells concentrated to $10^8$ CFU/l were shown to be decreased nearly to zero after operating the POTOK 150m-01 unit for 90 min. The result was predictable and repeatable.*

To conclude we present the cutting edge "POTOK" technology originated and developed to improve the health and quality of life by the Russian scientific firm "POTOK INTER" The POTOK INTER is a member of the Russian association ASENMCO.

# FABRICS OF CLEANROOM GARMENTS

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**Abstract**

Cleanliness, hygiene and quality are terms that are used more and more frequently in modern production, most often in the pharmaceutical and microelectronics industry. The main target is to obtain and to maintain a high level of cleanliness during production processes so that production will be optimized and defects in quality will be minimized. Personnel and their activities are one of the major sources of contamination. Cleanroom garments are used to minimise the number of contaminants dispersed by personnel – more specifically, skin scales and fibres from personal clothing. The use of protective garments in cleanrooms will set high demands for the fabric. Significant elements are fibres, yarns, weaving and finishing in the manufacturing process. Fabric should prevent particles passing through it into the surrounding atmosphere as well as prevent particles attaching fibre surface. Designer of the fabrics and clothes should be aware that although the best properties for contamination control would be at virtually 100% efficiency in filtration at a specified particle size, the resultant clothing could be highly uncomfortable and impractical for wear by personnel.

## 1. Introduction

The continual increase in sophistication and miniaturisation of biomedical and electronic devices makes it virtually essential that they are manufactured using cleanroom technology. The term contaminant has a very broad meaning and as a general definition it is material in solid, liquid or gaseous form. When considering medical and electrotechnical end products and when trying to

protect those products from surrounding contaminants, a well understanding of the fabric technologies and specific challenges of the individual end uses is essential. Microorganisms are of particular importance because they may be disease causing agents, be large enough to interfere with the fabrication or operation of a microelectronic component, or be the source for deterioration or adulteration of pharmaceutical products and medical devices. It has been verified that particle contamination is a major cause of defects in microelectronics.

*Table 1. Industrial particulate cleanliness classes (≥ 0.5 µm/m$^3$).*

| Field | EN-ISO 14644-1 | USA Fed Std 209E | Particle |
|---|---|---|---|
| Manufacturing of ULSI | 2–3 | 1–100 | 0.1–0.3 µm |
| LSI | 3–5 | 100–10 000 | 0.3–0.5 |
| IC | 4–5 | 1 000–10 000 | 0.5 |
| Electronic precision machinery | 5–6 | 10 000–100 000 | 1–5 |
| Optics, printing | 5–6 | 10 000–100 000 | 0.5–1 |
| Medical treatment | 3–5 | 100–10 000 | Virus, bacteria |
| Pharmaceutical | 3–6 | 100–100 000 | Virus, bacteria |
| Foodstuff | 5–6 | 10 000–100 000 | Virus, bacteria |

## 2. Fabric design issues

The material used for cleanroom clothing must conform to certain requirements, it should be comfortable to wear, it should provide an adequate barrier between the wearer and the environment and should not release any particles that may contaminate the production area.

The principal design features for cleanroom fabrics are barrier properties, strength, linting, breathability, comfort and sterilisation stability, of which the latest is primarily for medical surroundings. Among the properties required of

materials used for cleanroom clothing are, suitable length of fibres so as to reduce contamination by their dispersion into the air, resistance to wear, moisture absorbing capacity, ability to repel and also contain soil.

Characteristics of the fabric, threads and materials used in apparel and accessories may affect the performance of the gowning system. Fabrics can be grouped into types, such as woven, nonwoven and laminated structures. Evaluation of fabrics intended for use in cleanrooms may include, depending on the intended application, sectors such as cleanliness, electrostatic properties, biological properties, durability, comfort, opacity and chemical effect.

The material should withstand repeated laundering and sterilisation and have also suitable conductivity.

## 3. Barrier performance

With the rapid increase in blood borne diseases, such as hepatitis C and HIV, the need for medical workers to wear garments that provide a barrier to fluids such as water, blood and alcohol have become critical. The major requirements for barrier fabrics are that they resist the penetration of liquids, particular blood and at the same time be sterile, breathable, flexible and inexpensive. Barrier requirements can be partial resistant or total proof, ranging from particulates and bacteria to fluids and viruses. In general, a hydrohead of > 40 cm is required to compete in the medical product market.

There is a working group (CEN/TC 205/WG 14) which is planning minimum performances for surgical gowns, drapes, clean air suits and their both disposable and reusable textile materials. The project group has made round robin studies and it seems that high resistance to liquid penetration correlates in some way with high resistance to microbial penetration. Resistance to liquid penetration shall be required to be around 30 cm water column to establish also a suitable level of resistance to microbial penetration. Working environment of operation room is classified to standard and high performances. Products of both categories are divided into critical and less critical areas, but all activities happen in steril space.

In some cases special coating and breathable polymer films are being added to fibres and fabrics. In other cases ingredients are added directly into polymers being used to make fibres. In nonwoven structures, melt blown low denier fibres are being layered in the middle of spunbonds or used to make three dimensional molded shapes. Bocomponent fibres are also being used in the production of spunbonds, carded and thermal bonded nonwovens. Today totally barrier materials which means viral and liquid barrier are fabrics reinforced with impermeable polymer films. In operation theatres there are used reinforced drape materials which means three layer structures consisting one layer polymer film.

## 4. Strength

The strength requirements for cleanroom fabrics vary with the end use applications. General recommendations concerning fabrics to be used for clothing can be applicated with the exception of special needs. There are different kinds of strength characteristics like tensile, bursting and tear strength which all gives their own property to end products.

## 5. Sterilisation stability

When designing fabrics for sterilsation it is essential to understand the impact of sterilisation procedures on fabric performance features. Raw material of fibres, yarns and films should be chosen suitable for sterilisation type that will be used. Different sterilisation methods are used: hot steam autoclave (saturated water steam), gas sterilisation (ethylene oxide, vaporised hydrogen peroxide), hot air sterilisation or radiation sterilisation (alfa, beeta, gamma, electron beam). Required properties are sufficient tensile strength, tear and abrasion resistance and high filtration efficiency consisting bacteria and dust particles. Attention should be paid to raw materials, other chemical polymer selections and their convenience for different sterilisation processes.

## 6. Comfort and breathability

The comfort and breathability factor is usually considered as opposing the barrier function. The fabric designer have to focus very carefully on identifying

the trade-off resolution between needed comfort and barrier characteristics. The material should be strong and smooth and at the same time provide adequate water vapour permeability and proper filtration properties. For today versatile use it has been developed semipermeable mambranes which work as reinforcements providing enhanced fluid barrier performance while maintaining breathability.

## 7. Linting

The term lint generally means fibre fragments released from material during handling. The linting property of materials is becoming increasingly important as more companies build hardware that is sensitive to damage or degradation from both contamination and electrostatic discharge. Linting is obviously also an important consideration for hospital operating theatres. Therefore, it is critical to control the materials that come in contact with, or simply are near sensitive hardware or operating theatres. Most materials lose compounds by friction, but cleanroom materials should not release lint and other particles during handling. The textile material itself is a possible source of particle emissions.

A working group of CEN/TC 205/WG 14 recommends that, for catering particles from 3 to 25 µm in size, the linting index would be a maximum of 5.0 and the IPM would be a maximum of 4.5 for surgical gowns, drapes, equipment coverings and clean air suits. The Particulate Matter (PM) is a theoretically calculated amount of foreign matter and loose particles.

## 8. Electrostatic properties

Controlling electrostatic charge reduces surface contamination and electrostatic discharge. The characteristics of materials and fabrics to attract an electrostatic charge and to release that charge are intrinsic to their physical composition and surface characteristics. Very important is to be noticed that ESD (ElectroStatic Discharge) properties of a fabric may change after extended use and several washings.

The materials used for cleanroom garments, boots and shoes are often made from insulating materials. Electrostatic charges are generated both inside and

outside the material as well as induced on personnel wearing the garment. ESD protective fabrics incorporating electrically conductive fibres can safely dissipate harmful electrostatic charges when they are effectively grounded. Low charging fabric can also be manufactured by antistatic finishing treatments. If a finishing agent is applied to the surface, it should be strongly attached the way that the low charging properties remain after several washings and abrasion in use.

A large number of specialised fibres with conductive properties have been developed. They are moisture based antistatic fibres and electronic conductor based antistatic fibres. Improved moisture based antistatic properties arise from an increasing water absorption. Conductor based antistatic fibres are different types depending on conductive component and its location in a fibre. Main types are:

- Metal fibres which are cut to staple fibre lengths and blended with other fibres, for example polyester and polyamide.
- Conductive fibres containing metals, metal oxides or metal salts. Metal can be on the surface of a fibre, fibre can be so called bicomponent with a structure of a sheath-core fibre with a metal sheath, conductive metal particles can be co-extruded into a fibre polymer or metal particles can be adhesive bonded on the surface of a polymer fibre.
- Fibres containing conductive carbon are various, generally used and proved useful as an additive for making plastics and fibres conductive. The earliest fibres were made through coating the fibres with carbon particles. Later products have been based on incorporating carbon into the fibre. The whole fibre can be loaded with carbon or carbon can be incorporated into one component of a side-side or a sheath-core bi-component fibre or carbon can form few spots on a fibre surface.

A lot of research has been and will be made to develop fibres containing inherently conductive polymers. In principle it is possible to coat and impregnate conventional fibres with conductive polymers and product fibres from conductive polymers alone or in blends with other polymers. Polymers that conduct electric currents without the addition of conductive (inorganic) substances are known as intrinsically conductive polymers such as polypyrrole, polyaniline and polyethylene dioxythiophene. Still there are some problems to solve before these fibres are ready for wider applications in ESD protective fabrics. The recommended electrical resistance range for static dissipative materials is $1 \times 10^5$ ohms to $1 \times 10^{11}$ ohms (ESD STM 2.1 – 1997).

## 9. Measuring cleanroom required properties of fabrics

There are many different test methods for fabrics that are used to describe the cleanroom acceptability. Each fabric has unique properties and performs accordingly, therefor not all test methods may be applicable to each fabric type. For the same reason, results of a given test on different fabrics may not necessarily provide direct correlations for comparative purposes. To ensure the proper and reliable functionality of a fabric in a cleanroom, the real cleanliness measurement should be determined under real-use conditions and environment.

## 10. Acknowledgements

The author would like to thank to all participants of the EUREKA E! 2441 co-operation project which was one project of the Finnish STAHA (Managing Static Electricity) technology programme supported by Tekes, the National Agency of Technology.

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# BIOLOGICAL INDICATOR D-VALUES AND THEIR APPLICATION TO THE STERILITY ASSURANCE LEVELS

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## Abstract

A biological indicator (BI) is a device intended for use by healthcare providers or manufacturers of sterile medical materials to monitor adequacy of sterilization. The device consists of a known number of microorganisms, with a known resistance to a specific mode of sterilization. These microorganisms are typically bacterial spores and are placed in or on a carrier and enclosed in a protective package. Subsequent growth or failure of the microorganisms to grow under suitable conditions indicates adequacy of sterilization. The bacterial spores used for these BI's follow a predictable death rate when exposed to specified sterilization parameters. These spores have the capability to integrate all factors that affect sterilization. BI's are used to establish sterility assurance levels.

Biological indicators typically contain populations of one million spores. Biological diversity is similar to any population of one million living organisms of the same species. Recommendations will be made as to the methods and test instruments used to provide the most accurate and precise evaluation of process delivered lethality, thereby, providing the user with well-defined values that can be meaningfully applied to routine sterilization processes.

The resistance performance of a BI is characterized by the determination of a D-value. This is the most important criterion for the BI. The D-value is defined by the time in minutes that it takes at a specified set of conditions to reduce the spore population by one log or 90%. D-values can be determined by performing either fraction negative (quantal) survival/kill calculations or by enumeration of surviving spores determined using plate count recovery techniques. The enumeration analysis uses a graphic representation of spore inactivation against increasing exposure time to the specified conditions.

Methods for performing these two approaches are described in the European Norm 866, ISO 11138 and the US Pharmacopeia. These standards describe the different approaches but differ slightly in the use of this data.

These standards accept three different scientific methods for D-value assessment. Each of these methods uses different experimental data and yields a different value, which leaves the user to decide which value is most appropriate: a) survivor curve; or b) fraction negative approach. The survival/kill calculation requires a D-value, which is determined by either of the above mentioned formulas. The test instructions in the standard are not adequately defined to assure consistent data generation, further providing the user with potentially conflicting information.

The survivor curve method subjects multiple BI's (typically four) to a series of graded exposures. The BI's are enumerated using colony-forming units of direct plate counts. The number of surviving bacterial spores after each exposure is plotted. The survivor curve method depicts biological indicator resistance over a four to five log population decline. The D-value is read as the negative reciprocal of its slope. If the curve is linear, the D-value will represent the biological indicator resistance over this range of exposures. The disadvantage of this method is that it is not able to establish the time when all spores are killed; this is due to the sensitivity of the recovery method. The plate count technique requires between $10^1$ and $10^2$ CFU's pre sample. Many sterilization processes lose their linearity in the transition between very low numbers of spores and all spores killed. This has the possibility of understating the D-value, which results in understating the sterility assurance level (SAL). The standards only require the linearity to have a correlation coefficient to be 0.8. A correlation coefficient of 1.0 is a straight line. The acceptable correlation coefficient should be much higher than 0.8.

The fraction negative approach is the other method of estimating D-values. This approach relies on the proportion of non-surviving BI's. These fractional zones are also referred to as quantal data. For these test groups, groups typically twenty BI's are subjected to different exposure times then incubated for growth. This method is limited in demonstrating linearity of the process lethality since quantal exposures occur over an exposure range of less than two D-values.

The Holcomb Spearman Karber fraction negative method calculates the mean time to sterility from sets of quantal data. It then uses the shortest exposure time to achieve all spores killed to calculate the D-value using the initial population.

The Stumbo Murphy Cochran fraction negative method estimates the number of surviving organisms at any exposure time having a quantal result. If several quantal data points are observed, then these D-values are averaged. This method allows a D-value to be calculated from one quantal data point.

Fraction negative data approach is limited in demonstrating linearity of death kinetics because it affects data only over two D-values. Fraction negative data limitation can be compensated for by using exposure time intervals that are closer together thus providing an increased number of quantal data points and by larger numbers of BI replicates per exposure.

The standards require that two approaches be used by manufacturers to determine a D-value. This is intended to provide the user with better estimates of the biological indicator resistance to a specified sterilization process. However, in practice it has only created confusion and misunderstanding.

Even though the requirement is to obtain D-values by two different methods, only one of these values is required to be placed on the certificate of analysis, however, these two values have to be such that the one value is not more than 50% of the other. The standards also state that the D-values must be able to be reproduced with no greater difference than ± 20%. Taken to extremes with a 50% difference being one method and a ± 20% reproducibility allows a latitude of up to 90% difference in D-values for the same spore preparation. It is no wonder that biological indicators are not appreciated for their value. Users require consistent performance for their BI's. Without consistent performance, it is impossible to calculate meaningful SAL levels.

This paper proposes improved requirements for these standards that are readily achievable by manufacturers of biological indicators and their test equipment. This will provide users with more consistent data to be applied to their processes. These improved values will allow the user to more accurately assess sterility assurance levels.

# HYGIENIC EQUIPMENT AND SYSTEMS – REGULATIONS, ISSUES, STANDARDS AND GOOD PRACTICE

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## Abstract

A hygienic system, in the context of this presentation, is described as: "*An equipment or system that exploits appropriate materials, properly finished, carefully designed, fabricated and assembled with the objective of resisting corrosion, resisting adhesion of contaminants, and being easy to clean and inspect.*" Typical examples of systems are pharmacopoeial water storage and distribution, product vessels, hygienic piping, equipment surfaces and processing facilities. This paper will not consider rooms and architectural matters in detail.

The ultimate proof that a pure water system, CIP system, process vessel or machine is fit for purpose lies ultimately in successful testing and monitoring of the finished product quality. Such an "end product" approach can conceal hidden problems, and as we demand greater reliability from our systems in use, a more systematic quality assured approach to system design, installation, and start-up is required. The QA concept of *implementing validated processes* on *qualified equipment* with *trained personnel* precludes such high risk strategies, and ensures that process validation is a confirming activity and not an act of discovery of systemic deficiencies.

The essential requirements (GMPs) and design considerations (standards, regulations & good practice) that will influence the performance of an item of equipment, the process system or the facility housing it are described.

We begin with a reminder of the Pharmaceutical GMP objectives, backed up by information about available food industry guidance such as that from the European Hygienic Engineering & Design Group (EHEDG) and the new 2002 ASME Bioprocessing Guide. Some equipment details and system features will be used to illustrate essential principles of hygienic and the scope of the

guidance available. The principles of hazard and risk assessment are also discussed since this process is essential to helping us decide where to expend our efforts to best effect.

Since predictable cleaning and sanitising of surfaces is essential to the chemical, cosmetics, cosmaceutical, pharmaceutical, biotechnology, food and beverage industries, the paper will also consider the available standards and techniques available, and most importantly how acceptance criteria for cleaning might be determined.

The paper will include some thoughts about some common problems encountered, future requirements and opportunities for technical advancement that could be brought to bear in our quest for more reliable effective hygienic systems.

# 1. The GMP Regulatory expectation

It is essential to note some of the fundamental expectations in the GMPs. The following references are provided to both illustrate the objectives, and also to show the general nature of the requirements. The requirements are perhaps best considered as things to worry about and objectives to achieve, not how or what to do in detail. It is important to note that the GMPs are more explicit concerning facilities for sterile product manufacture and for pharmacopoeial water systems. Certain aspects of water systems requirements are dealt with later.

### 3 EU GMP

Premises and equipment must be located, designed, constructed, adapted and maintained to suit the operations to be carried out. Their layout and design must aim to minimise the risk of errors and permit effective cleaning and maintenance in order to avoid cross-contamination, build up of dust or dirt and, in general, any adverse effect on the quality of products.

### 3.10 EU GMP

Pipework, light fittings, ventilation points and other services should be designed and sited to avoid the creation of recesses, which are difficult to clean. As far as

possible, for maintenance purposes, they should be accessible from outside the manufacturing areas.

### 3.36 EU GMP

Manufacturing equipment should be designed so that it can be easily and thoroughly cleaned. It should be cleaned according to detailed and written procedures and stored only in a clean and dry condition.

### 3.37 EU GMP

Washing and cleaning equipment should be chosen and used in order not to be a source of contamination.

### 5.19 (a) EU GMP

Production in segregated areas (required for products such as penicillin's, live vaccines, live bacterial preparations and some other biologicals), or by campaign (separation in time) followed by appropriate cleaning.

### 5.19 (e) EU GMP

Using cleaning and decontamination procedures of known effectiveness, as ineffective cleaning of equipment is a common source of cross-contamination.

### 5.35 EU GMP

Before any processing operation is started, steps should be taken to ensure that the work area and equipment are clean and free from any starting materials, products, product residues or documents not required for the current operation.

### 5.45 EU GMP

Before packaging operations are begun, steps should be taken to ensure that the work area, packaging lines, printing machines and other equipment are clean and free from any products, materials or documents previously used, if these are not required for the current operation. The line clearance should be performed accordingly to an appropriate check-list.

### Annex 1 EU GMP

**Note!** The present guidance does not lay down detailed methods for determining the microbiological and particulate cleanliness of air, surfaces etc. Reference is made to other compendia such as the CEN/ISO Standards.

### 21 CFR 211.67 Equipment cleaning & maintenance

(a) Equipment & utensils shall be cleaned, maintained, and sanitised at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements.

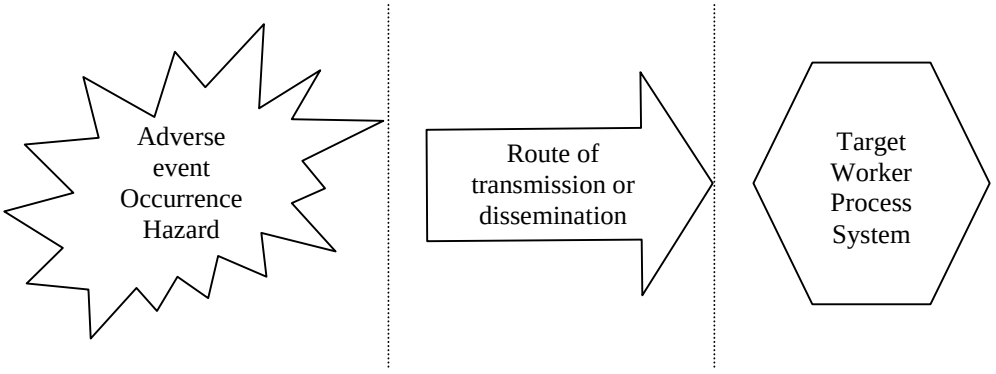
From these examples it is quite plain to see that GMP/cGMP is a philosophy, not a technical standard.

## 2. The principle of assessing risk

The processes of risk assessment influences virtually every walk of life today. To some it may seem like the documentation of common sense. Whether we like it or not, we must recognise that in quality and safety critical systems it is now almost certain that regulatory authorities will wish to look at the output from a risk assessment process, and increasingly, may wish to inspect the integrity of the process itself. This means that not only must the output be competent, but also that the technique employed must be undertaken in accordance with a predetermined discipline or protocol. Within our industry techniques such as HACCP (Hazard Analysis Critical Control Point) and FMEA (Failure mode and Effect Analysis) have gained broad acceptance. This paper will not explore the techniques specifically, but will identify how appropriate techniques might be applied to a system designed for the avoidance of contamination of a product or process. It is useful to recognise that a well organised risk assessment process can also provide a vital team building environment. The right environment ensures the development of technical consensus and buy-in to the solutions selected by the complete operational team within a firm.

For any quality of safety critical system following diagram represents how we might evaluate adverse events and occurrences, the route that might cause such

occurrences to cause a problem, and of course the target that might be adversely influenced by the event transmitted through a known route.




The need to assess and propose mitigation measures

| Assess Degree | Assess Degree | Assess Degree |
|---|---|---|
| • Amount<br>• Strength<br>• Frequency | • Length of route<br>• Ease of detection<br>• Attenuation & dilation | • Time<br>• Sensitivity<br>• Cumulative effects |
| Mitigation measure | Mitigation measure | Mitigation measure |
| • Change/remove material<br>• Change process<br>• Contain/protect | • Break route<br>• Extend route-attenuate<br>• Improve detection | • Reduce exposure time<br>• Remove target<br>• Harden target |

*Figure 1. Risk and its assessment.*

## 3. Some hygienic design objectives

The following sets out some more specific expectations of system performance that can be developed to expand upon the very general GMP aspirations.

Surfaces must be cleanable and must not present a toxicological hazard by leaching of components into the product when they are direct contact. All product contact surfaces must be resistant to the product, and to cleaning and

anti-microbial agents (disinfectants or sanitizers) under the full range of operating conditions. Product contact surfaces must be made of non-absorbent, materials and must satisfy the roughness requirements specified since this will have a major impact on cleanability.

Product contact surfaces must be free of imperfections such as crevices, therefore we must:

- Avoid direct metal to metal joints other than welding.
- Avoid steps due to misalignment in equipment and pipe connections.
- If seals or gaskets are used, their design must be such that no crevice exists where soil residues may be trapped and bacteria can accumulate and multiply.
- Use hygienic specifications of joint-rings and seals.
- Eliminate contact of product with screw threads.
- Corners should have a radius equal to or larger than 6 mm; the minimum radius is 3 mm. Sharp corners must be avoided.

All surfaces in contact with product must be either easily accessible for visual inspection and manual cleaning, or it must be demonstrated that routine cleaning completely removes all soil. If clean-in-place (CIP) techniques are used, it must be demonstrated that the results achieved without dismantling are satisfactory.

## 4. Surfaces of process equipment

It is very clear from the general GMP expectations that we should ensure that we specify, select and procure equipment that is fit for purpose, that will not harbour contamination and will not be a source of contamination in its own right.

In order to achieve this objective we would expect that equipment surfaces be cleanable, corrosion resistant and have an aesthetically pleasing appearance that engenders a desire to maintain a clean & hygienic state. However if we were to apply such broad requirements in a blanket way across all the equipment components it would be very likely that we would end up with a machine that was heavily over specified in some areas, and that would probably break a budget. In order to introduce some control and rationalisation to these demands and expectations, it is very useful therefore to develop a concept of criticality boundaries. These help relate the criticality of contact between the specific

equipment surface and the product. Some general performance expectations are clear:

**Cleanable** generally means smooth and crevice free.

**Corrosion resistance** must apply to appropriately to the normal atmosphere, cleaning agents and of course the product itself.

**Aesthetic appearance** means when clean it looks clean and engenders pride. This is a very intangible attribute.

*Figure 2. Appearance of equipment engenders different attitudes.*

One effective approach is to divide the potential for product of processed contact into the following three categories:

**Direct contact.** Where it is known that there will be a direct contact between the product, contact component surfaces and critical parts of the machine.

**Indirect contact.** Where it is known that contact is only made between the machine and external surfaces of product containers for example.

**No contact.** Where there is no direct contact between the machine surface and the product will process, and the only communication is that they are sitting in the same working environment.

An approach like this clearly allows us to differentiate criticality and decide upon the nature of the materials based on the assessed risk. Table 1 shows how this might be applied to a liquid filling machine that utilises CIP of the product fluid halfway, and topical cleaning of the equipment between batches.

*Table 1. The assessed risk of different material categories in a liquid filling machine, which is cleaned using CIP.*

| Part of machine | Indirect contact | Direct contact | No contact |
|---|---|---|---|
| Bed of filler | | | Product spills<br>Cleaning agent<br>Sanitising agent |
| Motor & drive access panels | | | Product infrequently<br>Cleaning agent<br>Sanitising agent |
| Cap hopper | | Caps<br>Sanitising agents | |
| Cap feeder | | Caps<br>Sanitising agents | |
| Filling pump & needle supports | | Product infrequently<br>Cleaning agent<br>Sanitising agent | |
| Fluid pathway | | Product<br>Cleaning agent | |
| Conveyor belts | Product spills<br>Cleaning agent<br>Sanitising agent | | |
| Star wheels | Product spills<br>Cleaning agent<br>Sanitising agent | | |
| Labelling system components | | | Specific cleaning techniques. |

In order to satisfy these objectives, we need to bring into play a full range of available materials. Most commonly a combination of stainless steel, plastics polymers and various elastomers can be used. Within the pharmaceutical industry a combination of 316 L stainless steel will be used for the most critical surfaces, and 304 stainless steel for the surfaces where purely cosmetic considerations are important. The selection plastics materials are more difficult

since many of them have the potential to leach compounds into the product, or as importantly absorb materials into their matrix. We will find from the food industry a substantial resource of information related to materials that are compatible with food products. Many, but not all of these are directly applicable to the manufacture of pharmaceutical medicinal products. Some areas where we have to take additional care when transposing food industry practice to pharmaceutical and life sciences include the increased aggressiveness of some of the processes, increased frequency of application and the impact of the regulatory demand for more validation evidence. Examples of differences are use of thermal depyrogenisation, and exposure to aggressive agents such as water for injections and pure steam.


*Figure 3. A typical pharmaceutical filling machine showing complex surfaces and the use of different materials.*

The following diagrams, taken from the EHEDG Guidelines illustrate principles of good practice that are readily translatable from the food industry to medicinal product manufacture.

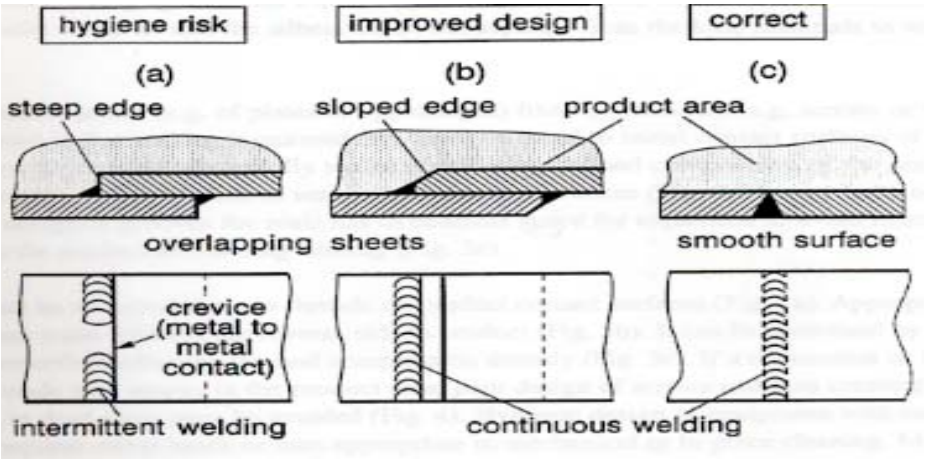



*Figure 4. EHEDG Comparison of bad and good practice for material joints on machines or equipment.*

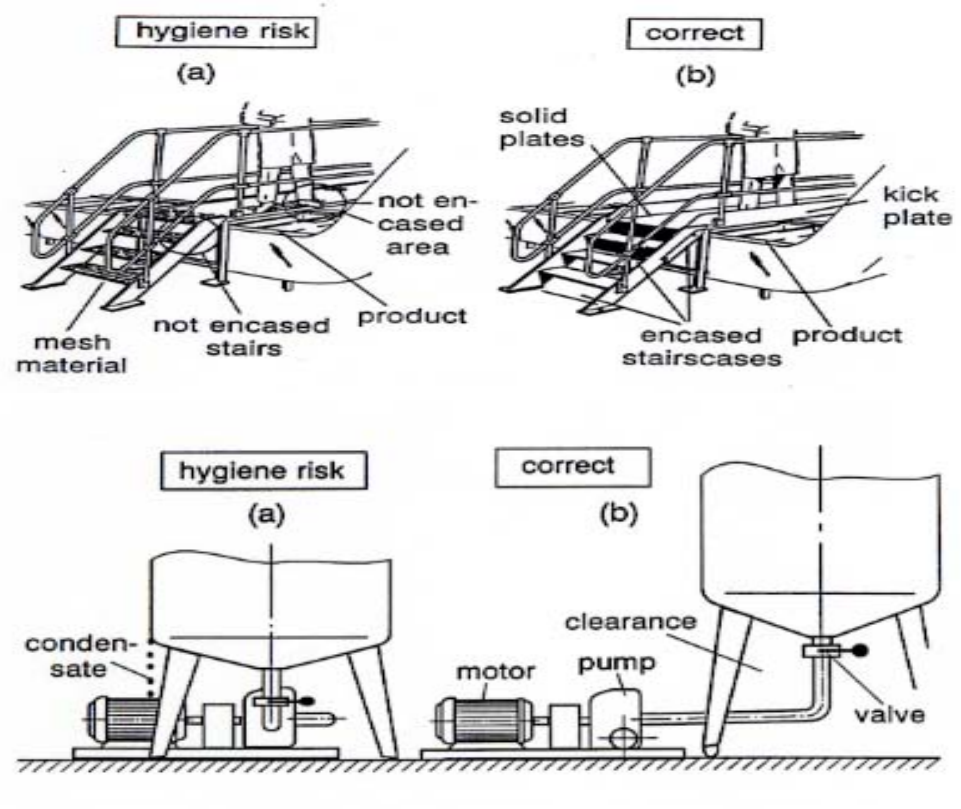



*Figure 5. EHEDG comparison of bad and good equipment access and platform practice.*

# 5. Some general material selection considerations

Material selection is an essential part of the design process and a requirement of the validation philosophy is to ensure that evidence exists that appropriate materials have been used in the correct way. The prime objectives in the selection of the correct materials are as follows:

- To ensure corrosion potential is minimised.
- To avoid any leachates from contact materials.
- To prevent the adherence and development of bio-films on the wetted surfaces.
- To avoid retention of cleaning or disinfecting agents within the systems.
- To ensure that surfaces are aesthetically appropriate and inspectable.
- To select materials that are easy to fabricate to the high standards expected.

## 5.1 Stainless steel

For the stainless steel components, avoidance of corrosion is a principal objective. Stainless steel corrosion is usually divided into four groups. These are general corrosion, pitting corrosion, crevice corrosion, and stress corrosion cracking. Of these, the latter three are the most likely to occur in pharmaceutical water, steam, and process systems. Correct material selection, carefully controlled welding, and post-installation treatment are all techniques that we should use to prevent these corrosion effects from causing system failure.

Surface roughness of stainless steel vessels and piping is a basic engineering specification that must be determined for quality critical water systems. The arithmetical average roughness, Ra, is the best method for defining basic surface roughness. The treatments applied to the surfaces of stainless steel are aimed at modifying the surface roughness and ensuring completeness of the oxidised protective surface. The less rough the surface the better will be its corrosion resistance and cleanability. Electro-polishing in particular gives the greatest surface improvement, and avoids some of the deficits that come from mechanical polishing methods. During the course of the fabrication and installation of a piping system, particularly where in situ assembling and welding is required, it is impossible to avoid some distress at the weld sites. Whilst every care must be taken to ensure that the welds are inert gas protected, tube ends prepared appropriately, and the welding process controlled very carefully, it is

still necessary to carry out post-weld improvements. The most common approach is to passivate the weld site chemically. Passivation is traditionally carried out using 15–20 % nitric acid at 50–60°C. However, recognising some of the safety and effluent disposal issues associated with nitric acid, several citric acid based passivation agent are now being utilised.

## 5.2 Plastics

Plastics materials are most commonly used within water treatment systems and for many parts of our process equipment. The following plastics are easy to clean, and may be used in hygienic equipment design:

- Polypropylene (PP)
- Unplasticised Polyvinyl Chloride (uPVC)
- Acetal Copolymer
- Polycarbonate (PC)
- High density Polyethylene (PE)
- Polytetrafluoroethylene (PTFE).

If you are considering the use of polytetrafluoroethylene (PTFE), it is important to be aware that PTFE can be porous and is usually difficult to clean. PTFE is an effective facing material for items such as diaphragm components.

Plastics materials are frequently reinforced to improve strength and pressure resistance. Material specifications and any material inspection should ensure that reinforcement material (such as glass or carbon fibres and glass beads) in plastics should not come in contact with the product and must not become detached and shed into the product. The plastics materials used in water purification equipment include:

- Unplasticised Polyvinyl Chloride (uPVC) – Used in piping for some pre-treatment system components.
- Acrylonitrile-butadiene-styrene (ABS) – Used in some old purified water systems and in low grade DI systems.
- Polypropylene (PP) – Being promoted in hygienic form for purified water systems.
- Polyvinylidenedifluoride (PVDF) – Heavily used in the microelectronics industry for high purity water systems (>10 megOhm), and now being seen in similar ultra-high purity systems needed in pharmaceutical research.

- Polytetrafluoroethylene (PTFE) – Starting to be promoted in special extruded form for some purified water systems.

## 5.3 Elastomer guidance

Many different types of elastomers are used in the food industry for seals, gaskets, and joint rings. The recommended choices are:

- Ethylene Propylene Diene Monomer (EPDM). (not oil and fat resistant applications)
- Nitrile rubber
- Nitrile/Butyl rubber (NBR)
- Silicon rubber**
- Fluoroelastomer (Viton)**.

**Also for high temperature application (up to 180 ºC).

Excessive compression will cause damage to rubber components and may cause the elastomer to extrude into the product zone, adversely affecting cleanability. Therefore, where an elastomer is used as a seal between solid surfaces, the compression of the elastomer must be controlled (also taking into account thermal expansion during pasteurisation or sterilisation of product or equipment). There are now several sanitary unions available on the market that have a mechanical stop to prevent over-compression of gaskets.

Adhesives used for keeping gaskets and seals in place are often ignored. These materials should always comply with the recommendations of the supplier of the equipment for which those gaskets are used. This is required to ensure that the adhesive will not lead to localised corrosion attack of the stainless steel of the equipment. All bonds must be continuous and mechanically sound, so that the adhesive does not separate from the base materials to which bonded.

# 6. Some available guidance and standards

There are an enormous array of guides and standards available for us to take advantage of. Ironically only two of these have been developed specifically for the biotechnology and life science industry. They are *BS EN 12296;*

*Biotechnology Equipment; Guidance on testing procedures for cleanability* and *ASME BPE 2002 Bioprocessing Equipment.* The other guidance considered here is that produced by the EHEDG based in Brussels.

## 6.1 ASME BPE 2002

ASME BPE 2002 was planned to be sympathetic the US and EU Dairy Standards and any available biopharmaceutical design and fabrication standards. The document was approved as a US national standard on January 24th 2002. The main elements of the standard concentrates on the following subjects:

- Some general considerations.
- Design for sterility & cleanability.
- Dimensions and tolerances related to welding of stainless steel.
- Material jointing.
- Stainless steel and metal finishes.
- Equipment seals.

Some of the experts contributing to the section on design for sterility & cleanability came from the US pharmaceutical industry. Overall it is a very detailed document that concentrates on all the features that might give rise to crevices and features that could harbour contamination, make surfaces difficult to clean or be sites for corrosion to take hold. The following list highlights some of the more interesting material in this standard:

- A drainability test.
- A CIP dye test with guidance about flow rate stability ranges.
- Various illustrated good practice details.
- Weld visual quality guidance.

The section on surface finishes is of particular interest. It provides guidance about the range of finishes available on pipe, tube, fittings and forged components. However it fails to specifically guide the reader on what to use when. The objectives for WFI and pure steam are very different from those of a product process vessel & piping system handing a protein biological product. The former requires corrosion resistance principally, whilst the latter also demands consideration of surface properties and their resistance to the adhesion of product residues.

## 6.2 BS EN 12296:1998 Biotechnology equipment- Guidance on testing procedures for cleanability

This CEN standard was published as part of the huge family of Biotechnology standards from CEN Technical Committee 233. It is an extremely simple short standard with a normative section that sets out the basic requirements for defining a method, the test procedure and the documentation of the results. A very useful decision tree is in informative Annex A, and informative Annex B has some example test methods. There are no limits set in the standard:

- Visual inspection.
- Swabbing surfaces.
- Sampling rinse water.
- Testing a following batch for residuals of the former batch.
- Direct monitoring of residual soil.

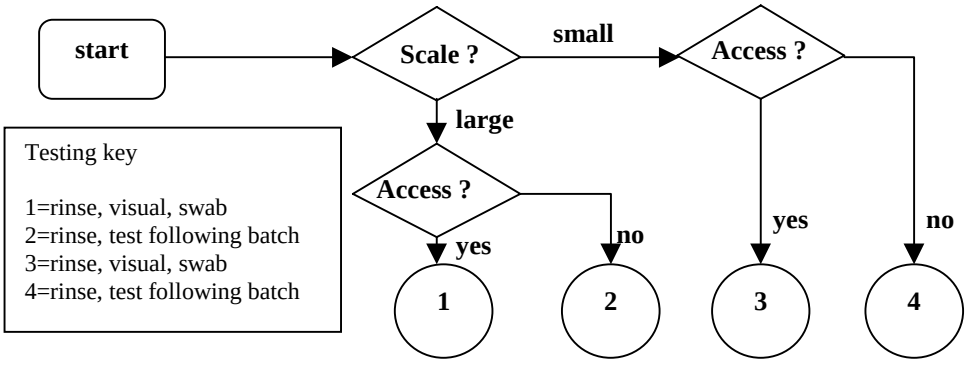



*Figure 6. Decision tree from Annex A, selection of cleanability test method.*

## 6.3 EHEDG Guidelines

European Hygienic Engineering & Design Group (EHEDG) Secretariat is based in Brussels, Ave. Grand Champ, 148, B-1150 Brussels, Fax: 32 02 763 0013, Tel: 32 02 7617408, Email: secretariat@ehedg.org. The website address is www.ehedg.org.

The EHEDG is a consortium of equipment manufacturers, food industries, research institutes, and public health authorities, founded in 1989 with the aim to promote hygiene during the processing and packing of food products.

European legislation requires that handling, preparation, processing, packaging, etc. of food is done hygienically, with hygienic machinery in hygienic premises (EC directives 98/37/EC and 93/43/EEC). How to comply with these requirements, however, is left to the industry.

To assist in the design of safe and hygienic machinery, the EU has mandated the Committee for European Normalisation (CEN) to produce standards. The EHEDG, through its published documents, feeds into these standards.

As food safety does not end at the borders of Europe, the EHEDG actively promotes global harmonization of guidelines and standards. The US-based organisations NSF and 3-A have agreed to co-operate in the development of EHEDG Guidelines and in turn, EHEDG co-operates in the development of 3-A and NSF standards.

The current titles in the EHEDG series of guides are:

1. Microbiologically safe continuous pasteurisation of liquid foods (12 p.)
2. A method for assessing the in-place cleanability of food processing equipment (15 p.)
3. Microbiologically safe aseptic packing of food products (11 p.)
4. A method for the assessment of in-line pasteurisation of food processing equipment (9 p.)
5. A method for the assessment of in-line sterilisability of food processing equipment (7 p.)
6. The microbiologically safe continuous flow thermal sterilisation of liquid foods (22 p.)
7. A method for the assessment of bacteria tightness of food processing equipment (7 p.)
8. Hygienic equipment design criteria (12 p.)
9. Welding stainless steel to meet hygienic requirements (19 p.)
10. Hygienic design of closed equipment for processing of liquid food (17 p.)
11. Hygienic packing of food products (15 p.)
12. The continuous or semi-continuous flow thermal treatment of particulate foods (24 p.)
13. Hygienic design of equipment for open processing (20 p.)
14. Hygienic requirements on valves for food processing (18 p.)
15. A method for the assessment of in-place cleanability of moderately-sized food processing equipment (11 p.)

16. Hygienic pipe couplings (23 p.)
17. Hygienic design of pumps, homogenisers and dampening devices (12 p.)
18. Passivation of stainless steel (11 p.)
19. A method for assessing the bacterial retention ability of hydrophobic membrane filters (6 p.)
20. Hygienic design and safe use of double-seat mixproof valves (19 p.)
21. Challenge tests for the evaluation of the hygienic characteristics of packing machines for liquid and semi-liquid products (32 p.)
22. General hygienic design criteria for the safe processing of dry particulate materials (20 p.)
23. Production and use of food-grade lubricants (19 p.)
24. Prevention and control of *Legionella* spp. (including *legionnaires* disease) in food factories (24 p.)
25. Design of mechanical seals for hygienic and aseptic applications (14 p.)

# 7. Good installation practice

The installation of hygienic systems can make or break our quality and integrity objectives. However good our materials are, they can be destroyed by poor installation practice. One of the most important aspects of any side assembly operation is the maintenance of a clean installation protocol, and of course documentation, documentation, documentation. Remember without documentation of a critical system its existence is just a rumour according to the FDA, and without a signature of record is just considered to be graffiti. This sort of remark may sound the little extreme, that they are provided purely to make a point of the importance of integrity of the whole story we assemble in regulated industries. Is not possible within the scope of this paper to go into all aspects of the philosophy of validation, but needless to say the comments made below should all be fully encapsulated within the validation carried out on the system in question.

There are many aspects of good installation practice that we must take into account, and we can now find more and more help from vendors with specialised products. For example, we are able to obtain now from specialised component suppliers, valve and piping arrangements that give virtually zero deadleg connections into our systems. Designs and specifications should look in detail at these configurations and take advantage of reducing site-based risks. Under the

banner of validation IQ, some of the most important basic tasks that we have to accomplish are defined. These include:

- Complete and effective documentation of the materials utilised.
- Dispensing of materials from stores to job-site.
- Clean installation protocol. Clean and controlled working practices that avoid introducing contamination into the system.
- Inspection of work in progress.
- Progressive sealing and tagging of the installation as it progresses.
- Where welding is carried out it is essential that this process is controlled and logs should be carefully kept related to operator qualification, equipment set-up, samples, and weld site identification. It will be common practice to inspect welds on a periodic basis. Only in critical very high pressure process systems or in the event of major problems being identified, would it be normal to instigate in-situ weld x-ray inspection.
- Detailed as installed records.
- Cleaning and passivation methods and records.

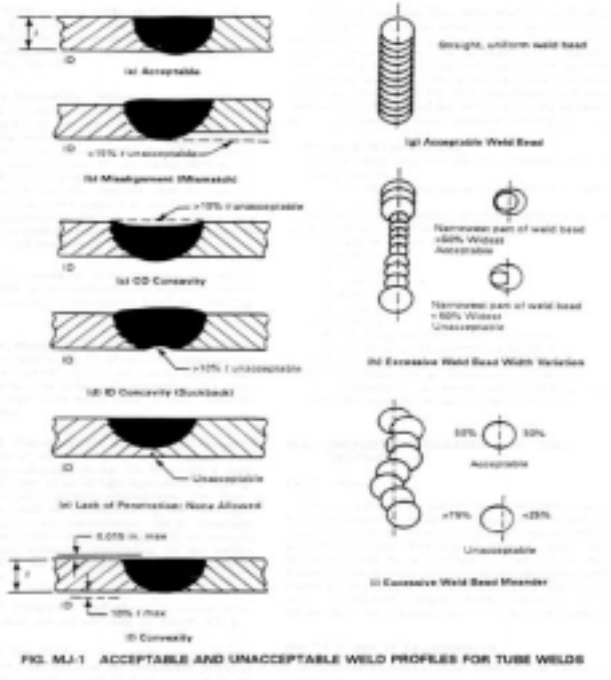



*Figure 7. Example of weld quality guidance taken from ASME BPE 2002.*

# 8. Some common problem areas

Finally some common problems are aired. Understanding of these issues can help us avoid problems or resolve them if they occur.

## 8.1 Rouging of stainless steel

Rouging is a phenomena that is very prevalent among hot (>60 degC) compendial water systems, and normally presents no obstacles to the passing of USP/EP water quality tests. It is discussed at some length in the ISPE Baseline Water and Steam Guide passivation section.

Rouge is a thin, brown reddish thin film deposited on the interior surface of piping, vessel walls and other interior of components of a system that are exposed to pure water (WFI and USP purified). The deposited rouge essentially consists of ferric oxide. It may also contain iron, chromium and nickel in various components. Rouge is colloidal rust that is removed relatively easily from the surface. The source of the rouge may be from the general surface of the piping or tubing or from the surface of a storage vessel, but most likely from the heat-affected zone of the welds. Stainless steel surfaces with high ferrite content may also be contributors of rouge. Please note that the Austenitic stainless steel consists of over 60% of iron and that hot pure (and of course WFI) water and clean steam are aggressive and have the potentiality of leaching out iron from the surface of the steel. The high sensitivity microelectronic industry does not use stainless steel but plastic materials for their piping systems.

Rouge can be removed by re-passivation. This will require a short term facility shutdown. Re-passivation will not solve the rouging problem. In one or two years rouge might reappear. It is very important to do the whole operation correctly the first time.

## 8.2 Access to the work during installation

The following picture is self-explanatory. In installations where falls, weld quality, ability to inspect, and tracablity are important, we must ensure we have thought about access. It is all too easy to forget the person on site working in the real world.

*Figure 8. Remember access to the work during installation.*

## 8.3 Other problems areas to avoid.

- Failure to request material certificates in specifications and at the time of placing an order.
- Using unqualified suppliers and contractors for critical systems or components.
- Buying on lowest price without evaluating the risks.
- Accepting impossible programme or timeline proposals from contractors.
- Failure to have clear and measurable milestones during the work.
- Failure to have clear acceptance criteria at Factory Acceptance Test (FAT) & Site Acceptance Test (SAT) stages.

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# RISK ASSESSMENT IN THE PHARMACEUTICAL INDUSTRY

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## Abstract

There are many risks involved in the pharmaceutical industry. These range from commercial risks to risks to the patient from failures of the quality system. It is not only risks in dosage form manufacture that can cause problems for the patient. Many risks exist from active raw materials, excipients and packaging materials. FDA has published a guideline for risk assessment and many other regulatory authorities are considering the assessment of risk to be an integral part of a quality system. Risk assessment, when used as a quality tool, will enhance the quality of medicinal products and is a major component of quality assurance. The historic statement that "quality cannot be tested into a product, it must be built in" is a real driver for risk assessment. Had risk assessment been carried out in the companies that have been forced to recall defective products in the last few years, many problems would have been addressed before they arose. This paper will address ways that companies can carry out meaningful risk assessment without incurring added astronomic costs. It will also warn against making risk assessment another tool that companies, consultants and regulatory authorities pursue beyond the point of patient benefit.

## 1. Background

The entire science of medicines is fraught with risks of many types. These range from financial risk, for example the risk that a new product in final stages of clinical trials gives rise to unforeseen and inexplicable harmful side effects. The risk here can be many millions of Euro, dollars, pounds or crones.

An even greater financial risk is the case of a product, which is licensed, put onto the market, and then produces unwanted side effects (e.g. Thalidomide, Opren).

This is not the type of risk that will be discussed in this paper. Nor will the risk of environmental damage be considered, although such risks can have a significant magnitude. It is intended to concentrate on the major issue of risk based approach to regulation of product quality.

Those of us who were present at the $R^3$-Nordic conference in Sandefjord last year heard an excellent presentation on Dispersion and Risk Assessment of Airborne Contaminants in Pharmaceutical Cleanrooms by Dr. Berit Reinmüller [1]. In Stockholm there was one paper by Dr. Anna-Maija Sjöberg from the University of Helsinki [2] on the role of pathogen monitoring in microbiological risk assessment. Another paper on the adaptation of the Hazard Analysis Critical Control Point concept to the pharmaceutical industry was given by Dr. Per Lantz from Pharmacia [3]. It is intended that this presentation will be a continuation of those themes, with an additional FDA position on risk-based approach to product quality regulation.

## 2. Risk assessment

Before commencing any risk assessment it is necessary to define the terms that will be used. Probably the most important definition is that of 'risk'. This has been variously described as:

"The possibility that something will go wrong to prevent, directly or indirectly the achievement of specific business objectives" [4], and "the potential for adverse impact of areas of uncertainty on a decision or action path" [5] and "the possibility of loss, injury, disadvantage or destruction". A more simple definition is the impact of performance variation.

Commonly two themes arise from the definition. The first is that risk is higher in times or situations of uncertainty and the second is that risk is almost invariably seen to be a negative event such that "something will go wrong".

It is unusual for the perception to include a positive motive such as "if we take this risk we may be able to improve" (the product/process). After research and development, and regulatory approval of a pharmaceutical product there are risks associated with the manufacture and control of the product. These risks can fall into four categories: human, procedure, control and product. Human risks are

those where the process operator has not carried out the procedures absolutely as defined in the standard operating procedures for the process. Procedure risks are those involved in a known or unknown deviation or intervention in the process. Control risks are those associated with any known, recognised or unknown, unrecognised deviation from an analytical or laboratory based procedure. Product risks are those associated with previously unrecognised side effect such as those found after the introduction of thalidomide and opren [6].

All these risks are in areas covered by regulatory authorities so it is perhaps pertinent now to review the FDA approach to risk. FDA has published a document [7]. This document describes a science and risk based approach to product quality regulation incorporating an integrated quality system approach. FDA uses a twin approach to the quality of “drug” products. Here “drug” is defined as human and veterinary medicines including human biological medicinal products but excluded veterinary feed products and some other medicated veterinary items. It is now almost twenty-five years since there was a major revision of cGMPs and during that time, the review of information submitted in applications and the inspection of manufacturing facilities have help to assure the quality, safety and efficacy of products in the markets regulated by FDA. Of course, other regulatory agencies have had the same objectives during that period and their efforts have served public health in a similarly effective manner. FDA now intends to introduce the most recent concepts of risk management and quality systems to assure continuation of product quality.

It is also intended to encourage the latest scientific advances in pharmaceutical manufacturing and technology. To enhance the approval process the submission review program and inspection program will be co-ordinated to operate synergistically. It has long been recognised that consistent application of regulations, manufacturing standards and quality approaches are consistent. FDA intends to use its resources most effectively and efficiently to address any significant health risks. The reasons for this novel approach to product quality have been quoted as the ever increasing number of medicinal products and their greater role in health care.

The reduction in number and increase in time interval of FDA inspections. FDA believes that it has learned from other quality initiatives and that there have been a substantial number of advances in pharmaceutical science and technology.

There is also an increase in the discovery and use of biotechnology derived products. The pharmaceutical industry has undergone globalisation and there have been significant advances in management of science and quality.

FDA has concluded that it must match its level of effort to the magnitude of the risk and that a more searching risk based approach is necessary. Many of the intellectual knowledge of the advances in pharmaceutical science and technology have been brought into the FDA arena and recent science can contribute to improvement of assessment of risk. One only needs to look at the increased risk to the criminal with the advent of DNA finger printing. FDA is also to evaluate interaction between the pre-market CMC review process and the application of cGMP requirements and bring them into an integrated system. ICH has brought out the best, in co-operative efforts on harmonisation and all regulatory authorities will work under the umbrella of ICH to protect public health globally.

Protection of public health is a major mandate for all regulatory agencies. From the FDA standpoint an external review of existing cGMP and product review processes has been proposed especially to view the inconsistencies at the interfaces. There will also be a re-assessment and re-evaluation of the existing scientific approach to product and cGMP to give harmonised, consistent, integrated approach to product quality regulation. There will be an emphasis on risk based control point analysis (similar to HACCP) to integrate with the most recent advances in pharmaceutical engineering.

How will FDA, in particular, achieve this? The steps FDA will take are divided into short term, intermediate and long term. FDA is using current regulations such as 21 CFR Parts 210, 211, 600 and 610, which it believes has sufficient flexibility to permit the change to a science based risk management approach. There always will be the possibility that cGMP amendments may take place, particularly in view of public and industry comments on the May 1996 proposals. As we all know 21 CFR Part II has seen changes in February 2003 with many sub parts being withdrawn. Amendments to 21 CFR Parts 600-610 should also be foreseen.

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# REGULATORY ASPECTS OF BIOMEDICINES

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## Abstract

Biological and genetic research is going forward rapidly and new developments are revealed almost every day. New information and knowledge is applied for developing new drugs and devices. These new biomedicines set specific requirements for regulation to ensure safety and efficacy of the products. Regulation is put into effect on the national level (National Agency for Medicines) and on the European level (European Agency for the Evaluation of Medicinal Products).

The definition of a biomedicine is given in the Directive 2309/93 in its Appendix Part it is either a recombinant-DNA-derived product or protein expressed in procaryotes or eucaryotes or hybridoma derived monoclonal antibodies. However, it is obvious that there is overlapping in the definition between a biotechnological and biological product which makes strict definitions and thus, regulation, difficult to set.

Development of biomedicine is a multi-step process. The first steps are usually done in research laboratories or small companies and further work in larger companies. Although manufacturing of drugs is one the strictest regulated area, the available regulatory documents are subjects to interpretation. Documentation of the early developmental phases, application of GLP or GMP, proper characterisation of the product at the different stages of development are some examples of the difficulties in developing a new biotechnological drug.

Specific consideration must be given to specifications and characterisation as well as to viral and prion safety of biomedicines. Viral and prion contamination rises from the biological reagents or substances used in manufacturing process. Possible sources of contamination are cell lines used for production of biomedicines, serum and other media as well as personnel. For application of marketing authorisation regulation is more accurate but variability of

biomedicines (between and within substances) still causes problems especially in case of comparability issues. New advanced therapies such as somatic cell therapy and gene therapy set specific future challenges for the regulation by means of definitions and safety.

# GMP IN VACCINE PRODUCTION

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## Abstract

The main principals involved in the process of manufacturing medicines are safety and efficacy. Vaccines are administered to healthy individuals as preventive medicine. This makes the safety of these products of the main concern. Mistake in these products could cause catastrophic consequences, as the vaccination of children and infants is systematic. In vaccine production toxins and microbes which cause dangerous infectious disease are widely used. This is very demanding for premises, activities and personnel. Safety of both product and personnel is a clear requirement during production. Basic safety concerns of premises and equipment can be eliminated by technical arrangements. Activities of personnel and high level of purity of premises and material are playing a major quality role as the vaccines are produced aseptically. In vaccine production management of every step of production should be well in place at all times, because at the end of the process, products are not sterilised in their final containers. The sterility test and other quality tests applied to the finished product should only be regarded as the last in a series of control measures by which quality is assured. Therefor cleanliness of the premises and good quality of performed activities including hygiene level and safety of personnel are verified regularly in sterile production. For safety and efficacy reasons also quality control methods including in-process control methods should be reliable and they should be verified by validation.

# MEDIPOLIS GMP, PILOT PLANT FOR BIOTECH COMPANIES

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Medipolis GMP Ltd, Oulu, Finland

## 1. Introduction

Medipolis GMP Ltd is creating partnerships in R&D and GMP pilot production with innovative pharmaceutical and biotech companies. We have state-of-the-art clean room technology, production equipment as well as dedicated personnel with profound understanding of cGMP.

## 2. Design principles

This pilot scale facility was designed to meet cGMP requirements (EU, FDA) and to have flexible production capabilities for bio products. The Pilot plant is a three floor building, technical area in the ground floor, clean rooms in the 1st and air handling units in the 2nd floor.

As the processes to be carried out in the facility were unknown, the design was based on series of concept processes with stated sizes, process steps and environmental requirements.

For flexibility reasons the containment level of the live area of the pilot plant was designed to be BL3, which means e.g. negative pressure and double air locks (one way in, other way out).

Utilities, such as purified water, clean steam, gases and chilled water are brought in to the clean room through utility panels. Piping for the panels is in the technical “fingers” which are between clean rooms. All the clean utilities are produced in the ground floor technical area and piped up to the clean rooms.

This way we have complete clean room fitted out ready to accept the specific process equipment required by any potential user. Also our aim was to have as little piping inside the clean room as possible.

## 3. Facilities

Total floor area for the building is 2300 $m^2$. Classified clean room 400 $m^{2,}$ class A/B, C and D (ISO 5 – ISO 8). In the clean room there is dedicated media and buffer preparation area, live area (fermentation), three separate purification rooms, including a +4°C cold room and final filtration and freeze drying area.

Main process equipments are Fermenters, capacity 10 to 500 l. For down stream processing there is Homogeniser, Chromatography systems, laboratory and pilot scale, Tangential flow filtration systems, bench and pilot scale, and Freeze dryer (15kg).

All the waste from live area is decontaminated, solid waste through decontamination autoclave and liquid waste through continuous decontamination system.

## 4. Air handling

Air handling of the clean room area is organised with five fresh air units. In the live area there is two fresh air units and no circulation of the air. Also there is terminal exhaust HEPA filters. Exhaust in the live area is secured with double funs.

For the rest of the clean rooms (purification suite, media and buffer preparation and final filtrationa/freezedrying area) there is three fresh air units with circulation. All the clean room air handling units are connected to buildings own standby power system.

Particle size requirements for the clean room are described in Table 1. Particle levels in the clean room are monitored with PMS (Particle Measurement System). Criteria for temperature in the clean room is 22°C ± 4°C.

*Table 1. Clean room particle size requirements.*

| | EU GMP (ANNEX 1)<br>0.5 µm | 5.0 µm |
|---|---|---|
| **ISO 5 M 3.5<br>A at rest/in operation<br>B at rest** | **3500 kpl/m³ (99 kpl/ft³)** | **0 kpl/m³ (0 kpl/ft³)** |
| **ISO 7 M 5.5<br>B in operation<br>C at rest** | **350 000 kpl/m³ (9930 kpl/ft³)** | **2000 kpl/m³ (57 kpl/ft³)** |
| **ISO 8 M 6.5<br>C in operation<br>D at rest** | **3500 000 kpl/m³ (99295 kpl/ft³)** | **20 000 kpl/m³ (567 kpl/ft³)** |
| **D in operation** | **not defined** | **not defined** |

## 5. Alarms Management System

Validated Supervisory Control and Data Acquisition (SCADA) system controls purified water loop and handles all classified alarms from clean room, equipment and systems. Alarms are also forwarded to mobile phone of the duty officer.

## 6. Validation

All the critical systems and equipments are validated according to cGMP.

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# THE VALIDATION OF A NOVEL RAPID STERILE TRANSFER PORT SYSTEM USED IN BARRIER FILLING LINES – AN IMPROVED STRATEGY FOR MATERIALS HANDLING IN THE PHARMACEUTICAL INDUSTRY

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## Abstract

Material transfer into Class A filling environments has been identified as one of the most common causes of aseptic processing failure. Traditional filling facility design layout has placed the filling machine in a Class B environment. Process operations require that sterile materials be transferred across the B environment to the Class A filling area. Keeping such a filling line supplied with components requires numerous operator interventions that risk contamination of the filling environment.

In recent years, the development of barrier isolator filling lines has made the challenge of supplying components to filling lines even more difficult. The most recent FDA draft concept paper on Aseptic Processing publication recognizes that it is not necessary to place a barrier isolator inside a Class B environment, providing that an adequately secure materials handling process is validated and in place. This paper reviews the challenges and options for material transfers including the validation of a new UV light-based rapid sterile transfer technology that reduces the risk of contamination from multiple material transfers into the Class A environment. By sterilizing the interface between the Class A, and Class C environment, this system provides a secure method of material transfer allowing barrier isolator lines to be placed in the significantly less costly Class C environment. This system, the Rapid sterile Transfer Port

system (RsTP), features a three minute UV sterilization cycle providing validation at the point of transfer. The UV light-based system design is novel, more robust, reliable, and easily validated as compared to previous applications of UV sterilization in the pharmaceutical industry.

# 1. Introduction

The heart of any liquid parenteral final fill and finish operation is the filling machine. The filling machine is a conveyor system responsible for bringing empty sterilized containers, glass vials, and syringes from a sterilization tunnel and delivering sterile filled and sealed parenteral dosage formats to a packaging line.

Certain dosage forms need to be assembled before the drug solution is filled, for example naked glass syringe barrels. Before the filling step, a sterile hypodermic syringe needle is assembled onto the glass syringe barrel, and then the container is sealed closed. Rubber closures, in this case plunger tips, are delivered by a hopper feed system.

The critical filling operations are identified as those where sterile liquid, containers, and components are exposed to the environment before sealing. These operations are conducted in highly controlled Class A environments.

Operators are the single largest source of microbiological contamination within the aseptic filling core. A recent PDA survey (Figure 1) also identified material transfer as a significant contributor to aseptic processing failure. In an 8-hour shift, it is typical to supply 10,000 to 100,000 components to the filling area. The number of components transferred at any one time can range from 2,000 to 5,000, and a typical filling run of 100,000 containers would require an average of 20–50 transfers. Each time a transfer is made, there is an increased risk of elevated microbial challenge to the filling environment. Obviously, the more transfers that need to take place the greater the practical risk.

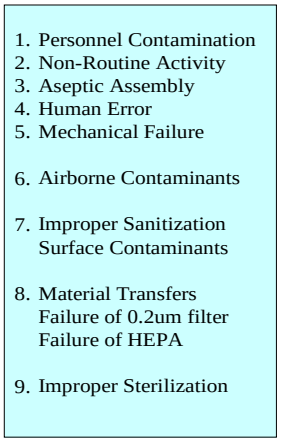



*Figure 1. Aseptic Processing Failure Summary (PDA Industry Aseptic Processing Survey 2001).*

## 2. Current practices in managing sterile material transfer

There is a broad range of current practices and applicable product technologies for managing sterile material transfer processes. The material transfer process depends largely on the details of facility design and manufacturing philosophy. Facility design and philosophy are illustrated by considering two extreme examples: (1) filling operations contained within a Class B clean room and (2) those contained in a barrier isolator filling operation.

In the first example, a Class B clean room uses wipe and pass methodology, as the pre-sterilized components are transferred from a Class C area into the Class B environment (Figure 2).

The primary concern with this transfer is to avoid contamination of the much cleaner Class B room with particles or microorganisms. Wipe and pass transfer techniques using a small chamber between the staging area and the clean room are often employed. An operator in the staging area removes a layer of protective packaging and/or wipes the external surface of the packaging with a cleaning and sanitizing agent. Once completed, the operator places the package in the chamber for passage to the clean room. An operator inside the clean room

removes the transfer container containing the package and places it on a captive trolley.

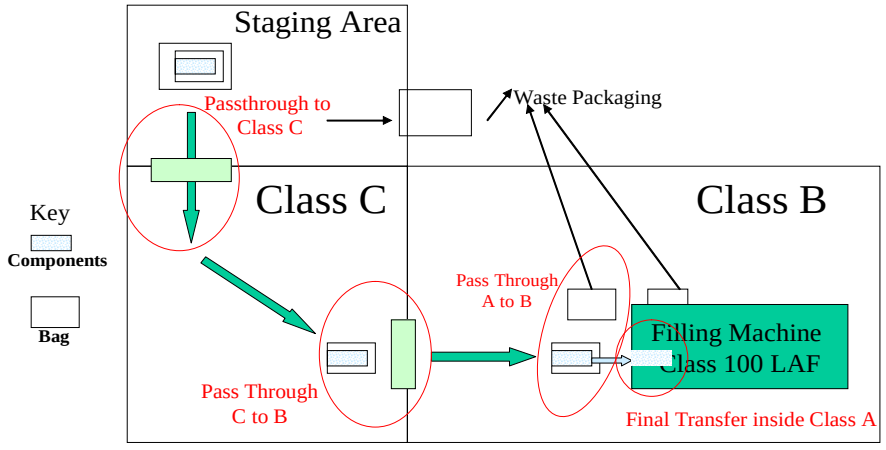



*Figure 2. "Wipe and Pass" method.*

The transfer container moves across the clean room to the Class A filling area. The challenge associated with this second transfer is doubled. The Class B environment has contaminated the external packaging of the components, so in transferring the sterile components, the operator again has to remove a layer of packaging and/or wipe down the external surface of the transfer container with a cleaning or sanitizing agent. The next task is to get the sterile components inside the Class A filling environment without compromising the sterility of the components themselves or the cleanliness of the environment immediately surrounding the exposed sterile components of the aseptic filling process. The component packaging cannot be opened in the Class B area, as this would compromise the sterility of the components. Therefore, the operator has to partially enter the Class A environment. In some processes, the operator stops the filling operation, cuts open the container packaging, and replenishes the component feed hopper. By entering the Class A environment, the operator is in close proximity to the sterile components and sterile liquid dispense heads resulting in possible contamination. By bringing the non-sterile transfer container into the Class A environment, the operator increases the microbiological challenge to the aseptic filling process.

Wipe down procedures are subjective and hence hard to validate. During media fill validation and revalidation each planned intervention needs to be included in the media fill protocol. Risk increases with the number and complexity of each transfer step. Transfers of this type rely on quality operator training and accurate SOP execution. Sterility failure can be catastrophic, even failure during a media fill will result in significant extra work and lost production time. Most significant costs are associated with manpower, sterilizing materials and space requirements.

In the second example, barrier isolator filling lines using Rapid Transfer Port (RTP) technology is used for transfer of sterile materials. RTP technology uses a flexible or solid container to transfer the sterilized components. These containers still need to be transferred from their staging area into close proximity with the isolator. The materials are then transferred from the Class B environment to the isolator.

Alpha/Beta RTP systems are a two-component port system. The Alpha component is a stainless steel port set into the wall of the barrier and sealed by a plastic door. The Beta component is a stainless steel flange attached to the transfer container and sealed by a plastic door. Neither door can be opened unless they are docked together. The exterior door faces the room environment and is exposed to contaminants. However, the port docking process seals the "contaminated" door faces together, which minimizes the risk of external contamination (Figure 3).

The primary benefits of RTP/Barrier technology include the following:

- Operator manipulations are managed only through an isolator glove port.
- Use of flexible RTP containers avoids the need to introduce packaging materials into the isolator environment.

Because of the primary benefits that RTP technology offers, it is widely considered to be "state of the art" for a filling environment.

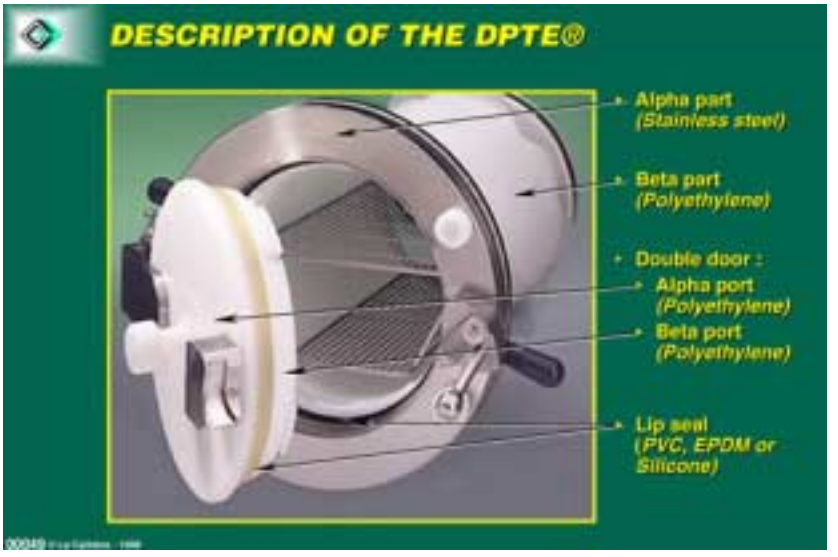



*Figure 3. Alpha/Beta RTP system.*

One significant limitation of RTP technology is that the transfer is aseptic rather than sterile. For the port to open, the canister cover must pass through the port opening. This opening action is facilitated by a small mechanical clearance between the outside diameter of the canister cover and the inside diameter of the port. This small exposed ring (termed the “ring of concern”) could contain contaminants. This risk was illustrated in 1995 by the BUGS Group using the "Toner Test". Copy machine toner was applied to the docking surfaces of the canister and port. The canister was docked to the port and the door was opened. When the port door was opened, two lines of toner were visible. One line appeared at the canister flange/port flange interface and the other line appeared at the port door/canister cover interface.[1] It is normal practice for the “ring of concern” to be sanitized with alcohol before starting a transfer.

## 3. Sterile materials handling, isolators and future process design

Trends in pharmaceutical manufacturing are requiring more process security, which includes minimizing or removing operator intervention in the process. This requirement has increased regulatory and industry support for barrier and isolator filling line installations. This trend brings has new challenges, such as how to design component supply processes to feed a barrier filling line that

ensures minimal risk and regulatory acceptance. The FDA preliminary concept paper, *Sterile Drug Products Produced by Aseptic Processing Draft*, evidences this trend. Relevant highlights from this concept paper include:

Recognition that barrier isolated filing lines appear to offer an advantage over classical aseptic processes. A Class 100,000 background is appropriate depending on manufacturing situation. The ability to maintain integrity and sterility of an isolator is impacted by the design and use of the transfer ports.

A reasonable conclusion is that well designed secure transfer port systems, which are vigorously validated will facilitate the use of isolator filling lines in Class C environments. One solution to executing such a strategy is RsTP. The major benefit of such systems, as compared to RTP counterparts, includes sterile rather than aseptic transfer, and a sterile transfer interface that is easily validated.

## 4. Overview of rapid sterile transfer port (RsTP) systems

One sterilizable transfer port utilizes the Alpha/Beta double door design and addresses the “ring of concern” by integrating dry heat sterilization into the Alpha port (Figure 4). An electric heater is installed in the port flange, which heats this area to over 200°C in three minutes. This temperature is held for 30 seconds followed by a three minute cool down period. The double door is opened and a shield moved into place. The purpose of the shield is to prevent damage of the components by the heated edges of the port.

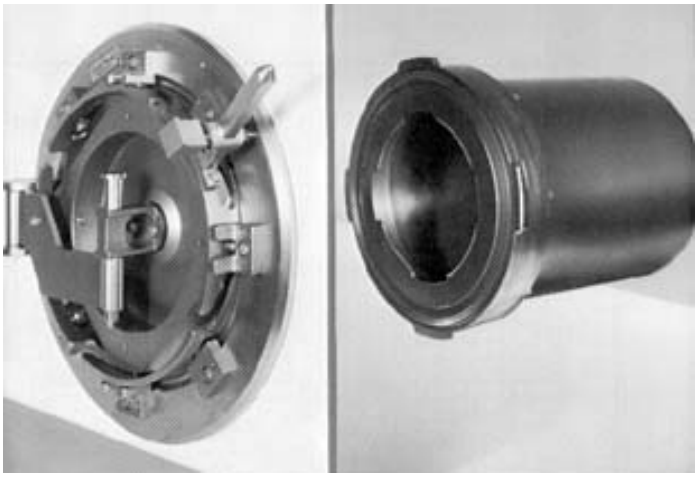


*Figure 4. Central Research Laboratories' transfer port and reusable container.*

More recently, a new ultraviolet (UV) light-based RsTP system has been introduced. This system is a double-door system, but is not dependent upon a mechanical rotating interlock. Operation of this RsTP system is simple. A filled, sterilized container is directly docked to the UV port (Figure 5).

*Figure 5. SafePass® UV sterilization port.*

A simple molded collar, integral with the flexible transfer container is pushed into place and locked by a cam and pin system. The operator starts the three minute UV sterilization cycle by pushing a button. This system is easy-to-use and delivers excellent economics, as there are no expensive Beta flanges requiring regular repair or replacement. This system uses flexible bags as the

sterile transfer container. The components are sterilized inside sealed transfer containers using either gamma or autoclave sterilization.

While the FDA preliminary concept paper, *Sterile Drug Products Produced by Aseptic Processing Draft*, leads us to a conclusion that RsTP systems are valuable tools in aseptic processing. It should be noted that this paper questions the use of UV light as an acceptable sterilizing technology for transfer port technology, but at the same time the authors of this paper believe that a thorough review of UV science, past application and rigorous validation of this technology is merited. The rest of this article is dedicated to a discussion of UV suitability, repeatability, and validatability as it applies to a UV light-based Rapid sterile Transfer Port System.

## 5. A review of ultraviolet radiation

UV light exemplifies a specific range of radiation produced by the sun. Although, UV only represents about 10% of the radiant energy produced by the sun, much of which is absorbed or reflected back into space, it has a large impact on biological activity. Of particular note is the ability of UV to destroy the reproductive capabilities of microorganisms including viruses, mycoplasma, bacteria and fungi.

There are several texts that provide valuable information about radiation biology, photobiology and the physics of light, and the way light and radiation interact with biological and chemical systems.[2–5]

Two main groups of radiations exist: (1) corpuscular and (2) electromagnetic wave radiations[6]. Corpuscular radiations are composed of streams of atoms, electrons and protons and are not discussed in this article. Electromagnetic waves or non-ionizing radiations travel from a source in packets in the form of quanta or photons with each quantum having an associated frequency and wavelength. Ultraviolet light is the portion of the electromagnetic spectrum that lies between X-rays and visible light, having a wavelength between 100 and 400 nm (Figure 6).[7]

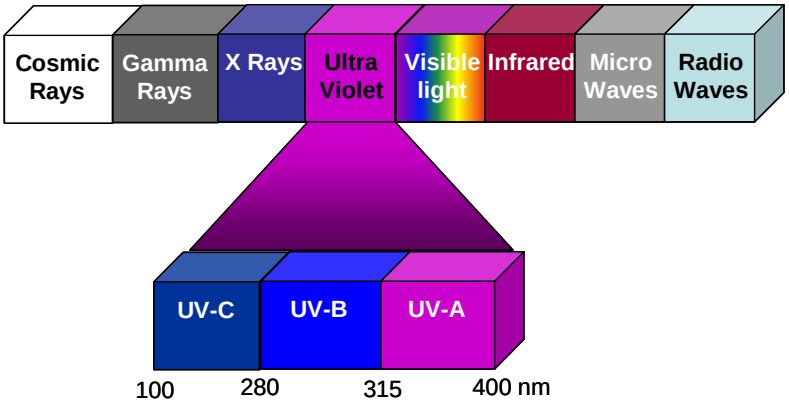



*Figure 6. Electromagnetic spectrum.*

The UV spectrum is well understood and is divided into three basic categories:

UVA 315–400 nm

Photochemical light that is associated with sun tanning and tanning salons.

UVB 280–315 nm

Erythemal light that is used for the treatment of skin disease.

UVC 100–280 nm

Germicidal light, which includes the wavelengths for microorganism inactivation and disinfection.

The most common source of UV light is medium and low-pressure mercury lamps.

## 6. Mechanism of ultraviolet sterilization

The biological effects of UV light and the mechanisms of action are well understood.[8–10] It is in the region of 290–315 nm, the UV absorption of most nucleic acids decreases rapidly.[2] Nucleic acids show a maximum UV absorption wavelength between 260 and 265 nm.[11] The bactericidal effect of a low pressure mercury UV lamp is a direct result of its emission wavelength intensity, and the

absorption by the bacterial cell's nucleic acid.[4, 5] The absorption of germicidal UVC light interferes with the replication of DNA and RNA, and consequently disrupts normal cell function necessary for survival.

UV radiation has long been used as a fairly selective tool for causing cell damage. Understanding the effect of UV irradiation on the vital cellular processes also led to the observation of recovery of previously "dead" cells. The term photoreactivation describes recovery of cells rendered non-viable by UV irradiation. It has been observed that the viability of irradiated *Streptomyces griseus* was increased by 10-fold following storage for 1 to 2 days. Subsequent studies demonstrate that exposure to visible light was in part responsible for photoreactivation. Experimentation showed that exposure of UV irradiated suspensions of *S. griseus* to visible light resulted in an increased recovery of viability of 100 000 to 400 000 fold.[3] The following three different repair mechanisms may occur depending on the organism.

## 7. Photoreactivation or Photoenzymatic Repair

DNA photolyase, activated by absorption of light between 300 and 500 nm, found in microorganisms, plants etc is capable of binding and splitting dimmers.[4] Exposure to light within two to three hours after UV irradiation is required for recovery.[7]

## 8. Excision repair

Found in most organisms, this repair mechanism is independent of the presence of light. Repair involves cleaving and excision of damaged DNA by nucleases and repaired by new DNA strand synthesis by means of a DNA polymerase.[3, 5]

## 9. Post replication repair

Damaged DNA is replaced with parental DNA sequence from either multiple replicate DNA or complement DNA strands.[3, 5]

The cellular capacity for viability recovery may partially explain apparent discrepancies in laboratory results with respect to the use of UV light as a sterilizing source.[3] It also suggests the need for consideration during validation study design.

## 10. Factors contributing to the success of UV sterilization

UV light has been widely used to control microbiological contamination.[6] The most notable limitation has been the ability to develop applications where UV light can be reliably harnessed to provide validatable sterilization for use in the pharmaceutical industry.

Germicidal use of UV light has been employed in the health industry for over 60 years. In most of these applications the action of the UV light has been termed as sanitization or disinfection and rarely as sterilization. The major reason for this dichotomy rests with the choice of application. In almost every documented use of UV light for microbiological control, the target subject has been large in size or volume, distant and diffuse with respect to the UV source. Shechmeister[6] provides an overview of how UV light has been used to sanitize air, surfaces, and water within the hospital, laboratory and beverage markets. In each of these applications the UV sterilizing source has typically been mounted on walls or encased in quartz glass placed around the transport pipes.[12] In these applications the intensity of the UV radiation, and hence its "killing power," delivered to the target is diminished. Distance between the sterilization target and the UV source, the presence of moisture, and particulate contamination combine to reduce the effectiveness and predictability of the UV dosage.[13] These factors make sterilization unlikely and validation difficult.

## 11. Novel rapid sterile transfer port technology

The sterilization performance and validation of a RsTP system incorporating an UV sterilization source can be directly attributed to the design of the UV source and RsTP/barrier interface as an integrated system. The critical design characteristics of such a system need to ensure a consistent delivery of a known intensity of 254 nm UV light.

The UV RsTP has been designed to meet these criteria. It consists of two components: (1) a sterilization port and (2) a sterile transfer container (see Figure 5). The sterilization port is installed into the barrier/isolator wall and is sealed from the outside environment by means of an internal door. This door houses the UV sterilization source. The components to be transferred are sterilized in the transfer container bags by gamma, autoclave or ethelene oxide (ETO) sterilization.

In isolator applications, the port is sterilized by opening the UV door and inserting a plug in the open port. All internally exposed areas and the UV door are decontaminated during the isolator vapor phase hydrogen peroxide (VHP) sterilization cycle. The control systems of the port and isolator are linked, ensuring a full decontamination cycle has been completed. Once all the hardware is sterilized the transfer system is ready for use.

A number of safety features, including a series of interlocks and alarms, prevent the inside of the isolator from being exposed to the outside environment by the premature opening of the door. Similarly, the UV sterilization cycle is closely monitored and alarmed, and is stopped should an alarm condition occur. In such an event, the door cannot be opened. The sterile transfer container is removed from the port, and the alarm condition corrected, only now can a new transfer cycle be initiated.

From the component supplier perspective, the RsTP transfer container is a simple packaging container; there is no fundamental difference in supplying in this type of container versus a standard autoclave bag. One European closure vendor is supplying gamma pre-sterilized closures in this container, which included a complete supply chain validation to ensure the secure delivery of a pre-sterilized component. A US closure vendor also recently published a paper in which they described a similar program to provide pre-sterilized closures in a bag container.[14]

Pre-sterilized components, especially gamma-sterilized components, offer the pharmaceutical industry an attractive economic option, which enhances process security by increasing the number of components per transfer, thus reducing the number of transfers per process. The direct cost of packaging, indirect cost of autoclave investment, and validation may be an important consideration for new

facility planning. Reducing the number of transfers increase process security by minimizing the number of operator interventions in the filling area.

## 12. Designing a UV light sterilization interface for validation

Validation of the sterilizing capability of UV light has been conducted by a direct microbiological challenge. To meet the validation requirement, the design of the novel RsTP system needed to meet the following criteria:

Ensure a consistent level of energy output from the UV light source.

Ensure a consistent level of UV radiation is present at the sterilization site.

Ensure a consistently low bioburden level is present at the sterilization site.

## 13. Ensure a consistent level of energy output from the UV light source

The effectiveness of the mercury vapor lamp decreases as the temperature of the lamp increases. The temperature of the low-pressure mercury lamp rises because infrared (IR) light is produced along with the UV light. As a result, the longer the lamp runs the more IR is produced resulting in greater heat output and a lower 254 nm UV intensity. Two critical design features have been enhanced. The UV source is designed to run at 40–45° C, its optimum temperature for maximizing the UV output.

Consistent UV intensity is key to the reproducibility and validation of the UV sterilization process. Consistency can only be achieved if the UV lamps are functioning correctly. A lamp monitoring circuit integrated into the starter design monitors the amount of current drawn by each lamp, which ensures functionality of the UV source. Such a change would equate to a reduction in UV intensity and generate a system alarm.

## 14. Ensure a consistent level of UV radiation is present at the sterilization site

sterile UV light intensity decreases with distance from the source. The Inverse Square Law is often used to compute the intensity of light at any distance from a lamp but is inaccurate in the near field where most of the germicidal effect occurs. Other models, such as the radiation view factor equation, provide a more accurate prediction of the UV intensity at small distances.[15] This is one of the key reasons why the use of UV in this application is significantly different compared with other disinfection applications. In these applications the validation approach has been complicated and performance compromised because the UV source is a long way from the sterilization target. In the transfer application the UV source is placed very close, 0.185inch (4.699 cm), to the transfer collar interface to be sterilized. By tightly controlling the dimensions of the transfer container collar, its position in the port and only allowing the sterilization cycle to begin once the collar is locked into place; a consistent distance is maintained between the interface and the sterilization source.

Shadowing can also reduce the effectiveness of an ultraviolet sterilization system and make validation more difficult. Shadowing occurs as a result of the ultraviolet rays being blocked or obstructed by foreign objects such as particulates or crevices. The transfer container collar profile is designed to minimize the effects of shadowing. Additionally, a removable collar cap protects the collar face from scratches or particles, which may collect on the collar face. Manufacturing the transfer collars in a Class 1000 environment further minimizes particulate contamination.

## 15. Ensure a consistently low bioburden level is present at the sterilization site

A low bioburden at the site of sterilization is ensured in the following four ways:

**Design of the collar interface:** A tight fitting protective cap that permits gamma or autoclave sterilization covers the entire transfer collar interface surface.

**Manufacturing practice:** A Class 1000 molding environment minimizes bioburden contamination prior to sterilization.

**Pre-sterilization of the collar transfer interface:** The transfer interface collar is autoclave or gamma sterilized along with the components.

**On-site SOPs (standard operating procedures):** The protective cap is only removed, inside a Class C/B environment, seconds before it is docked into the UV RsTP sterile transfer port. Risk assessment studies[16] have concluded that a five minute exposure in a Class C room would result in a maximum contamination rate of 92 cfu on an exposed 100 mm ($79cm^2$) interface collar.

# 16. Validating UV light sterilization in the novel RsTP system

For any sterile transfer system to be of value to the pharmaceutical industry, its functionality needs to be validated in terms readily understood by the industry and acceptable to the regulators. In determining an acceptable validation standard for sterile transfer, the starting point has to be the application. For example, the purpose of the RsTP system is to create a sterile transfer interface between a Class A environment (barrier isolator) and the surrounding Class B or C environment. In looking for an acceptable standard of sterility, the obvious candidate is the sterility assurance associated with autoclave sterilization. The expectation is that an autoclave cycle would be capable of sterilizing a microbiological challenge of $1x10^6$ cfu, giving a theoretical assurance of a sterility level of 1 in 1 000 000.

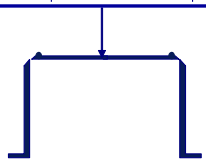
Scientific data suggests that Tobacco Mosaic Virus (TMV) has a maximum resistance to UV requiring a dose of 440 mWatts/$cm^2$ to achieve a 100% kill of the TMV while *Bacillus anthracis* spores require a dose of 46 mWatts/$cm^2$ to achieve sterility. The total dose delivered during a 90 second cycle using the RsTP UV sterilization source was at least 1000 mWatts/$cm^2$, more than twice the minimum required dosage for TMV. The cycle requiring validation had a duration of 180 seconds, ensuring a larger dosage than is required for sterilization.

The sterilizing capability of the UV cycle was validated by a direct microbial challenge. This bacterial challenge was inoculated directly onto samples of LDPE (low density polyethylene) material used to manufacture the transfer interface collars. An approximate concentration of $10^6$ to $10^7$ spores of *B. pumilus* (ATCC 27142) was used. Complete sterility of the LDPE collar interface was shown after a minimum of 45 seconds exposure to UV (Table 1).

Photoreactivation is considered as an integral component of the sterilization validation protocol. To assess the sustained lethality of the UV irradiation dose associated with the novel RsTP sterilization cycle, a validation protocol was developed that would assess the ability of UV irradiated organisms to repair themselves through photoenzymatic or dark repair mechanisms. The possibility of photoenzymatic (photoreactivation) repair was excluded by exposing irradiated organisms to monochromatic and broadband light. Similar the possibility of excision dark repair mechanisms was excluded by incubating irradiated organisms in the dark. Photo protection with preceding illumination was not investigated.[2] It was considered that the inoculation organisms were already exposed to light before irradiation, and any recognized benefit was included in the original sterilization validation.

*Table 1. Microbial Challenge data.*

| Inoculum (HDPE) | Ave. Conc. | Exposure Time | CFU Per Slide | % Recovery |
|---|---|---|---|---|
| *Bacillus subtilus* | $5.6X10^6$ | 60 Sec | 0 | 0.0% |
| *Bacillus pumilus* | $6.3X10^6$ | 180 Sec | 0 | 0.0% |
| *Bacillus stearothermophilus* | $6.8X10^6$ | 60 Sec | 0 | 0.0% |
| *Deinococcus radiothurans* | $4.5X10^6$ | 60 Sec | 0 | 0.0% |
| *Pseudomonas aeruginosa* | $7.5X10^6$ | 60 Sec | 0 | 0.0% |
| *Staphylococcus epidermidis* | $7.7X10^6$ | 60 Sec | 0 | 0.0% |



The study, using *B. pumulis*, was conducted three times on separate days and 36 gamma irradiated 2 x 2" LDPE slides were tested with an inoculum size of approximately 1.2 x $10^6$ to 1.6 x $10^6$ spores in 40% alcohol. Each slide was

exposed to UV light for 180 s. For enumeration of the recovered bacteria post-UV exposure, four exposed slides were incubated in 100 mL of TSB (tryptic soy broth) at 30–35 °C for 14 d. Furthermore, for photoreactivation, two procedures were employed. In the first procedure, 16 slides were placed in glass petri dishes and stored under fluorescent light (light intensity: 500 ± 40 Lux) for 7 d at 2–8°C, 20 ± 1°C, 24 ± 1°C and 35 ± 1°C.

In the second procedure, another set of 16 slides were incubated for 7 d in the dark followed by 7 d exposure to fluorescent light (light intensity: 500 ± 40 Lux) at 2–8°C, 20 ± 1°C, 24 ± 1°C and 35 ± 1°C. The results showed that all 36 slides exposed to UV light for 180 seconds were sterile indicating that *B. pumilus* spores (ATCC 27142) did not undergo photoreactivation after exposure to UV light.

## 17. Conclusion

Rapid sterile Transfer Port systems offer potentially significant benefits to the pharmaceutical manufacturer over the existing aseptic Rapid Transfer Port systems. Most notably, a decreased risk associated with component transfer to a high speed filling line. In this situation, operator intervention is required several times each hour to feed more components to the filling line. With each successive transfer there is a small but real risk of an increased microbiological challenge to the filling area, either from the operator or from the transfer process. Until recently, the only available sterile transfer system was based on old mechanical Alpha/Beta RTP technology and dry heat sterilization. UV light has been successfully developed, validated and used as a sterilizing source for a new rapid sterile transfer process. To make UV sterilization practical the design of the transfer port interface has been optimized from a complex alpha/beta flange system to a simple docking collar and door assembly. The simplicity of the one-piece molded collar design facilitates using UV sterilization. Logistical problems associated with the cleaning, repair tracking and replacement of the beta flanges and their gaskets has been replaced by the single use nature of the one-piece molded collar interface. The increased security to the transfer process facilitates the development of a more robust material handling processes. These improved processes permit the placement of isolators in less costly, less environmentally controlled areas thus reducing capital investment and running cost.

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# DISPOSABLE SYSTEMS AN INTERESTING ALTERNATIVE FOR ASEPTIC MANUFACTURING

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## Abstract

A completely disposable system for manufacturing of sterile products is being developed in co-operation between Pharmacia, Millipore and STEDIM. The integrated disposable system covers the manufacturing steps of formulation, sterile filtration and filling. The potential advantages of the system are increased product safety, less investments and costs and increased productivity. The disposable system does also create a flexible and simple production environment.

The concept is divided into four activities. The first activity is to implement the use of a pre-sterilized disposable mixing bag, STEDIM, for preparation of manufacturing formulation. The second activity is the use of a pre-sterilized disposable holding bag, STEDIM, with sterile filter, Millipore, for diluents or final product to be filled. The third activity is the use of a pre-sterilized disposable filling system, the Acerta®, Millipore. The Acerta®, filling system may be integrated with new or existing filling lines. The fourth activity is to implement a process and work flow, which combines all these three technologies into one aseptic system making it completely disposable from preparation of manufacturing formulation to filling of final product.

The system creates a complete disposable contact surface for the product that eliminates the need for conventional equipment cleaning and thereby minimizes preparation time between campaigns. The system also offers added product and operator safety by complete containment of the drug. Cost savings are achieved by shorter preparation and installation time, more flexible production and the elimination of cleaning. The use of disposable systems is also a flexible

alternative for R&D for faster development time with potentially simpler validation requirements.

The presentation will describe the functions of the system and present data from the on-going experiments and validation studies. The issues of extractable and adsorption of compounds into the disposable material will be discussed.

# VALIDATION OF A NOVEL ASEPTIC CONNECTION DEVICE

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## 1. Aseptic connection methods

Aseptic processing continues to play an important role in the manufacture of pharmaceutical products especially with the increasing role of biotechnology products. Aseptic connections are critical links made between two sterilized components and can be made at several stages of the manufacturing process. With as many as 25,000 aseptic connections made in a year by a single pharmaceutical company, the equipment used to make these connections is a vital component of the processing operations.

Quick connectors may be used to reduce the time and complexity of the procedure but must take place in a HEPA-filtered laminar air flow hood because the connector is open to the surrounding environment when the connection is being made. Highly trained operators are essential to make this connection. In addition, the laminar flow equipment needs to be maintained and appropriate records kept. Tubing welders use disposable wafers or blades to perform a thermal weld of two pieces of tubing and do not require a laminar flow hood. Operator skills are reduced but the equipment requires qualification and maintenance and may have limited use in cramped environments. This paper describes the validation of a novel aseptic connection device which eliminates the need for porous wraps and special facilities such as laminar flow hoods or tube welders and can be performed with less dependence on operator skill.

## 2. Aseptic connection device

The device essentially consists of a male and female part where each of the ports is protected with a strong hydrophobic strip (Figure 1). During a connection, the two ends are locked together, making a joint that is effectively permanent. The

two strips are then peeled away simultaneously, bringing the two sterile faces together and sealed by two grommets. The sterile fluid path is completed by pushing the integral tapered hollow plunger into the tapered female side of the device (Figure 2). The fluid contact path through the device is all polycarbonate and the device can be gamma irradiated or steam sterilized by autoclaving.

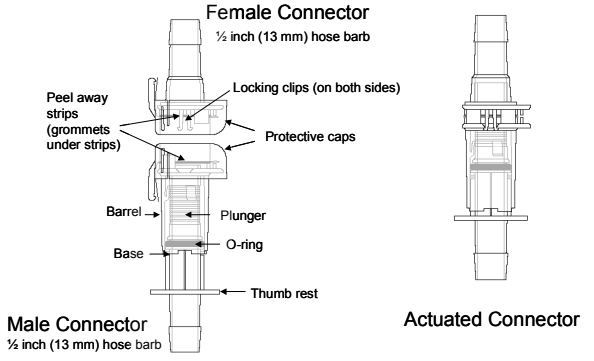


*Figure 1. Male and female parts of an aseptic connection device.*

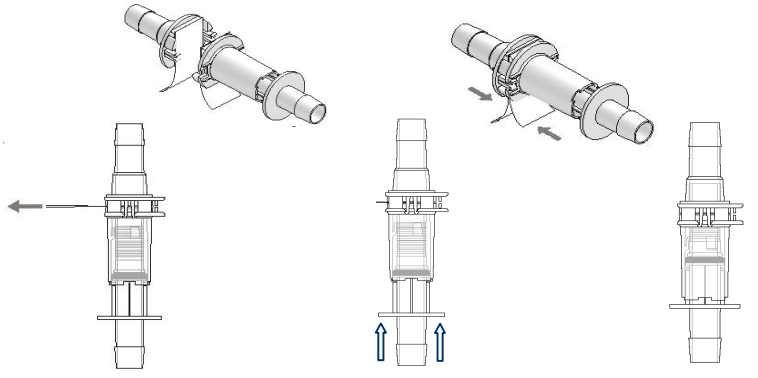

*Figure 2. Mounting of an aseptic connection.*

# 3. Validation – Functional soiling test

A validation test, which represented a worst-case scenario, was developed to demonstrate the ability of the device to produce a sterile connection after intentional contamination with a high level of bacterial spores.

The male and female connectors were soiled with a minimum challenge level of $10^6$ cfu of *Bacillus stearothermophilus* spores per device by submerging them for 30 seconds in the spore suspension containing 4% carboxymethyl celluose (CMC) solution to ensure adhesion. The soiled devices were then dried overnight at ambient temperature.

The number of spores adhering to the device was verified by placing samples of the soiled units in sterile phosphate buffered saline (PBS), sonicating the liquid and then performing a viable count on the liquid. The ability of the soiled devices to maintain a sterile path was determined as shown below (Figure 3).

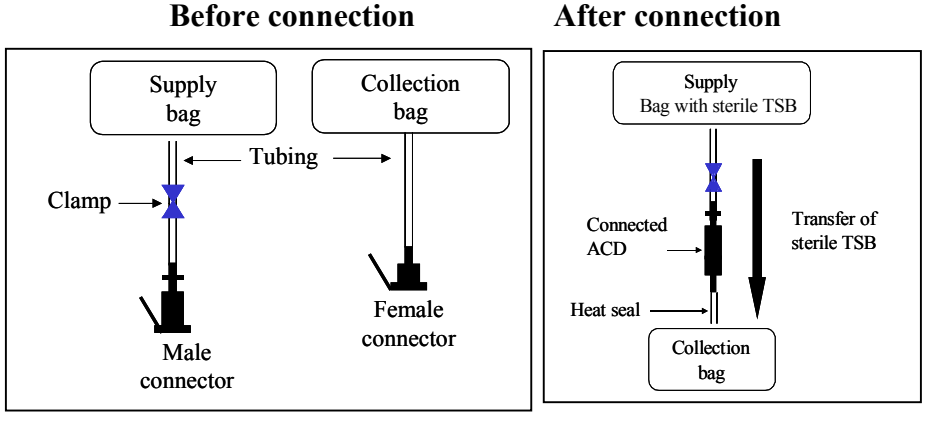


*Figure 3. The abitity to maintain a sterile path in a soiled device.*

The supply bag containing sterile trypticase soy broth (TSB) was connected to the collection bag through the soiled male and female connection. The bag containing the TSB was incubated for 7 d at 55 ºC and then checked for sterility. Positive and negative controls were also included as part of the soiling tests.

*Table 1. Sterility testing of broth transferred through a soiled connection mounted of male and female parts.*

| Challenge | Number of units | Viable count of transferred TSB* | Number of spores per device | Sterility Maintained? |
|---|---|---|---|---|
| 1 | 8 | 0 CFU/mL | $>10^6$ | Yes |
| 2 | 14 | 0 CFU/mL | $>10^6$ | Yes |
| 3 | 13 | 0 CFU/mL | $>10^5$ | Yes |

# 4. Conclusion

Results summarized above show that sterility was maintained in all tests performed and confirm that the device can produce a sterile connection even after deliberate exposure to high doses of *B. stearothermophilus* spores.

# 5. Other validation studies

An extensive and comprehensive range of additional validation tests were performed to confirm other critical functional requirements including:

- **USP Biological Reactivity Tests Class VI 121$^0$C**
- **USP Physicochemical Tests for Plastics**
  Non-volatile residue (NVR)
  Residue on ignition
  Buffering capacity
  Heavy metals
- **Extractables**
  Quantitative NVR
  Qualitative FTIR
  Total oxidisable carbon
  UV absorbance

- **Vacuum leak test for closure integrity**
  Microbial penetration test
  Dye penetration test
- **Burst pressure**
  Pressurisation tests up to at least 19.3bar
- **Creep rupture test**
  Burst testing after continuous pressure up to 10.3bar and 168hours
- **Tensile strength test on connection**
  Pulling force tests of typically 276N
- **Steam autoclave tests**
  Maximum temperature and time studies
- **Biological indicator tests**
  Steam sterilisation studies with *B. stearothermophilus*
- **Shelf life after gamma irradiation**
  Burst pressure, soiling tests and extractables.

More detailed information on all the validation studies is available in a published validation guide from Pall Life Sciences.

## 6. Summary

The validation studies show that the aseptic connection device can provide a functional, secure and sterile connection under worst case conditions and offers opportunity for safer management of aseptic processing.

# SESSION ON CONTAMINATION CONTROL IN MICROELECTRONICS AND $R^3$ TECHNOLOGY

# PRODUCTION ENVIRONMENT FOR NEW INTERCONNECTION TECHNOLOGIES

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## Abstract

Lighter, smaller, faster and cheaper are the magic words used to describe the trends in modern electronics. The miniaturization is a must in the market of the portable devices. Beside of the mobile phones there are several other application areas like smart cards, smart cloths, portable minicomputers, cameras, entertainment electronics and so on. The big challenge is to find the most effective technology to produce all those devices with competitive prices. The key factor is the interconnection technology. When the size and weight is decreasing, the density of interconnections must increase. This is possible either by making the packages of integrated circuits smaller or by using bare naked dies without any package (called flip chip technology) to decrease the assembly volume. The reflow soldering process has limitations with very dense interconnections especially when the pitch is under 200 µm. Then one possible solution is to use anisotropically conductive adhesives, which are a mixture of epoxy base adhesives and very small conductive particles. Since the adhesive interconnection is based on mechanical interconnection between the conductive surfaces, it is much more sensitive for contamination and impurities than soldering process. The cleanliness level of the whole production environment must be improved. Since the clean room area is very expensive and only one part of the manufacturing process is critical compared to normal surface mount assembly (SMA) process, there should be an alternative to move a part of the production in clean room environment. It can be a local arrangement restricted only to few process steps and equipment. Also the cleanliness level should be tailored for the manufacturing process.

# 1. Introduction

Future trends in miniaturization of electronic devices need a new approach in terms of volume and weight reduction in portable products. If we look closer at the miniaturization trends and life cycles of integrated circuits in the future (Figure 1), we can clearly see the tendency to smaller components forcing companies to innovate and improve their tools and processes or fade into obscurity.

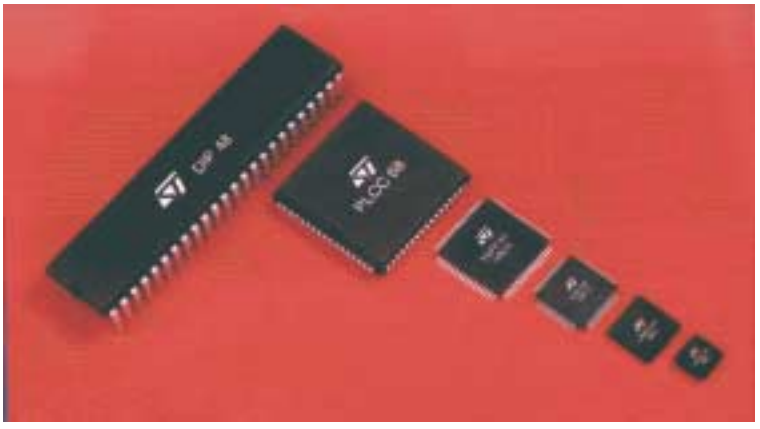

*Figure 1. Future trends of integrated circuit packages.*

The major trend in the ICs used in the traditional soldering process is going from the larger DIL (Dual In Line) packages to QFP (quad flat pack) and further to BGA (ball grid array) and to the smallest CSP (chip scale package).

In QFP, COB (chip on board) and in many BGAs the chip is first connected by wire bonding. When the connection density has increased the direct flip chip connection has become more interesting. The savings in assembly volume are especially large as can be seen in Figure 2. If the protective plastic package was left away the volume could be decreased tenfold [1].

The flip chip technology clearly has great potential to even smaller volumes when the silicon chip is thinned down to 10–50 μm (Figure 2). If the chip is further connected to the thin flexible substrate (thickness of 25–50 μm), the total volume can be decreased.

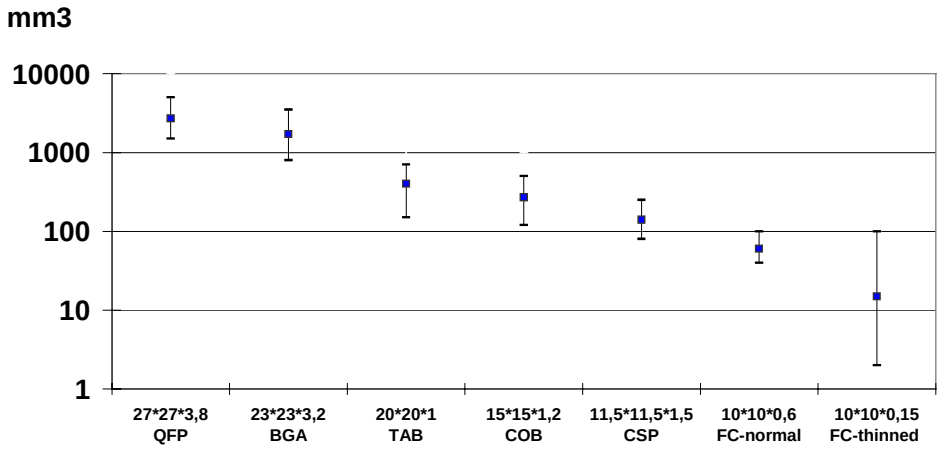


*Figure 2. Assembly volume needed in different technologies.*

One problem when using the soldering process is the dramatic decrease of interconnection volume when the pitch becomes smaller. Because the reliability is strongly dependent on the interconnection height and solder volume, the smaller pitch is causing more and more problems with the smaller height and solder volume (For example: pitch 1 mm→0.1 mm; solder volume 0.065 $mm^3$→ 0.0005 $mm^3$). Therefore in small pitch applications (<0.1 mm) the use of so called anisotropically conductive adhesive (ACA) has became very popular. In Figure 3 shows the main principle of ACA interconnection.

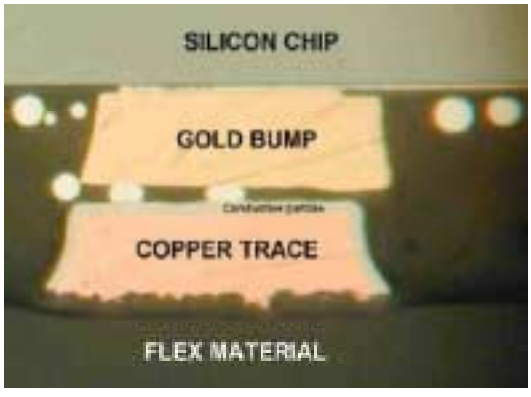


*Figure 3. Cross section of ACA-contact.*

Anisotropically conductive adhesives are composite materials containing typically epoxy-based matrix and small amount of conductive particles. The adhesive itself doesn't conduct before processing unlike isotropically conductive adhesives, because the volume fraction of the conductive particles is relatively low and the particles are not in contact with each other. The adhesive begins to conduct in z-direction after the IC is pressed against the pads on the substrate. After the bonding process part of the particles are stuck and squeezed between the pad and the bump. These squeezed particles form an electrical conductive bridge between the IC and the substrate [1]. Unlike in soldering flip chip process the electrical contact between the pad and bump is purely mechanical. The newest adhesives can be used in very small pitch applications down to 40 μ.

Conductive particles are typically metal flakes or spheres made of silver of nickel (hard particle) or metal coated plastic balls (soft particle). Soft and elastic metal-coated (nickel/gold) polymer particle provides reliable electrical contact between the bump and the pad since it deforms under pressure and provides large contact area [2]. Hard metal particle doesn't normally deform so much but it can penetrate through the oxide layer on the conductor and form a good contact. Typically the particle diameter is 3–10 μm, and the particle density varies from 2000–3000/mm$^2$ up to 20 000/mm$^2$.

## 2. Nature of ACA contact

It is important to understand the difference of the electrical contact made by ACA process compared to soldering process. In soldering process the contact is a metallurgical contact, which means solid metal structure between the contacts. It is normally very rigid. When using ACA process the contact is a mechanical and the contact surfaces are pressed together, since the adhesive is shrinking when it is cured Figure 4.

The contact resistance is a sum of particle resistance, constriction resistance ($R_c$) and film resistance ($R_f$). Thus the total contact resistance for an adhesive with "n" particles in contact between materials 1 and 2 can then be described by the formula:

$$R= \frac{1}{n}\times [R(particle)+R_c(interface1)+R_c(interface2)+R_f(interface1)+R_f(interface2)] \quad (1)$$

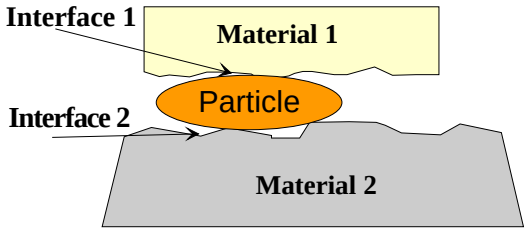


*Figure 4. Mechanical contact formed by small particle between two surfaces.*

The number of particle will directly affect the value of contact resistance. The constriction resistance is caused by the limited area of the contact surface. The film resistance can be very large in the case of contamination or oxide on the contact surfaces [2].

## 3. Requirements for environment

The environment of the modern factory for electronic production is normally described with requirements as temperature 22 ± 2 °C and relatively humidity (R.H.) 40 ± 5%. However the filtration of the fresh air has been done with EU7-type filter. This is classified as an intermediate filter with efficiency of 80–85 %. The situation is common in normal SMT-production, where the critical aspects are focused to the temperature and relatively moisture. This is especially true in printing process of solder paste. But in future when the pitch is coming smaller the particles and contamination of the surfaces is coming a more critical issue. The deposition of particles from indoor air on circuit boards in electronic equipment reduces the electrical isolation between conductors sometimes causing electrical short circuits. To keep spaces clean, air-handling systems have to remove large (2–20 μm) particles, including fibers, which are generated by mechanical wear on materials or derived from biological sources. Smaller particles (<2 μm), generated by combustion or photochemical gas-to-particle conversions, often pass through filters and are carried into production environment, may not be removed efficiently by the standard air filters. Small particles produce a greater threat to electronic production, because they can be deposited on both vertical and horizontal surfaces. Moreover, such particles tend to be hygroscopic – i.e. able to attract or absorb moisture from the air. When the environment reaches a critical relative humidity (50–65%), the deposited particles become like conductive liquid because of the absorbed moisture [3].

# 4. Practical solutions for sensitive production

For soldering process the larger particles are directly harmful since they cause short circuits. Also the small particles are harmful since they are difficult to observe with naked eye. In normal SMT production the particles smaller than 10 µm doesn´t exist since they are invisible for eye. The clean room culture is related to the careful measurement of the particle levels in different processes and environments. The key factor here is that the particle counters used in clean rooms are not suitable for the environment of high particle counts. When the distribution of the particle size is unknown, it is very risky environment for new technology. Therefore the solution is normally to remove the sensitive production line to the clean room – especially if ACA process is used. The second issue is the contaminants, which can cover the sensitive surfaces with isolated layer. This is typically impossible to happen in clean room environment since the strict regulations concerning the materials and chemicals, which are acceptable to bring into clean room. In normal SMT environment there are plenty of lubricants and oils available. Especially the silicon oil can easily spread out and damage the manufacturing facilities forever.

In Figure 5 is shown an example of ACA production line in clean room environment. The particle size of conductive particles in adhesive is 5 µm and after connection it will deform to 2–3 µm. So the harmful particles are larger than 2 µm and the class of clean room is 10 000. It has been built up around the production line with easy removable structural elements.


*Figure 5. ACA production line protected with class 10 000 clean room.*

An important question is related to the mix-technology, where one part of the production is done in normal SMT line and the rest of the process use ACA process (Figure 6) [4].

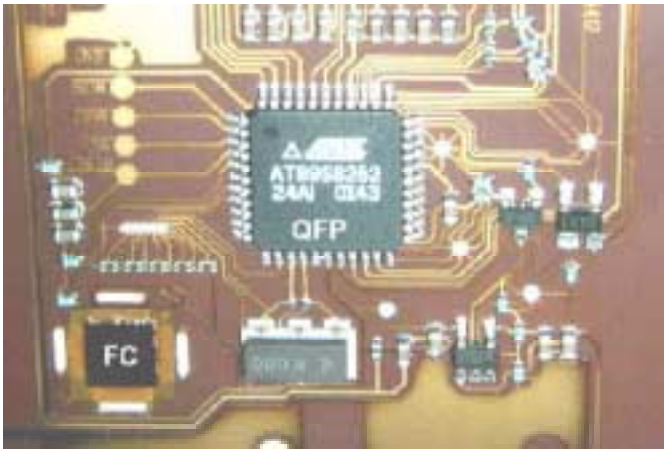


*Figure 6. Mix-technology process consisting SMT and FC (Flip Chip) components.*

One solution is to connect the bare silicon chips first in clean room and then make the rest of the process in normal SMT environment. This is not always possible since the solder process includes also a printing step of solder paste and that is difficult to do with the pre-assembled components. So in many cases the process flow is opposite and the cleanliness after the SMT process is an important issue. Especially if the clean surface area of the interconnection is large as in many display applications.

## 5. Conclusions

Future trends in miniaturization of electronic devices need a new approach in terms of volume and weight reduction in portable products. Therefore the interconnection density is increasing and the cleanliness level of the whole production environment must be improved. Since clean room area is very expensive and only a part of the manufacturing process is critical compared to normal surface mount assembly (SMA) process, there should be an alternative to move a part of the production in clean room environment. It can be a local arrangement restricted only to few process steps and equipment. Also the

cleanliness level should be tailored for the manufacturing process. The lack of particle counters suitable for high particle levels is a drawback in many applications.

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# CLEANLINESS DEMANDS FOR PRODUCTION ENVIRONMENTS OF FUTURE MINIATURE ELECTRONICS

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## Abstract

The main trend especially in telecommunication electronics is miniaturisation; the products will be smaller, lighter and still of high performance and low price. In addition to electronics components, such products include, among others, photonics, sensors, MEMS and are built on integrated, 3-dimensional multilayer substrates. In miniature devices the packaging aspects are emphasised: one has to know new materials, components and interconnection methods. In addition, it is important to understand surface phenomena, the ways of producing dense integrated substrates and to master design and reliability aspects. The control of contamination in all stages of manufacturing processes becomes more and more important.

The production of advanced miniature electronics is moving into clean rooms, because bare semiconductor chips are used, the packaging density is increased with smaller dimensions (< 50 μm) in conductor line widths and spaces, via diameters etc. In this presentation a short review is given on the trends in electronics production technologies together with the related cleanliness demands for production processes.

# 1. Introduction

Recent advances in leading technologies of the 21st century including information technology, biotechnology, and nanotechnology are based strongly on the miniaturisation of characteristic feature sizes to the level of micro/nanometer order. The development of microfabrication processes allows production of products that will be smaller, lighter and still of high performance

and low price. In addition to electronics components such products include, among others, photonics, sensors, MEMS (Micro Electro Mechanical Systems) and are built on integrated, 3-dimensional multilayer substrates. This system-on-package or micromodule approach is regarded as one of the most promising technologies for many of these multi-functional products.

In miniature devices the packaging aspects are emphasised: one has to know new materials, components and interconnection methods. In addition, it is important to understand surface phenomena, the ways of producing dense integrated substrates and to master design and reliability aspects. All this puts more demands for manufacturing facilities, and production of highly integrated miniature products is moving to clean rooms.

## 2. Component and substrate technologies

The main driving force for miniaturisation comes from the huge advances in semiconductor components, especially in integrated circuits (ICs). The development during the last 30 years is best seen in the famous Moore's law. It states that the industry would be able to double the number of transistors per chip every 18 months (Figure 1).

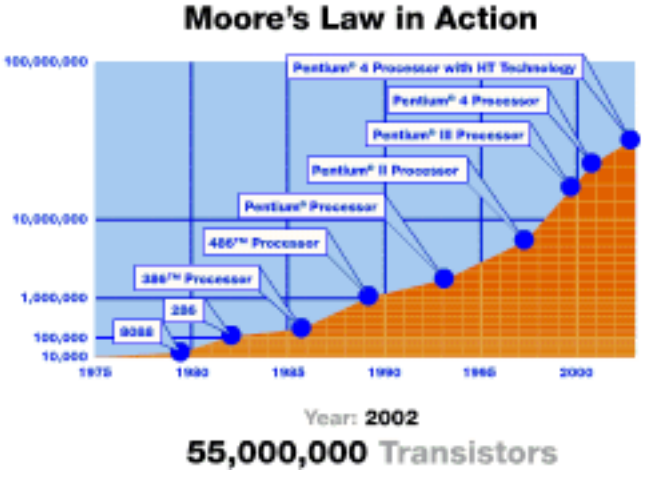


*Figure 1. Moore's law is showing the number of transistors per chip from the year 1975 [1].*

The increase in the number of transistors in ICs has consequently lead to increase in the number of I/Os from the chip (Figure 2). This has changed the format of IC packages from the early peripheral pin type packages to area array packages with the outputs in matrix format. These vastly increasing component types include Ball Grid Arrays (BGA), Chip Scale (Size) Packages (CSP) and unpacked chips (the so-called flip-chip components). The interconnection of all these components to substrate is made by bumps (usually by solder balls). The number of the bumps may be hundreds, even more than one thousand, in a fine pitch format [1, 4].

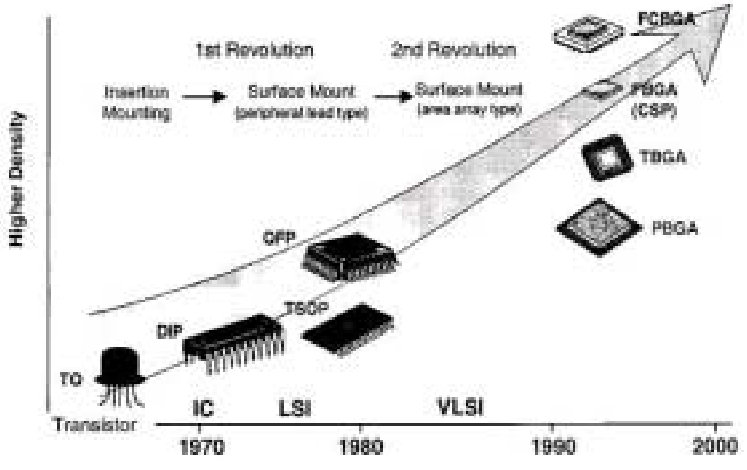


*Figure 2. IC package growth trends [2].*

The development described above has direct consequences in the substrate technologies. The dense, fine line and small interconnection pad structures cannot be produced by conventional printed circuit technology. By the so-called build-up-technology (BUT) new dielectric and conductor layers are manufactured using technologies coming from the fine-line thin film multi-chip module technology. It combines some aspects of the PCB technology e.g. electroless and electroplating and the multichip module technology e.g. via and dielectric formation) to form new high-density micro-via technology. For dimensional trends in advanced PCBs (Table 1).

*Table 1. Summary of the general roadmap, which shows the wirebonding pitch coming down to 50 micron and flip-chip to 50–100 microns. The next generation BGA and PCB board approaching 10–20 micron technology [1].*

**Global EPA Roadmap**

| **Wirebonding** | **1997** | **1999** | **2001** | **2003** | **2005** | **2007** | **2012** |
|---|---|---|---|---|---|---|---|
| Pitch (ball) (μm) | 70 | 50 | 50 | 50 | 50 | 50 | 50 |
| Pitch (wedge) (μm) | 60 | 45 | 45 | 45 | 45 | 45 | 45 |
| **Flip chip substrate** | **1997** | **1999** | **2001** | **2003** | **2005** | **2007** | **2012** |
| Flip chip pad pitch (μm) | 250 | 180 | 150 | 130 | 100 | 70 | 50 |
| Pad size (μm) | 100 | 70 | 60 | 52 | 40 | 25 | 18 |
| Array (max. pads/chip edge) | | | | | | | |
| Handheld | 28 | 38 | 46 | 53 | 701 | 100 | 140 |
| Cost performance | 48 | 66 | 80 | 92 | 120 | 171 | 240 |
| High Performance | 68 | 94 | 113 | 130 | 170 | 242 | 340 |
| Automotive | 28 | 38 | 46 | 53 | 70 | 100 | 140 |
| Wireable array depth | | | | | | | |
| Handheld | 3 | 3 | 2 | 2 | 2 | 2 | 2 |
| Cost performance | 4 | 3 | 3 | 4 | 4 | 3 | 3 |
| High Performance | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Automotive | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| PCB base substrate (1 line/pads) | | | | | | | |
| Line width (μm) | 50 | 34 | 30 | 26 | 20 | 15 | 10 |
| Line space (μm) | 50 | 38 | 30 | 26 | 20 | 15 | 11 |
| Thin film base substrate (2 lines/pads) | | | | | | | |
| Line width (μm) | 30 | 22 | 18 | 15 | 12 | 9 | 6.1 |
| Line space (μm) | 30 | 22 | 19 | 16 | 12 | 9 | 6.6 |
| Thin film base substrate (4 lines/pads) | | | | | | | |
| Line width (μm) | 16.2 | 11.7 | 9.5 | 8.2 | 6.2 | 5 | 3 |
| Line space (μm) | 17 | 12.6 | 10.4 | 9 | 7 | 5 | 4 |

Ceramic technology, especially LTCC (Low Temperature Cofired Ceramics) is growing very fast in applications, where high reliability and performance are required. Due to good high frequency properties and high packaging density based on multilayer structures and possibility to integrate passive components inside the substrate, LTCC technology is gaining ground also in telecommunication products.

Currently, screen-printing is used for conductor patterning, but technology that combines screen-printing and photo imaging is entering the market and allowing formation of finer structures in LTCC multilayers. Also new fine-line printing technologies have been developed at Microelectronics laboratory in several international and national projects. Direct printing methods have cost advantages over photo processed techniques in mass production. This leads to smaller size circuits or reduced layer count, both of which in turn lead to reduced costs. High conductivity silver, gold, and mixed silver and gold conductors are currently used in the production.

Besides higher packaging density, the accuracy of conductor lines in these methods enables improved high frequency properties and smaller tolerances for integrated passive components. The biggest interest lies in production of narrow line conductors, capable of at least 40 μm lines and spaces in a multilayer structure. The manufacturing and cost efficiency needs to be developed and the quality of circuits demonstrated before this new technology can be used in large-scale production.

## 3. Micro- and nanotechnology

The advances in microtechnology extend naturally to the development of microsystems that can be achieved through integration of the core techniques of both hardware and software. Microelectromechanical Systems (MEMS) can be realised in the form of sensors, actuators or mechanical structures. MEMS find widespread applications in military/aerospace, industrial and process control, energy management and many other application areas. One expanding area is in telecommunication applications. The advantages offered by microsystems in terms of reduced size, power consumption and compatibility for integration are currently being investigated for the development of miniaturised RF components such as filters, micro-switches and antennas.

The semiconductor industry is already operating in the deep nanometer scale and 1.2 nm gate oxides are in production today. Operation of transistors down to 15–20 nm has been demonstrated (2–3 generations ahead). The challenge is to successfully integrate new materials and technologies.

## 4. IC assembly

In the IC assembly migration from wire bond to flip chip will continue as the technology matures. In Appendix 1 is a summary of trends in wirebonding and flip-chip bonding with fine line and space microvia technology that will need to be developed to support the high pin count, fine–pitch, flip chip devices. The migration from aluminium and silicon dioxide to Cu/low k on chip interconnects will present a significant challenge to the packaging community for wire bonded and flip chip die. Materials compatibility issues will need to be addressed [1].

With increased demands for higher and higher packaging density especially for portable telecommunication products, the lateral packaging is not always enough. This has brought various 3-dimensional procedures including the use of the mechanical parts of the device as wiring substrates. One growing technology is chip and module stacking. Some examples are shown in Figure 3 illustrating the interconnection methods for bare chips and CSP components.

## 5. Reliability concerns

The increased packaging density, use of bare chips, new micro-systems, photonic and sensor components and finally the integration of nano-structures to assemblies put great demands on manufacturing facilities. It does not concern only particle contamination, but also other environmental contamination and factors including temperature, humidity and vibration. The practices in all phases of manufacturing processes have direct effects on the productivity and reliability of the final product.

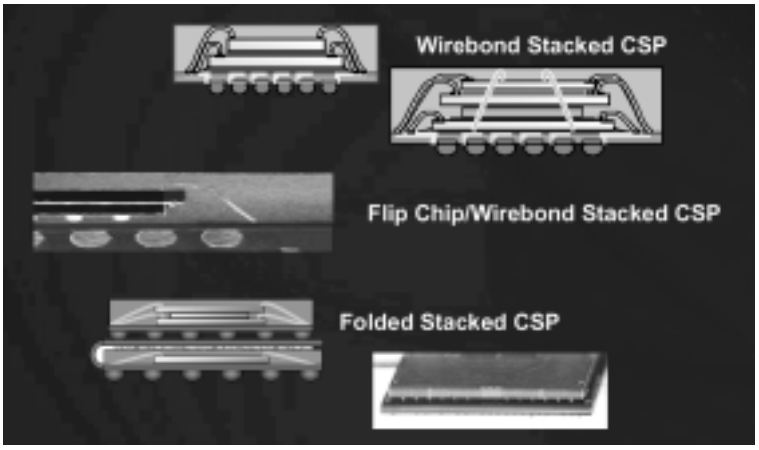


*Figure 3. Cross-sectional views of stacked chip and CSP structures [4].*

In the following are listed some of the main factors that must be in good control in various steps of manufacturing processes:

*Organic and ceramic fine line substrates*
- Particles
- Contamination
- Humidity
- Temperature

*IC assembly*
- Particles
- Contamination
- vibration

*Integrated substrates with new micro/nanotechnology components*
*Sensors*
contamination
environmental interactions
*MEMS*
- contamination
- particles
- cleaning
- outgassing

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# NEW MICROELECTRONICS CLEANROOM FACILITY IN OTANIEMI – USER'S VIEW ON THE BUILDING PROCESS

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## Abstract

Design and construction of a cleanroom is always a very demanding and expensive project. In order to ensure that the cleanroom fulfils the specified requirements it is necessary that the end user participate in all stages of the project. This is especially important when the cleanroom is built for research purposes, in which case the cleanroom should be both flexible and easy to convert in case new demands arise.

The end user's contribution is necessary in the design stage, when requirements are set for the cleanroom classes, temperature and humidity variation and vibrations as well as the basic layout. For the dimensioning of the ventilation, cooling, DI-water, gas and other systems the end user must provide the information of process tool requirements to the cleanroom contractor. During the construction stage the end user's comments should be taken into account and of course the end user plays an active role during commissioning and user training.

## 1. Micronova cleanroom – general description

The Micronova cleanroom (Figure 1) is built for the purpose of research, development and small-scale production of micro-, opto- and nanoelectronic components. It is used jointly by the Technical Research Centre of Finland (VTT) and Helsinki University of Technology (HUT). The total area of this cleanroom, which is actually an extension of an older cleanroom in use by VTT, is 1500 $m^2$. The cleanroom is of the clean bay – service chase type and the ventilation is based on filter fan units. The classification ranges from ISO 4 in the lithography areas to ISO 7 in the assembly and training lab areas.

The cleanroom and its technical spaces occupy four floors of the building. The cleanroom is on the second floor, below it is a technical area including also some laboratories, right above it the plenum and on the fourth floor the plant room for all air handling units and exhaust fans. The construction of the cleanroom and the adjoining office and laboratory building began in April 2001 and was completed in October 2002. The cleanroom was carried out as a design-build project with ABB (ABB Airtech, Sweden and ABB Installations, Finland) as the cleanroom contractor with the HVAC design performed by JP-Talotekniikka. The whole project was managed by CM-Urakointi.

During the whole process of designing, building and commissioning the end users were actively involved both as a source of information to the cleanroom contractor and as a party in the approval of different solutions. During the building stage the end users' representatives also had a role in the supervision of the installations. All requirements and opinions presented by the users were considered by ABB.

# 2. Cleanroom design

## 2.1 Basic requirements

The basic requirements defined in the tender invitation were:

- Cleanroom classification: ISO 4–ISO 7 (clean bays)
- Temperature 21 °C ± 0.5 °C
- Relative humidity 45 % ± 5%
- Vibration < 100 μm/s, 0–100 Hz
- Pressure difference
  Clean bay to ambient 50 Pa
- Raised access floor in the whole area.

## 2.2 Layout

General considerations in designing a cleanroom layout are:

- Functional needs, knowledge of processes and process equipment
- Personnel and material transport routes
- Cleaning of materials and equipment

- Sufficient air locks and gowning areas
- Need for other auxiliary space; laundry, house cleaning, chemicals storage rooms etc.

In addition to these in this special case the following requirements also had to be fulfilled:

- The area should be divided equally between VTT and HUT, without access to the other party's clean bay area
- Both parties should be able to use the transport elevator serving the old cleanroom
- There should be an easy access from VTT's new area to the old cleanroom
- The entrance and air lock should be in common use in order to save space.

After an iterative process in close Cupertino between ABB and the end users the above layout was accepted. Because the clean bays are not in common use by VTT and HUT some areas, e.g. lithography and wet processing are duplicated. There is a broad service and transport corridor surrounding the clean areas. This corridor is not isolated from the inner part of the cleanroom but directly connected to the service chases and can be used also for storage and secondary equipment. This solution arises from the need to save space and sets some quite stringent requirements of the quality of the wall structure. Structurally the surrounding corridor is, however, isolated from the inner part by a vibration joint.

## 2.3 Infrastructure and media

During the initial stage of the planning process the following media were defined to be accessible in the cleanroom as well as the laboratories on the first floor:

*Exhaust from process equipment*

- exhaust for acids and basic chemicals (equipped with water scrubber)
- solvents exhaust
- exhaust for toxic, corrosive and explosive gases
- exhaust for vacuum pumps.

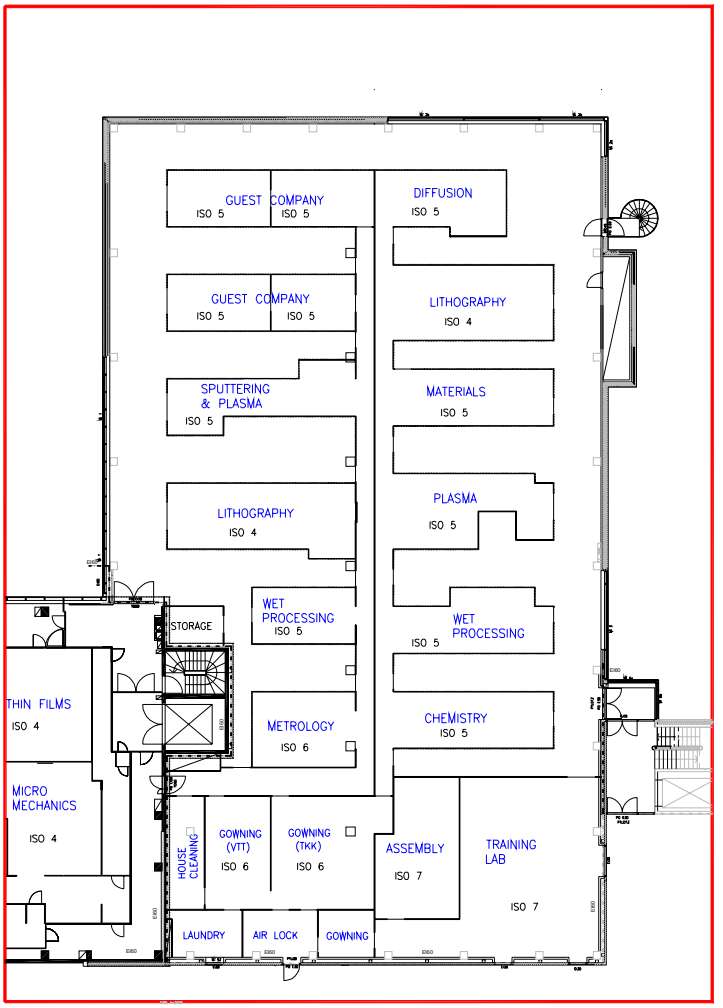


*Figure 1. Layout of the new part of the Micronova cleanroom.*

*DI-water*

*Drains*

- neutralisation drain (to own neutralisation plant)
- solvent drain (to tank)
- drain for hydrofluoric acid (to tank)

*Cooling water*

- process equipment cooling (closed loop)
- vacuum pump cooling (closed loop)

*Bulk gases*
- compressed air (dew point – 70 °C)
- utility nitrogen (from tank, 5.0)
- process nitrogen (purified, 7.0)
- oxygen (5.0)
- hydrogen (5.0)
- argon (5.0)

These gases are available in the ceiling of each service finger.

*Process gases*
Approx. 20 different gases fed from the gas cylinder to the point-of-use

*House vacuum*

*Bus bar electrical power supply system*

*Data network*

*Central vacuum cleaning system*

*Sampling fire alarm system*

*Cold and warm city water, ordinary drain* (laundry, house cleaning, air lock)

The DI-water plant and distribution system was not included in the main cleanroom contract, but was delivered by Hyxo. The gas system was delivered by Micro Gas Systems.

## 2.4 Tool list

In order to configure the capacity of these systems as well as the dimensions of pipes and ducts a tool list (Excel sheet) was set up and maintained by ABB. The

end users provided as accurate data as possible on the requirements for media supplies, e.g. electrical power, cooling water pressure and flow, compressed air, DI-water, gases etc. for all process equipment. This information was used by the HVAC-designer to calculate heat loads, exhaust and supply air needs etc, both for each service chase and the whole installation. This tool list also served as the basis for the detailed drawings of pipework and ducts for all media.

The tool list contains the data for approx. 150 different process tools and rew to a considerable size. For this reason it was not an easy task for the end users to fill in all the data and to update it or for ABB to maintain it and introduce all changes. In our opinion a data base program would have been easier to handle.

### 2.5 Cleanroom structures

The ceiling grid and the wall profiles were delivered by ABB. The wall panels are mainly aluminium honeycomb as are the blank ceiling panels. Where transparent walls are needed polycarbonate panels have been used. Filter fan units were evaluated as a separate item, and were ordered from M+W Zander.

## 3. Cleanroom building process

### 3.1 Foundation

During the construction of the foundation of the cleanroom building the supervision was mainly focussed on vibration isolation and damping. Also, as the building was an extension much attention had to paid to the effect of the construction work on the existing building and the sensitivity of the processing work performed there.

### 3.2 Framework

The cleanroom has outer walls on three sides. The overpressure and high relative humidity requires a very tight wall structure with low leakage. The tightness requirement was expressed as a leakage maximum of 2 $m^3/s$ at an overpressure of 50 Pa. This represents 10 % of the maximum make-up airflow, which is a

considerable loss. The wall is humidity insulated and all leaks have been tightened. Measurements during the construction period and preliminary measurements after the installation were taken into use show that the requirement is fulfilled.

The concrete slab, which constitutes the foor of the cleanroom, was cast in a cup-moulded shape. As many as 1400 holes were drilled in the slab to facilitate the feed through of all media as well as pumping lines etc. for the process equipment.

## 3.3 Ventilation

A necessary condition for a functioning cleanroom is a well designed ventilation system in the installation of which all cleanliness and other requirements have been taken into consideration. The ventilation provides the necessary flow of filtered air with specified and stable temperature and humidity, and also removes the heat load from process equipment and particles generated inside the cleanroom.

The main components of the ventilation system for the Micronova cleanroom are two make-up air handling units, six recirculation air handling units, three main exhaust fans and of course the filter fan units (approx. 210 pcs). In the make-up air handling units the incoming air is filtered, heated or cooled and excess humidity is removed if necessary. The recirculation units are equipped with cooling, heating and spray humidification. In a climate with a long humidification season a considerable amount of electricity can be saved by the use of spray humidification instead of steam humidification. In the steam mode electrical power is used to some extent for the heating in stead of the much cheaper district heating. Additionally the introduction of steam actually cools the air stream, which again reduces the need of cooling produced by electricity.

The last filter in the recirculation air units is class EU11 (HEPA class), which means that the air supplied to the plenum has a low particle concentration. The actual HEPA-filters attached to the filter fan units are class EU15. Because the ventilation is of significant importance to the cleanroom the end user took part in the choice of FFU’s, factory inspection of air handling units and choice of filters.

### 3.4 Media and infrastructure

The materials, dimensions and routing of all ducts and pipework of the various systems were calculated and designed based on the information given by the end users in the tool list. The draft drawings made on this basis were sent to the end users for comments. Several joint design meeting were held during this process. The updated drawings were delivered to the users for final acceptance.

Additionally the users participated in the evaluation and choosing of the supplier of some specific systems, e.g. neutralisation plant and chemical exhaust scrubber. During the installation phase the end users participated in the supervision of the installation work.

## 4. Vibration measurements

ABB Airtech performed vibration measurements of the cleanroom concrete slab at four different occasions. The first two were during the construction of the framework, the third after the completion of the cleanroom (acceptance tests) and the fourth when the cleanroom had been in full operation for some time. At the third occasion the vibration of the raised access floor was also measured.

Vibrations were measured in 11 points of the cleanroom, three of which were located outside the vibration joint and connected to the framework of the building. The other 8 points were on the vibration isolated concrete slab. The two first measurements were done to establish a baseline for the vibration level of the slab and the corridor area. The results for two points are shown in Figure 2.

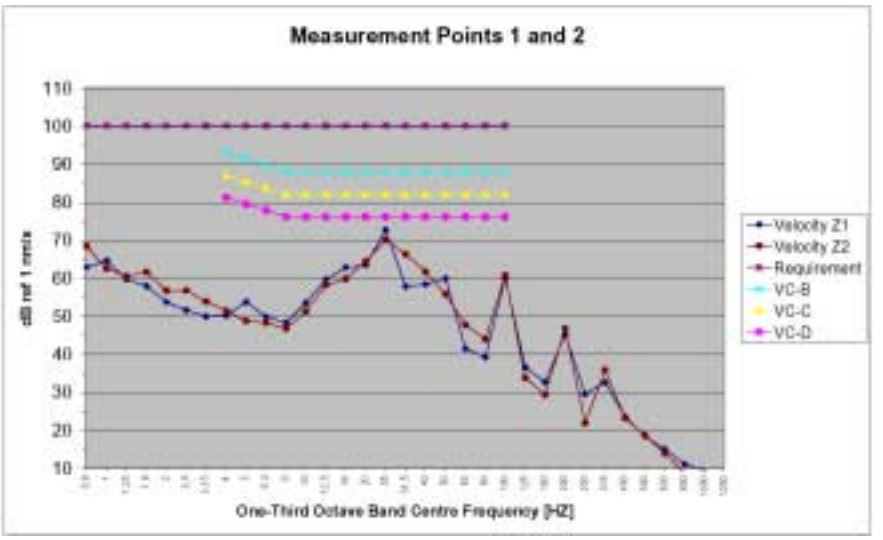


*Figure 2. Velocity levels at measurement point 1 (outside vibration joint) and point 2 (lithography area on concrete slab) [1].*

Both curves are well below the VC-D curve and the required level (100 μm/s). To get some information on the vibration isolating properties of the structure a small air compressor was started in the basement area below the cleanroom. As a result the peak in the velocity level at point 1 at frequency 25 Hz was increased to the VC-B curve level while the level at point 2 was only slightly increased. Vibration measurements during the acceptance tests were made at two different conditions; all air handling units and compressors running and most of the machines turned off. As shown in Figure 3. The Z-velocity is increased to the level of the VC-C curve at 25 Hz when all machines are running, but still well below the given specification.

With machines off the level was well below the VC-D curve. When measured on the raised floor the velocity level was considerably higher but still below the VC-B curve. It should be noted that no specifications have been given for the vibration level of the raised floor.

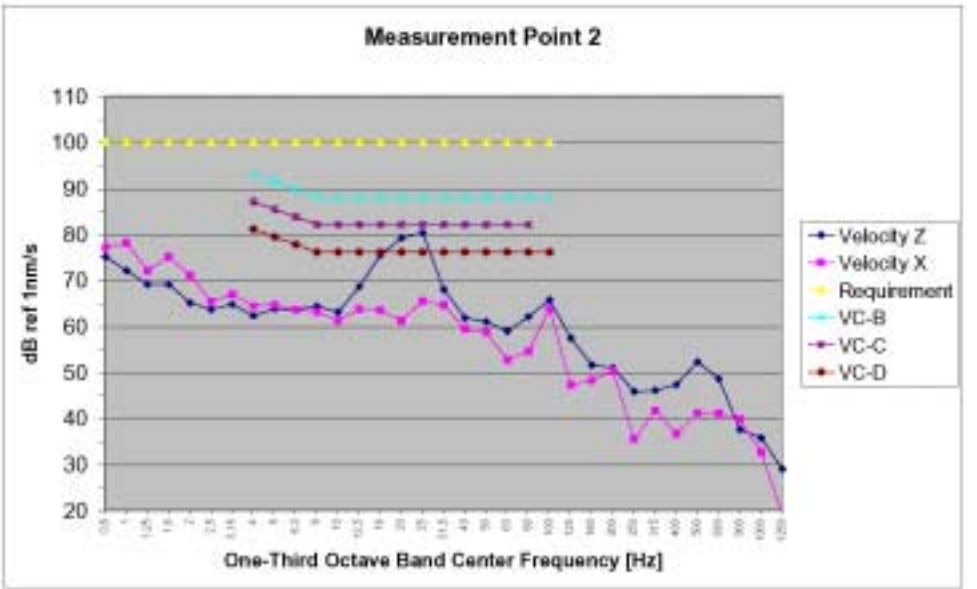


*Figure 3. Velocity levels at measurement point 2 with AHUs and compressors running [2].*

## 5. Commissioning

The commissioning (Figures 4 and 5) was done according to the ISO 14644-4 standard [3]. All systems have been tested to specifications and running tests have been performed. Personnel from VTT's cleanroom services group have participated in a training program concerning the running of the installation as well as maintaining it. Acceptance of the cleanroom installation was done after the following measurements had been performed:

- Cleanroom classification (particle concentration) in all classified areas
- Pressure differentials
- Temperature and humidity
- Air flow velocity (HEPA filters)
- Filter leakage
- Sound level
- Lighting intensity in cleanroom and plenum
- Conductivity of surface materials.

*Figure 4. View of surrounding corridor showing diffusion furmaces.*

*Figure 5. View of clean bay for plasma and sputtering.*

According to the standard the documentation should include:

- Functional description and drawings of the whole installation
- Instructions of use for each system including instructions for start-up and run-down in normal as well as failure conditions
- Maintenance instructions.

The documentation was far from complete at the time of the delivery, which has been an obstacle in the start-up, and running of the installation.

## 6. Summary

An active participation by the end users is necessary in order to ensure that the cleanroom is functionally and operationally well suited to the needs of the users. This however, requires that the participating persons are well aware of the general principles of cleanroom and solutions to specific problems. Experience of using, running and maintaining a cleanroom is of great advantage. The most important stages of participation is setting up the specifications and basic requirements of the cleanroom, providing detailed information on the process equipment and the commissioning process.

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# RESEARCH AND DEVELOPMENT ENVIRONMENT FOR MICROMODULE TECHNOLOGY AT VTT ELECTRONICS

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## Abstract

As a part of the ongoing development activities at VTT Electronics new clean rooms were built during 2002, which doubled the total clean room area to about 1000 $m^2$. The new clean rooms are a part of an environment designated for the development of micromodule and optoelectronics component technologies and product applications which forms the largest optoelectronics research facility in Nordic Countries. The environment supports especially development of new manufacturing technologies and businesses as well as spin-off companies by offering to industry, in addition to R&D services, access to clean rooms and special equipment, help in personnel training as well as prototype and small-scale manufacturing services.

## 1. Background

The main R&D activities of the VTT Electronics Optoelectroncis research area are focused on module manufacturing. The micro-module technology is growing to be a significant factor in electronics manufacturing, as it is covering gap between semiconductor technology and electronics assembly techniques (like surface mount technology). Using just printed circuit boards is in many cases not possible due to the large size and lack of environmental durability. Cost issues, like need for big production volumes, fast production cycles, and low design and production risks mean that the use of System on Chip (SoC) solutions is usually not favorable. In the micromodule technology the packaging density can be increased using integrated substrates and the active components can be attached on them before encapsulation (SoP - System on Package concept). It gives also the SMEs possibilities to participate electronics manufacturing by specializing

for certain applications and technologies. To operate with the whole variety of electronics manufacturing technologies, the initiation costs are huge.

Although the micromodule production costs are lower than in traditional semiconductor technology, the investments are, however, significant, especially for SMEs. Some special equipment are needed, and many processes can be only be done in a clean room to ensure the reliability, yield, quality and functionality. In addition to the ongoing R&D work and development activities of VTT itself there has been established a development project in the Oulu area to create new environment within VTT in order to help the companies in the start-up phase. Electronics designated for the development of micro-module and photonic component technologies and product applications (especially for wireless and optical telecommunication), optical instruments and sensors (for process industry) and biomolecular recognition, and polymer opto-electronics (for product packages, printed matter and electronic products).

Special attention has been paid to that the environment is designed to support development of new manufacturing technologies and businesses by offering the industry laboratory room and rights to use also other VTT facilities, personnel training and prototype in small-scale manufacturing services as well as product development and research co-operation. The services provided to the industry decrease the need for investments especially in the testing and development phase. One of the goals of the activities, thereby, is to support new spin-off-companies. We have also efforts going on to build a micro-machining incubator within this region.

The core of the environment is the VTT's new clean room, which combined with new equipment, VTT's own research and development work performed by skilled personnel ensure that the forefront of the R&D community can be kept. Furthermore, high level services can be supplied to our customers also in the future. The project has been supported by the European Regional Development Fund (ERDF).

# 2. New clean rooms

The project to build new clean rooms started in summer 2000, when the actual planning of the possible activities within the micro-module technology development environment started. Preliminary requirements, layouts, systems, were drafted, with the cost estimates. As the variety of services was going to widen, special attention was paid to the new contract models and especially to the security issues – as there was going to be personnel from several different companies to be using of these facilities.

The building project were divided to two contracts: Actual constructing of the body of the building, including e.g. intermediate floors and coatings of the inside of (outer) walls, floors and ceilings. The building contract was defined to include:

- storage spaces, work premises and restrooms as well as hoisting machines, electricity and heating for the duration of the project
- determining and relocating the existing cables etc.,
- constructional work
- steelwork fire protection, fire doors, smoke venting hatches
- moisture barriers, (epoxy) coatings, paintings, sealings, supporting structures, lead-throughs
- intermediate floor mounting reserves for the clean room elements.

The other contract, the actual clean room installation project, included detailed design of clean rooms and adjusting them to the building construction. The contract also included e.g. security and cleaning issues, as well as protective housing during the building phase. Purchasing the clean room equipment was on VTT's responsibility. The installing, with relating electricity, plumbing, etc. was performed by the contractor.

During 2001 the plans were finalized, environmental surveys were made as well as the bids for contracts. Actual building started in November 2001, and took almost a year. Project manager appointed by VTT coordinated the work, which was conducted by companies selected on the grounds of competitive bidding.

After the acceptance inspections the new facilities were taken into use in November 2002. This doubled the total clean room area at VTT Electronics to about 1000 $m^2$ as the new clean rooms are 520 $m^2$ of classes 100 and 10000, and

the old ones 500 $m^2$ classes 1000 and 10000. In the new facilities there are also new chemistry laboratory and office rooms. The environment development project include also acquiring various equipment and tools which allow state-of-the-art research work and services. The last purchases associated directly with this project will be done during spring 2003 and will contain equipment for:

- processing of polymer and glass ceramic films
- test and characterization of films, components and modules
- characterization and packaging of micromodules
- fabrication of printable optics and electronics
- automatic packaging and alignment of optical components.

## 2.1 Technical description

In the extension building there are storage and office rooms, chemistry laboratory, actual clean room is located in the ground floor and the technical premises (esp. HPAC) in the second floor and in the basement. The new clean rooms were built as an extension to the old ones, but they are not directly connected. The air condition systems are separate and the common entry chamber for the both parts was built between them. This made it possible to continue to work in the old parts during the whole construction phase.

The operative clean room floor level is 600 mm above the building floor, and on the same level as the old clean room floor. It is constructed from (600 x 600)$mm^2$ easily removable and replaceable panels (Clean Room Access Floor System PRODATA 6500/Ventilation Panel System PRODATA 6500 ALUVENT supplied by Reinraumtechnik Fellbach GmbH) and the feedings (electricity, water, plumbing, gas lines and terminals) are all located below it. The clean room height is 260 cm, which is the same as in the old part. The ceiling is a metal grid with filter units hanging from the second floor PAC room floor (LUWA RERA cleanroom ceiling system). The inside is based on (1200 x 1200)$mm^2$ modular structure (cleanroom walls and doors from Ritterwand), and its' classification can be easily changed without major structural changes, just adding the filter area (Figure 1).

*Figure 1. Clean room construction.*

The lithography room is isolated from the rest of the building to minimize vibrations. The other parts of the floor are also isolated from the frame of the building (the demand for the vibration amplitude is assumed to be 0.1 mm/s with over 1 Hz frequencies, as the linewidth minimum is about 0.5 μm).

### 2.1.1 Air conditioning

The air-conditioning plant as well as the water cooling systems with auxiliary devices and the DI-water system are located into the second floor PAC room. Incoming air is prefiltered, dried, then there is heat uptake, heating, cooling fine filtering and steam damping before it is fed to the clean room air conditioning system. As a backup system, can also be the PAC room air to be temporarily used to ensure the overpressure conditions. Then, however, the temperature and humidity is allowed to drift from outside the limits.

The first floor over the actual clean room is an open space where incoming air comes from the PAC room and is conducted down to the laboratory facilities using filter fan units. It is also classified as clean room and there is 10–15 Pa overpressure compared to outdoors. In the clean laboratory room (desired overpressure 30–40 Pa compared to outdoors) ceiling are mounted the U15 HEPA filters and LUWA FFU-SMART Fan Filter Units from where the air goes through perforated floor to the service aisle and further back up. The FFUs are controlled by a graphical user interface.

*Figure 2. Clean room ceiling & air conditioning system.*

The particle concentration is measured in the ISO5 rooms every 6 months and in other rooms once a year (Met One Model 237 laser particle meter, resolution down to 0.5 μm). Measurements can, of course, be done also more often if needed.

The lithography demands set the values for the acceptable temperature and humidity margins: temperature 21 ± 0.5°C, relative humidity 45 ± 5%Rh. In other parts the target figure for temperature is +21–23°C, and for humidity 35–55%.

### 2.1.2 DI-water

The DI-water system is located in the PAC room and serves both the old and the new clean rooms, as well as the chemistry labs. In the water purification system (PURITA) raw water route is: 90 μm filter, softener, activated carbon, NaOH dosage, 0.6 μm filter, reverse osmosis (RO), Electrodeionization (EDI), 2.5 $m^3$ storage tank, loop pump station, UV sterilisator, mixed bed 0.1 μm filter and DI supply. Unused water circulates back to the storage tank. Core of the system is the EDI-unit (Electropure EDI XL Series) which uses an electric field to remove ions and polar species from an aqueous stream.

*Figure 3. DI-water system.*

The water production capacity of the system was defined to be 10 $m^3$/d of 18 MΩ-cm water and 4 $m^3$/d of RO water based on the DI-water consumption estimate:

| | |
|---|---|
| – 'Old' processing equipment | 1200 l/d |
| – Old clean rooms, rinsing | 1000 l/d |
| – New clean rooms, processing equipment, rinsing | 7000 l/d |
| – Air conditioning moisturing | 2000 l/d |
| Which makes the estimated total need of | 10 000 l/d. |

### 2.1.3 Main other systems

The main gases used are nitrogen and compressed air (which is served through lines from compressors). Where any special gases (like helium, argon and oxygen) are needed, bottles are brought into the maintenance area as close to the point of usage as possible.

Neutralization of the waste water is done by Labko neutralization system which is located in the basement.

## 2.2 Security and conducts

As mentioned, thanks to the new rooms, VTT could improve our services to the companies. In addition to the product development and research co-operation,

we rent laboratory room, or and rights to use VTT facilities and equipment, we can train their personnel in clean room conducts and use of the equipment.

In the design of the rooms special attention was paid to the security issues. Rooms are modular in nature; the clean room area is divided to several smaller rooms, walls can relatively easily be added or moved. In every door there is an electric lock so that the right of access can be defined separately for each room and person. Every person working within the laboratories has to go through a training course to maximize the safety of all personnel, the quality of work, purity of facilities and functionality of equipment. As there has been a significant increase on the rooms and equipment, the organization of the maintenance has also been rethought. Responsibilities have been defined more accurately, and more resources has been designated for these tasks. New guidelines have been defined and rule books written.

## 3. Micromodule center activities

The VTT Electronics Micromodule Center facilities form an unique entity – the largest optoelectronics research facility in the Nordic Countries – which allows versatile manufacturing of optics, electronics and optoelectronics, combined with the design, simulation and characterization services. They include clean room facilities for component and module assembly and packaging, glass and optics workshop, 5-axis mechanical manufacturing as well as versatile design, simulation and characterization tools. These enable services in innovative electronic and optoelectronic integrated module and instrument R&D using modern optics, electronics and manufacturing technologies. The main customers are in process, instrumentation, telecommunication and electronics industries

With thin and thick film processing equipment new thin film, polymer and hybrid materials are manufactured using evaporation, sputtering, spinning or sol-gel processing. They have many application fields, such as communication, chemical and biosensing, microfluidistics and optics and electronics packaging. The applications manufactured include protective materials for electrical and optical components (including scratch-resistant, EMI and EDS coatings, hydrophobic coatings) components for micro-optics (waveguides, OLEDs, diffractive and integrated optics devices). As new research item, there has been introduced printing technologies in optics and electronics, which allows fast and

cost effective production of these materials and structures on flexible substrates (plastics and paper).

Interconnection and packaging technologies are used e.g. for micromodule encapsulation. There is work carried on with photonic modules, MEMS packaging, RF and microwave modules for telecommunication electronics. The services include design and implementation of electronic and optoelectronic modules as well reliability testing. Basic technologies used are:

- Bare chip interconnections by wirebonding and flip-chip technologies
- Conductive adhesives
- Soldering
- Hermetic sealing of metal and ceramic packages
- Hermetic glass to metal seals for windows, lenses and feed through pins
- Nd:YAG laser machining and welding
- Dicing of ceramic substrates and semiconductors (DISCO saw).

Low Temperature Co-fired Ceramics (LTCC) manufacturing line allows design and implementation of electronic and optoelectronic modules by using multilayer ceramic technology. The services include – in addition to the R&D co-operation – prototype and small-scale manufacturing of LTCC substrates & modules for electronics and optoelectronics as well as sensor packaging.

For characterization and testing there are versatile facilities, for environmental and electrical testing, characterization of optical devices. There are also tools for optical spectroscopy, machine vision laboratory

Within the area of precision machining VTT Electronics has versatile environment for various processes. There are glass and optics workshop (cutting, polishing, rounding, drilling equipment), optical clean room; pneumatically suspended optical bench, high precision alignment station, optical design, modeling and simulation tools as well as mechanical design tools and workshop containing e.g. state-of-the-art 5-axis CNC milling machine.

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# NANOFAB – A FLEXIBLE PRODUCTION SYSTEM IN CLEANROOM ENVIRONMENTS

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## Abstract

NanoFab describes a modular, scalable and flexible automation solution for manufacturing of nanotechnology products in cleanroom environment including control methods for the manufacturing. In view of the fact that many process techniques are identical and the demands on cleanliness and precision are the same, the concept is also applicable to other manufacturing typical in cleanroom environment e.g. MEMS, opto-component and microelectronics manufacturing. This document contains the functionality specification for NanoFab. Technical solutions to the specification are in most parts intentionally left out since it relates directly to a customer specification and other parties involved in an installation.

NanoFab is described as being composed of three parts; Equipment and devices of the manufacturing system, control system and manufacturing execution system. Specific production processes, process tools or process areas are, however, not included as a part of NanoFab and therefore not at all discussed in this document. The automation solution of NanoFab is in principle concerned with all parts and details between specific process tools/areas and is intended for manufacturing fabs that include multiple products as well as multiple processes. This consequently demands competent process, product and production engineering as well as efficient collection and management of process data. The manufacturing automation as described by NanoFab is for every manufacturer intended to have an impact on yield learning curve as well as provide manufacturing process improvement by enhanced equipment utilisation, less downtime, reduced operational costs which ultimately leads to better control on lead and cycle times.

# 1. Background

NanoFab is dealing with automation of manufacturing in cleanroom/controlled environments. The concept describes a modular, scalable and flexible automation solution for manufacturing of nanotechnology products in cleanroom environments including control methods for the manufacturing. In view of the fact that many process techniques are identical and the demands on cleanliness and precision are the same, the concept is also applicable to other manufacturing typical in cleanroom environment e.g. MEMS, opto-component and microelectronics manufacturing.

Cost and quality are significant parameters for all type of manufacturing. In the production typical for NanoFab the efficiency of production is commonly defined in terms of yield, lead time and number of released production orders (wafer starts). Each implementation of a new production process has a unique characteristic production yield learning curve. Given the costs of the fabrication facility and the economics of production efficiency a small improvement of the production process yield learning curve will have a substantial impact on the revenue and profitability. The manufacturing automation as described by NanoFab is intended to have an impact not only on the learning curve but also to provide manufacturing process improvement by enhanced equipment utilization, less downtime, reduced operational costs which ultimately leads to better control on lead and cycle times.

NanoFab is moreover intended for manufacturing fabs that include multiple products as well as multiple users/companies in order to maximize the use of process tools and machinery and ultimately reduce the production costs. Consequently, efficient process, product and production engineering as well as efficient collection and management of process data are important parts of the final solution.

## 1.1 Nanotechnology

Nanotechnology is a much used word these days. It is covering a large number of technologies as well as applications. Hence, it cannot be referred to for a particular production method. To understand nanotechnology always refer back to the basic application like sensors, MEMS, biotechnology, fine powders, etc.

The production processes for some of these applications are generically developed from processes used by the semiconductor industry.

There are some basic definitions of nanotechnology which are useful to keep in mind:

- A method of large scale generation of materials or systems built bottom-up atom by atom or molecule by molecule.
- Typical dimensions are in the range of 1–100 nm. When dealing with details of this size quantum effects can be utilised.

MEMS, sensors, opto components, but not front-line microelectronics, are still larger than larger than these dimensions. However, nanotechnology is a useful buzz word.

## 1.2 MEMS and Sensors

MEMS and sensors are very small mechanical and/or electronic systems frequently manufactured from silicon. Other materials used are glass, plastics or compound semiconductors. Typically the base material is being used either as a support or as an active mechanical or electronic part of the components, i.e. silicon is being etched to cantilevers, membranes or simple electronic circuits.

## 1.3 Opto

Various passive or more frequently active electro-optical components are being manufactured from compound semiconductors. Typical examples are lasers or detectors attached to the ends of optical fibers. These assemblies are very tedious to align and fix. This problem has opened for another family of MEMS components, sub-assemblies and fixtures for optical fibres.

## 1.4 Microelectronics

A presentation of large volume production of components with extremely small critical dimensions is not complete without mentioning integrated circuits – microelectronics. This topic will basically be left out of this presentation,

though, because most wafer fabs are very large facilities dedicated to specific process families and a limited number of products. NanoFab is designed to handle the production of mixed processes, products and volumes without losing the economy-of-scale.

### 1.5 Biotechnology

A MEMS component is usually part of a biosensor or part of miniaturised laboratories. A biosensor is often a silicon substrate with precise electrical, optical or mechanical properties, which as a final step is sensitised by a relevant biological compound. Extremely small laboratories are manufactured as CD discs or chip arrays with minute channels, wells and reaction chambers. A successful design relies on a thorough knowledge of micro fluidics. Typical substrate materials are silicon or poly-carbonates.

## 2. NanoFab

NanoFab describes an automation solution for cleanroom environments applicable to nanotechnology manufacturing as well as MEMS and semiconductor industries. Typical for this type of manufacturing are the complex process flow with a large number of process steps and troublesome re-entrants of work in process in the process flow, as seen in 0. In addition, expensive process tools require an optimization of the tool utilization in order to obtain a higher productivity and consequently drive down costs. Figure 1 shows that there are lots of feedback loops. Besides the described feedback loops, transportation and buffering between the processing steps are another problem making the control of the manufacturing system complicated. One feature of the manufacturing typical for NanoFab is that the topology of the process network is not pre-defined. Rather, it is dynamic and each product desire distinct requirements.

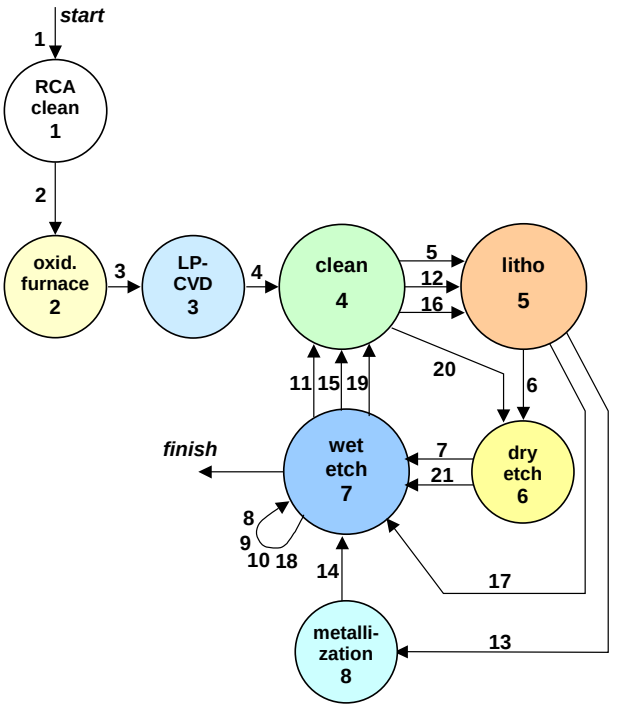



*Figure 1. A fab graph describing the process flow of a hypothetical Si membrane.*

Taking the cleanroom and its infrastructure for granted, NanoFab could be described as being composed of three separate parts, as schematically shown in Figure 2. The foundation of the automation solution consists of equipment and devices of the manufacturing system. Equipment and devices include process tools, material transportation devices, cassettes, boxes etc. However, specific production processes, process tools or process areas will not be discussed as a part of NanoFab. The control system level typically includes control of equipment and devices in terms of programmable logic controls and various types of drivers. Important parts to take into account are data communication and interfaces. The last part includes a manufacturing execution system, which is a software system that monitors and controls the activities in the fab. Important features of the manufacturing execution system in NanoFab address the need for recipe management, possibility for statistical process control, real-time monitoring of equipment status and material handling systems.

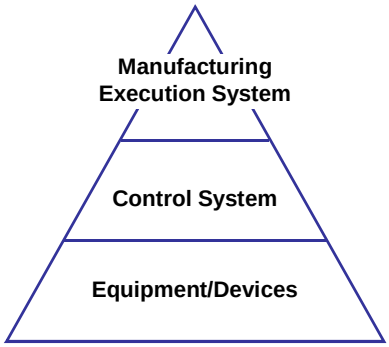



*Figure 2. The three main parts of the automation solution for NanoFab.*

The basic function of NanoFab is that lots to be processed are transported around to different process tools/areas according to the process run-sheet as defined by the recipe for that particular product. Depending on the level of automation, the transport can either be in form of an automated transport system or carried out by persons. At each process tool/area, the lot has to be loaded into the equipment in order to be processed. In order to have a system that can run independently from a central computer, a carry-on memory has to follow the lot. The carry-on memory contains information about recipes.The concept is intended to be flexible in the sense that new process tools and customized process areas could easily be installed and implemented in the production.

## 2.1 Managing the material flow

Managing the material flow of multiple product environments is a highly demanding task. Typically the material flow is not linear from front to back. Lots can revisit some areas a large number of times. The main task for the transportation system is to execute a material flow that is defined by recipes/fab graphs and transferred to the software controlling the material flow. The recipes define the order in which operations and processes are performed to navigate batches though the fab. Routing can be seen as the sequence of workstations passed through by a lot. Routings begin at a stocker point and end at another stocker point. A schematic product and resource portfolio is shown in Figure 3.

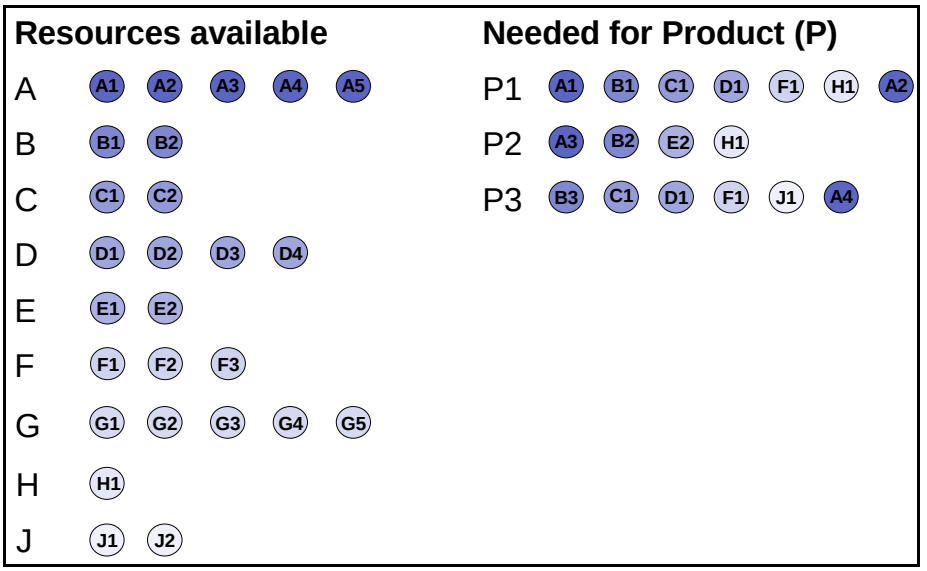



*Figure 3. Schematic representation of resource and product portfolio.*

## 2.2 Original material in processing

The basic material in the NanoFab processing is in the form of wafers. A number of wafers starting at the same time and to follow exactly the same process flow is defined as a batch. The process tool interfaces are the interfaces to the NanoFab and have to be considered as boundary conditions while designing other parts of the system.

## 2.3 Level of Automation

In NanoFab a number of different levels of automation can be identified. The automation level ranges from a fully automated to a manual solution with three intermediate steps, as shown in Table 1.

Note, for all automation levels a manufacturing execution system and a batch identification system is required. Also, when a standardized load port is present at any process tool/area SMIF-pods are required. In the **fully automated solution** the material handling system handles all batches and the production control is managed by the manufacturing execution system. Necessary equipments are SMIF-pods and standardized load ports including automated

loading systems at each process tool/area. To manage the production a batch identification system is required. In Figure 4 an example of the fully automated concept level is shown.

In the **semi-automated solution** batches are either transported by the automated material handling system or by PGV:s. If standardized load ports are available at each process tool/area the solution must necessarily use SMIF-pods. In case of manual load ports other type of (non standardized) boxes or pods can be used. The production control is managed by the manufacturing execution system, which requires a /batch identification system. An example of a semi-automated fab is shown in Figure 5.

*Table 1. The different levels of automation in NanoFab.*

| Solution | Transport system & controls | PGV* | ID** | Loading system | SMIF-pods and SMIF load ports | Other boxes/ pallets | Cassette load ports | MES |
|---|---|---|---|---|---|---|---|---|
| **Fully automated** | X | | X | X | X | | | X |
| **Semi-automated I** | X | (X) | X | | X | | | X |
| **Semi-automated II** | X | (X) | X | | | X | X | X |
| **Semi-automated III** | | X | X | | X | | | X |
| **Manual** | | X | X | | | X | X | X |

(**X**) Optional requirement

*****PGV, Person Guided Vehicle

******Lot/batch identification system

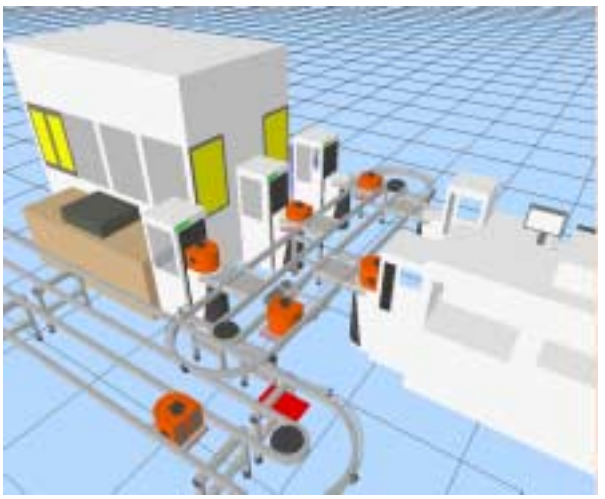


*Figure 4. An example of the fully automated fab.*

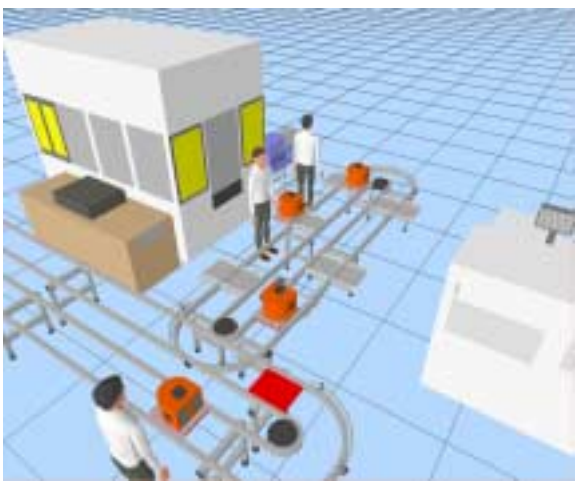


*Figure 5. An example of the semi automated fab.*

In the **manual solution,** operators with PGV:s transport the batches. The batches are contained in boxes or pods. The production control is managed by a manufacturing execution system and a batch identification system is required. An example of the manual automation level is shown in Figure 6.

*Figure 6. An example of the manual fab.*

## 2.4 Production Control Methods

The method of production control is an important factor in the productivity of a fab. Shorter lead times have direct implications in lowering costs and descending the learning curve as well as reducing the time to the market. Optimizing the whole system and avoiding optimization at local points is the only way to achieve this.

## 2.5 CONWIP

CONWIP (CONstant WIP) is a -loop batch release control mechanism. Its basic idea is to control cycle times by regulating work-in-process (WIP) for a factory (Figure 7). The mechanism requires a WIP threshold for each product. As soon as this threshold is met, new batches from the order pool are only allowed to enter the fab when batches of the same type leaves the fab. The mechanism is conceptually simple and easy to implement. The main concern is to find the appropriate WIP threshold. During simulations simple algorithms were found that are suitable for CONWIP in multi-product manufacturing environment.

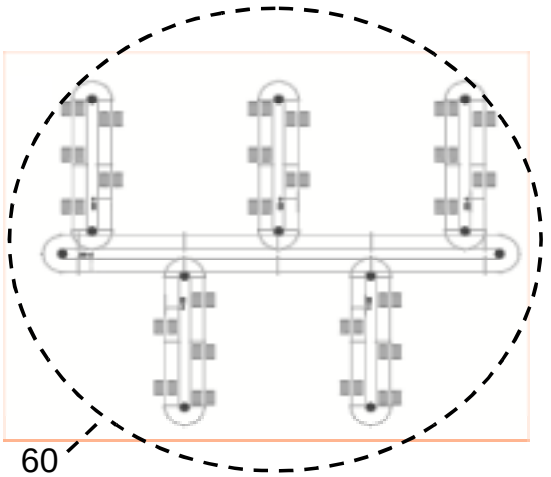



*Figure 7. CONWIP, the number of lots in the whole system is controlled.*

## 2.6 Loopwise CONWIP

It is possible to apply CONWIP to each loop of the fabrication process instead of considering the whole fab, as shown in Figure 8. Now, it is possible to control how the batches are distributed among the loops and how they progress. The control is enhanced in comparison to the complete fab CONWIP.

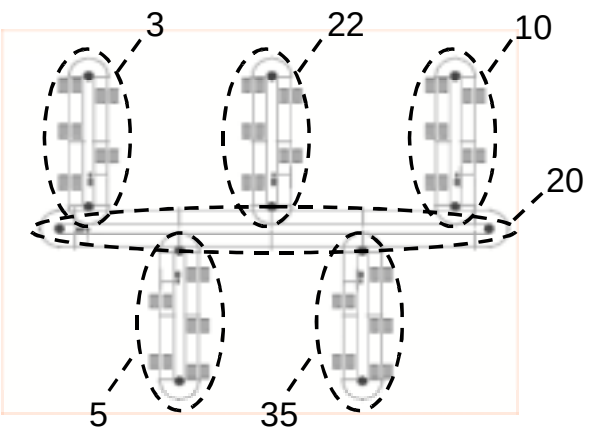



*Figure 8. Loopwise CONWIP, the number of lots in each loop is controlled.*

## 2.7 CONLOAD

Another solution that can be applicable in NanoFab t is CONLOAD. Instead of controlling the WIP directly, this method is intended to control the average bottleneck load. One needs to specify a target load for the bottleneck, say 90% of the productive time. Each batch entering the fab increases the total load of the fab by its sum of bottleneck processing times divided by the average cycle time of this batch type. New batches are only allowed to enter the fab if the total load, i.e., the sum of all batch loads in the fab, is less than the target load multiplied by the number of bottleneck machines (Figure 9).

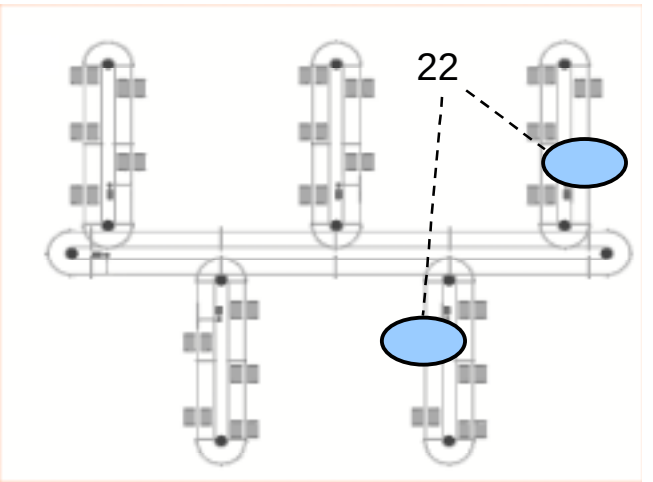



*Figure 9. CONLOAD, the controlling by the total amount of bottleneck process equipment available.*

## 2.8 Suggested control method: CONWIP

The first alternative to test for production control is the simplest one: CONWIP. The starting point is to define the CONWIP size, which is the number of batches in processing. The Total Throughput Time is then the CONWIP size multiplied by the total process time that one batch spends on the bottleneck. The used factor could be something between two and five, and starting will be done at values given by results from simulations. That will give the number of batches needed in the system, i.e. CONWIP size. Permission to release a new batch is given

when a batch passes the bottleneck for the last time, as schematically shown in Figure 10.

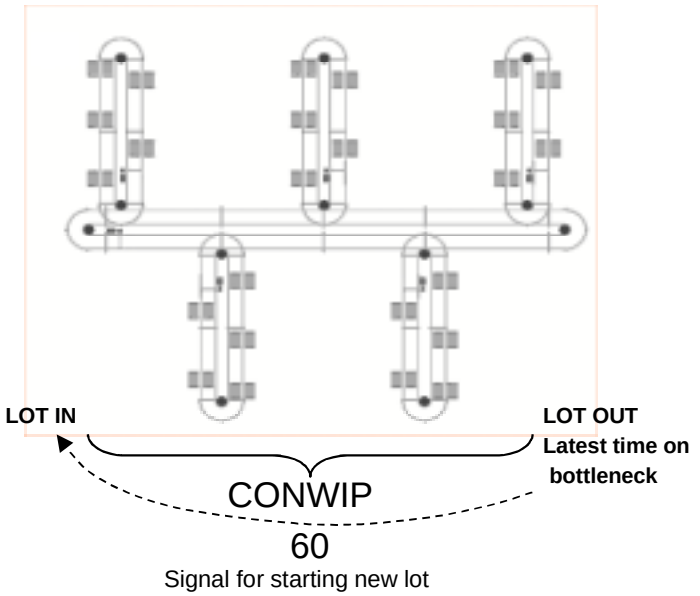



*Figure 10. CONWIP and releasing new products.*

When changing the product mix there is a risk that material from the bottleneck of previous product mix will disappear before there is material in the front of the bottleneck of the new product mix. That period of time will be avoided by releasing new mix faster than 'one out one in' rule to make sure that material of new mix will catch up with the bottleneck of previous product mix.

## 2.9 Manufacturing execution system

Manufacturing Execution System (MES) is a commonly accepted notation for a software system that monitors and controls the activities on a shop floor. Important features of the NanoFab MES address the need for recipe management, statistical process control, real-time monitoring of equipment status and material handling. A modular solution of the MES is demanded since it is required that the whole or only parts of the system according to customer specification shall be possible to put into operation.

### 2.9.1 MES and automation levels

For NanoFab different levels of automation are to be possible to implement. Hence, it is required to be possible to add or remove modules/components to the software in order to meet the requirements of a particular customer software solution. NanoFab will have to handle the following automation levels and its implications on the MES systems:

1. **Non-automated intelligent fab**. Operators are instructed by a software system what to do to each product and when. Operators carry out all activities outside the tools. Batch identification hardware is used to correctly present the batch to the system. The system provides the tools (electronically) or the operator with the correct work instructions.
2. **Semi-automated intelligent fab**. Automated material handling systems, controlled by the software system, attend to transporting the batch to the correct areas in the fab. An operator carries out loading and unloading of the batch between the automated handling system and the tool. Otherwise, the fab functions as in item 1.
3. **Fully automated intelligent fab**. In addition to item 2, the automated material handling system provides batch transport into and out from the tools. Ideally, the process between batch start and product shipment does not need human intervention.

### 2.9.2 Recipe management

The recipe management is an important part of the MES of NanoFab. Editing recipes and keeping track of recipe revisions is required features of the recipe management. Recipe modules build up the product recipe (Figure 11):

1. Recipe identification: Each recipe unit needs an identification that makes it unique and also gives information regarding revision number etc.
2. Process parameters: Process parameters are the necessary information to describe the processing in detail.
3. Operator instructions: Instructions to the operator about the particular process step.
4. Manual or automatic: The automation level of the process equipment is directly related to the work of operator and the exchange of information between tool and system.

5. Process step description: A description of what the process step does and who is responsible for the particular recipe.

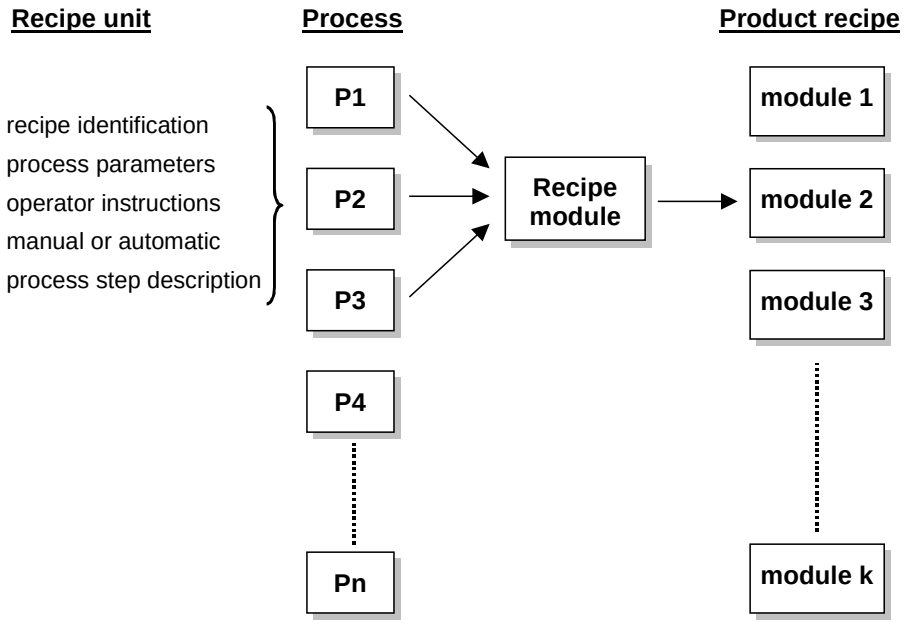



*Figure 11. Schematic view representing the recipe management. At each process step a recipe unit can be defined and they can be combined into recipe modules, which are the building blocks of the product recipe.*

### 2.9.3 Transport System

The main task of the NanoFab transport system is to control the material flow. The material flow is defined by the process recipe and executed by controlling software for the material flow. The recipes define the run-sheet and the order wherein operations and processes are performed to navigate batches through the fab. An important characteristic of the transport system is the throughput capacity. Figure 12 shows an example of a configuration of the transport system. A typical object to be transported:

- Maximum size: $0.5\text{m} \times 0.5\text{m} \times 0.5\text{m}$
- Maximum weight: 10 kg

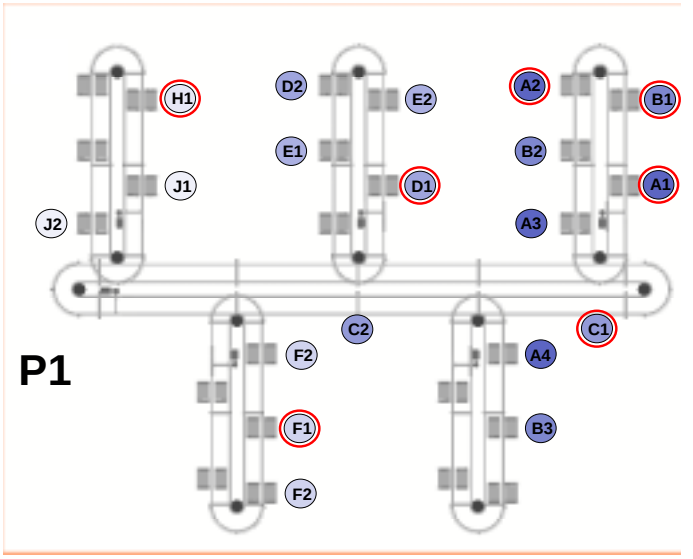



*Figure 12. Schematic layout of transportation system. Positions for process equipment (A1, B1, C1) are shown for an hypothetical product P1.*

### 2.9.4 Basic transport rules

Diverting a pallet is not possible unless process equipment or workstations can be bypassed. Hence, a basic rule is that pallets can bypass all existing operating stations. The cyclic dependency (each equipment waiting for another equipment to be ready) must be avoided. That will be one of the tasks of controlling software.

### 2.9.5 Transport / Material flow

The basic processing flow in the manufacturing can be described in three steps; Initialization of batch/lot, execution processing and finishing the processing (Figure 13).

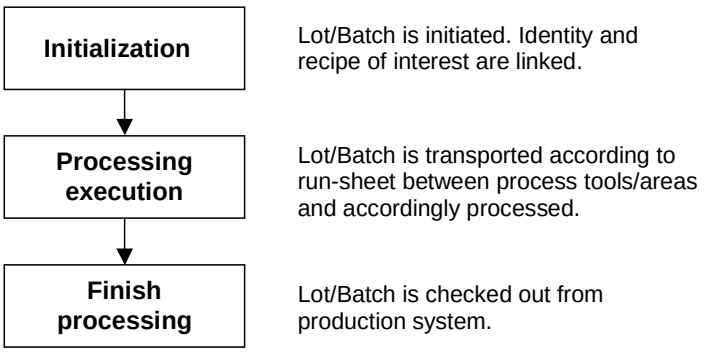



*Figure 13. Flow chart describing the three basic parts of the flow in production.*

The main purpose of the initialization phase is to let the MES recognize and initiate the processing of a new batch as well as to identify the individual wafers. It is important to link the identity of the batch to the recipe of the product to be produced. Then the batch is placed in the material handling system and released to production.

The next step is the processing execution, which includes two parts: transportation and the processing. The transportation system moves the batch around in the fab according to desired rules. At each process equipment/module the batch tag is read and decisions are made if it is the correct equipment and if it is available. If so, the batch will be loaded into the process equipment. If it is not the right equipment or the equipment is busy the lot will continue on the transportation system until these conditions are met.

When the batch has proceeded through all process steps, it will be moved to the exit buffer where similar conditions as for entering process equipment/areas are used and then check it out. The batch should then also be removed from the work in process list.

### 2.9.6 Process documentation

It is required that data from the processing is documented and stored in an electronic batch record. The main objective of the process documentation is to store events and data that is related to each wafer/batch. The documentation is

meant to give the possibility for analysis as well as tracking. There are at least three different logs that can be identified:

- **Event log.** This log contains notes related to events that occur during the processing.
- **Process data log.** In the process data log process data and relevant information about the processing shall be stored.
- **Production system log.** The main objective of the production system log is to record events that occur in the production system as well as typical status information

### 2.9.7 Material buffering positions

There are four types of material buffering positions in the system:

1. Transportation system.
2. Loading and unloading stations; including all kind of process ports.
3. Process equipment.
4. Stocker at bottlenecks.

Basically, the manufacturing system is based on the principle that all needed material is buffered while in use.

### 2.9.8 Visual overview of process flow

To ensure transparency of the process flow a visual overview of the work in process is essential. Hopefully, the operators can visually see where materials are at every moment and situation during processing. In addition, it is desired to be able to run the production manually if a major error occurs at an upper control level.

### 2.9.9 Overall demands on transport system

The basic functionality of most basic conveyors will meet the requirements of NanoFab. The overall demands for the transport system concerning contamination, vibrations, reliability and degree of maintenance are:

- All moving components (bearings, drive units, etc.) must be lubricated and sealed. Oils and greases are not allowed in order to avoid outgassing.
- Minimize the number of components for reliability and reduced maintenance.
- Strict specifications on materials; corrosion resistance, etc.
- Cleanroom classification has to be matched.
- Conductive from pallet to ground.
- The transportation system is to be modular; it should be possible to easily add and remove modules from the system
- Modular design and infrastructure.
- Non-contact queuing.
- Possibility to walk through transport system must be easily arranged; bridges, ports, lifts, elevator, etc.

## 2.10 Additional hardware/software items

### 2.10.1 Boxes/Pallets/Inserts/Cassettes

In the typical infrastructure for highly automated manufacturing in cleanroom environment standardized Standard Mechanical Interface (SMIF) pods (boxes) or Front opening Interface Mechanical Standard (FIMS) pods (boxes) are required. Especially, the standardized pods are necessary when using a standardized process tool load port. The pallet works as a fundament on which customized inserts that contain the cassette/pod/box is placed. Typically, the pallet is required to fit transport system specification. In Figure 14 the pallet, insert, box and product system for NanoFab is shown. General demands for the box/pallet/cassette in NanoFab are:

- Adapted to the specification of the transport system.
- Customized to the level of automation.
- Cleanroom class has to be matched.

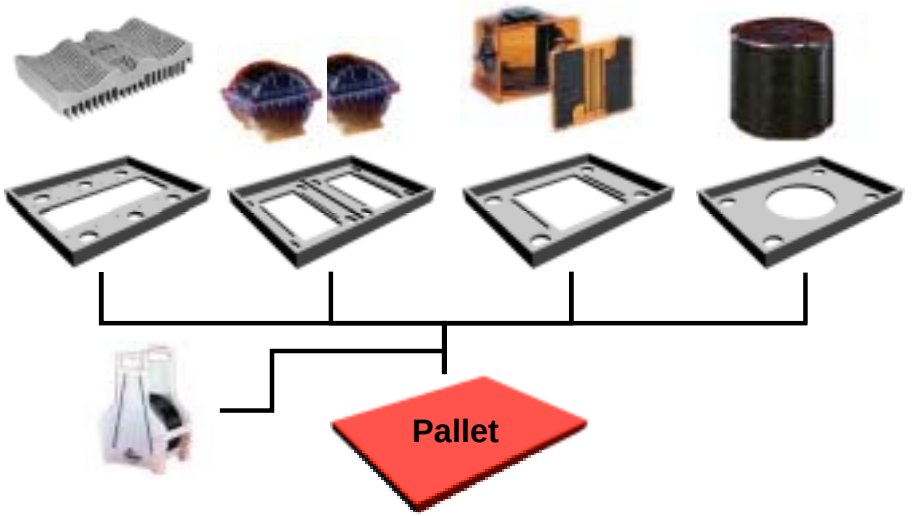



*Figure 14. Pallet and inserts for the fitting different level of automation and different shaped products. The system can also be used in manual operations as delivering product to the right place at the right time.*

### 2.10.2 Load ports/Process tool interface

For NanoFab a well-defined interface to the process equipment is required and has to be considered as an important boundary condition while designing other parts of the system. The requirements of the process interface are to comply with the three basic levels of automation:

- Fully automated
- Semi-automated
- Manual

The process tool interface, using automated loading, is in the most cases defined by the SEMI standards and described as a Standard Mechanical Interface (SMIF) or Front opening Interface Mechanical Standard (FIMS), which requires SMIF-pods or FOUP-pods for automatic loading. A number of suppliers of SMIF/FIMS load ports and pods exists on the market. Further requirements on the load port are:

- The process tool interface shall be cleanroom compatible
- Cycle time, particles/wafer and air flow according to specification.
- Safe loading
- Compatible with transportation systems, stockers and process equipment.

### 2.10.3 Tool communication/drivers

The industry specific process tool communication is defined by the SEMI standard in Semi Equipment Communications Standards (SECS) and Generic Equipment Models (GEM). The tool communication has to be handled by NanoFab. A number of suppliers of toolboxes for SECS/GEM communication are available at the market The SEMI automation software standard ties all parts of Computer Integrated Manufacturing (CIM) together.

- SECS-I (Semi Equipment Communications Standard I, E4)
- SECS-II (Semi Equipment Communications Standard II, E5)
- GEM (Generic Equipment Model, E30)
- HSMS (High-Speed SECS Message Services, E37). HSMS is a development of SECS-I that allows SECS-II messaging over high-speed networks using TCP/IP. With HSMS, the physical carrier is no longer important since the connections rely on TCP/IP, which means that there is no limit to the connection speed.

### 2.10.4 Batch ID system

At each station, data from previous process stations is read from the ID tag and new data generated at that station is written to the ID tag. The data travels with the lot throughout the entire manufacturing system. Data is updated at each station, and the information compiled on each tag directs what action will be taken at each station, including on- and off-line test areas.

Active tags are small sealed devices for remote read/write communication. Passive tags operate without an internal battery, deriving the power to operate from the field generated by the reader. Passive tags are consequently much lighter than active tags, less expensive, and offer a virtually unlimited operational lifetime. The trade-off is that they have shorter read ranges, less data storage capacity, lower noise immunity, lower data transmissions rates than

active tags and require a higher-powered reader. NanoFab will need an escort memory system for identifying the batch. Escort memory is also needed for routing information and process data collection. A basic requirement of high product mix fabs will be wafer-level material tracking and control.

### 2.10.5 Stockers

Stockers might be necessary at some locations along the production flow. Most probably stockers will function as buffers in front of bottlenecks in the manufacturing system. The type of stocker will be dependent on the level of automation. In the fully automated fab automatic stockers are necessary contrary to the manual or semi-automated fab where the stocker could be a simple shelf.

## 2.11 Standard compliance – SEMI

The SEMI standard covers most aspects of semiconductor manufacturing and includes: Equipment Automation (Hardware and Software), Facilities, Gases, Materials, Microlithography, Packaging, Process Chemicals, and Traceability. Guidelines also cover safety related issues. In addition, standards are published for Flat Panel Displays. The publication catalogue provides a listing of the available standards and guidelines. NanoFab is dependent on a small subset of these standards. The master outline of the SEMI standard with boldface entries indicate substantial applicability to NanoFab:

- **Equipment Automation Hardware**
- **Equipment Automation Software**
- Facility Standards and Safety Guidelines
- Flat Panel Display
- Gases
- Materials
- Microlithography
- Packaging
- Process Chemicals
- **Traceability**

### 2.11.1 Cost level

The price level of an automation systems offered to an end customer is of large importance. NanoFab focus on startup companies interested in small to medium sized fabs. If these companies should buy an automation system the price have to be substantially lower than the price paid by typical large fabs in the traditional semiconductor industry. The cost of an automation system depends strongly on the level of automation, but also on the chosen technical solutions.

### 2.11.2 Manufacturing execution system

The standard solutions used in the semiconductor industry will cost 300–500 kUSD for a software license for a smaller installation. On top of that a large cost for system integration and installation have to be allocated. This installation cost can be as large or even larger than the cost of the software. An example of a MES solution on the low cost side is in the price range of 10 kUSD and upwards. It is unclear how much work that has to be included to integrate these products in NanoFab.

### 2.11.3 Transport/Conveyor system

If a conveyor system from the industry outside the semiconductor business are chosen the price level is in the range of 2 kUSD/m. The cost of a control system and a full electrical installation is estimated to 15 kUSD for each installation. Thus the total cost of a 50 m long conveyor system would be 115 kUSD. Extreme cleanroom conveyors are substantially more expensive.

### 2.11.4 Other hardware costs and systems integration

Costs for computer terminals, PLC’s, batch identification systems etc will not dominate the price of the automation concept. However, it is important to estimate the cost of the system integration which might be a large part of the over al cost of our concept.

# 3. Profit analysis of NanoFab

With the purpose of identifying some of the benefits of NanoFab a profit analysis has been performed by ABB Cleanroom. The profit analysis is performed with realistic assumptions concerning present customer and products for ABB.

## 3.1 General conditions and assumptions for the profit analysis

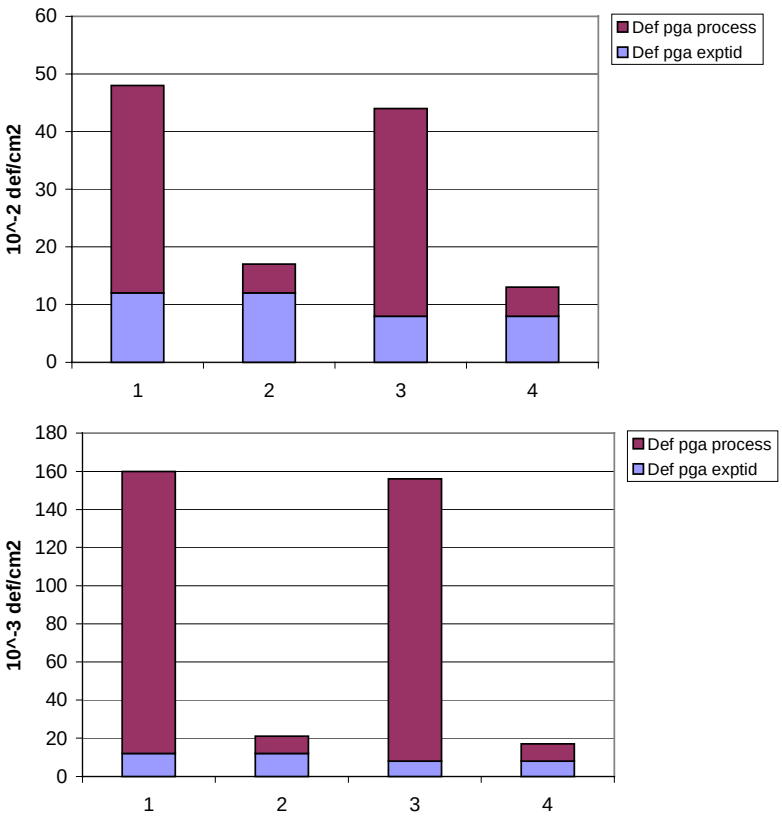
- The manufacturing is intended to produce MEMS or IC circuit using ordinary processing techniques.
- The chip area is 100 $mm^2$ and 9 $mm^2$ for IC and MEMS, respectively.
- The manufacturing volume is 20 batches each including 25 wafers per week.
- The price of one wafer is $1000
- Lead times are reduced by 25% with NanoFab:
- For the MEMS production the lead time is decreased from 4 weeks to 3 weeks.
- For the IC circuit production the lead time is decreased from 8 weeks to 6 weeks.
- Descending the learning curve:
- For the MEMS manufacturing the goal is to go from 35% to 70% in yield.
- For the IC circuit manufacturing the goal is to go from 45% to 90% in yield.
- Half the lead time has to be passed in order to render the possibility to evaluate and improve the manufacturing process.
- At each time twenty batches are evaluated.
- It takes 100 batches to descend satisfactory along the learning curve.

## 3.2 Profit due to descending the learning curve

The profit for MEMS is $ 262 500 and for IC circuit: $ 675 000.

## 3.3. Profit due to yield enhancements

The yield for IC:s is as a result increased by 2%. During one year of production these numbers means a reduced cost due to lost components by $1 250 000. Corresponding numbers for MEMS gives an improvement in yield by 5%, which results in reduced cost of $500 000.




1. *Before* improvement, *long* lead time.
2. *After* improvement *long* lead time.
3. *Before* improvement, *short* lead time.
4. *After* improvement, *short* lead time.

*Figure 15. Defect density for MEMS (upper picture) and IC circuits (lower picture) before and after descending the learning curve and with short and long lead time.*

# Definition of used terms

**Batch** A number of lots aimed to produce a given product type. Smallest possible batch is the same as one lot (see lot).

**Bottleneck** The process tool/station in the process flow with the highest long-term utilization. In simple flow lines without yield loss, where the arrival rate of jobs to every station is the same, the bottleneck is the station with the slowest average rate. However, in systems where yield loss exists, rework, or complex routings make the arrival rate different at different stations, the slowest station may not be the busiest. Since it is the busiest station that acts as a primary constraint on system performance, we designate the highest utilization station as the bottleneck.

**Cassette** The physical gadget containing wafers. See also Batch, Lot.

**Chip** A piece of a wafer containing a circuit. See also Die.

**Cluster Tools** Process equipment capable of performing a sequence of processing steps without leaving a controlled environment.

**Component** A packaged chip.

**Cycle Time** The average time between the release and completion of a job along a given routing. Includes all process time, setup time, transport time and wait time.

**Device** A single transistor, a chip or a component.

**Die** A piece of a wafer containing a circuit, which has not yet been packed. See also Chip

**Equipment Set** Consists of all hardware and associated software used during wafer fabrication, including processing equipment, materials movers, and automation systems.

**Fab Graph** A graphical mapping of a process flow onto an equipment set.

| | |
|---|---|
| **Hub** | Process equipment that performs more than one process step, even within a flow. |
| **Lead Time** | The time designated for a job to traverse a designated portion of the production process. Customer lead times are the times allotted to fill a customer request. Notice that lead times are management constants (i.e., set by policy), while cycle times are attributes of the system itself, see Cycle Time. |
| **Lot** | A group of substrates gathered as one unit to transverse the factory. |
| **MES** | See Manufacturing Execution System |
| **Manufac-turing Execution System** | A commonly accepted notation for a software system that monitors and controls the activities on the fab floor. |
| **Process Flow** | The totality of the sequence of process steps and recipes required making a working device |
| **Process Step** | A physiochemical method for transforming the object under consideration. |
| **Process Equipment** | The equipment that performs the transformation of the object under consideration. See also Process Step. |
| **Processing Time** | The total number of working hours required to process a batch, assuming all equipment available as needed = theoretical minimum cycle time.<br><br>Cycle time – Processing time = Wait time or time spent in queues. |
| **Recipe** | A collection of recipe units put together in a sequence in order to produce a device or component |
| **Recipe unit** | Recipe at one particular process (usually one tool/equipment) that includes a number of for that process typical parameters |

| | |
|---|---|
| **Run-sheet** | The physical incarnation on paper, data file or escort memory of the recipe for a particular lot. The run-sheet contains information about where the lot should be transported and what will happen to it. |
| **Setup** | The machine preparation that must be performed when the next batch to be loaded is from a different process than the one last that last used the machine |
| **Throughput** | The average output of a production process (tool, station, line, plant) per unit time. At the firm level, throughput is defined as production per unit time that is sold. However, since plants frequently do not control what is sold, a more conventional definition at the plant level is the average number of good (non-defective) parts (chip/wafer/lot/batch) per unit time. |
| **Wafer** | A near circular slice of a desired material. Usually made of silicon. |
| **Wafer Foundry** | Fab that manufactures circuits to customer specifications – contract wafer fab. |
| **Work-in-Process** | Inventory (wafer/lot/batch) between the start and end points of a routing. |
| **WIP** | See Work in Process/Work in Progress. |
| **Yield** | The fraction of functioning chips/items per wafer |

# MANAGEMENT OF STATIC ELECTRICITY AND ESD IN CLEANROOM PRODUCTION OF ELECTRONICS

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## Abstract

Control of static electricity and ESD in cleanroom production of electronics is becoming more and more important along with the ongoing reduction in device and electronics dimensions. Two major problems result from failing of electrostatic charge control in cleanrooms: product contamination due to electrostatic attraction with bonding of particulate to critical surfaces, and direct product failures due to electrostatic discharge. In this paper basic methods for static electricity and ESD control are presented. Novel solutions related to cleanroom garments as well as automated assembly are discussed in more detail. Finally, a concept of electrostatic control program for cleanroom production of electronics is given. The purpose of such a program is to minimise risks associated with implementation of new technology into a product or production equipment.

## 1. Introduction

Modern manufacturing of electronics requires often contamination free environment. In addition to the classical wafer level semiconductor production, cleanrooms are more and more frequently used in the production of plastic sub-parts for products, in the production of optoelectronics and in electronics assembly. The ongoing reduction of semiconductors elements dimensions and the use of microtechnology in assembly will emphasise the future need of contamination free production environment in several stages of electronics production. Sometimes the clean production may happen in small volume cleanroom-cells instead of large cleanroom production halls. But from the technical point of view, same cleanroom control methods are valid both for large volume rooms as well as for small cleanroom cells.

Electrostatic charge control has become a necessity in cleanroom production of electronics. Almost all operation in cleanrooms involves contact and separation of materials. That generates static charge. The ongoing reduction in device and electronics dimensions as well as other advances in the technology of products produced in cleanrooms has made the products more sensitive to failures of electrostatic origin than ever before. Accordingly the static charge control is more important than ever. Failing in the charge control would result in two kinds of major problems: 1) product contamination due to electrostatic attraction (ESA) and bonding of particulate to critical product surfaces, and 2) damage done directly to products by electrostatic discharge (ESD). Less frequent, but still possible, is the malfunction of production equipment caused by ESD-generated electromagnetic pulses. Damaged or defective product and inefficiencies in the production process due to problems of electrostatic origin show up as manufacturing yield losses, but even more serious may be the impacts on long term reliability of the product. In this paper I review basic problems related to ESA and ESD and present selected novel solutions for the management of static electricity in the cleanroom production of electronics.

## 2. Electrostatic fundamentals

Charge is a property of elementary particles – electrons (negative charge) and protons (positive charge) – that make up the atoms of materials. Normally charges are present in a material nearly equal quantities at any location. However, under some circumstances there can be an excess of one polarity at one location. Then electrical effects of the charges are no longer balanced and a net electrostatic charge exists at that location. A major way in which an excess of charge can arise is when two different materials (can be any materials), that are initially in contact, are separated. The amount of charge as well as their polarity depends on the materials, surface conditions, contaminants and humidity. Materials may be arranged in a table according to the polarity of charge they take in contact with other materials, called the triboelectric series, Table 1. Separation of materials on the table may also reflect the amount of charge generated during triboelectrification.

Any charge has a region of influence around it, which is called the electric (or electrostatic) field due to the charge. In the electric field a charge experiences an

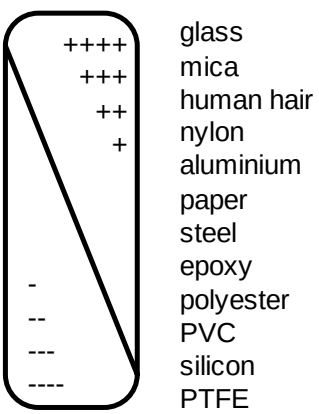
electric force acting upon it. The force $F$ experienced by a charge $q$ in an electrostatic field $E$ is

$$F = qE.$$

In the electric field like polarity charges are repelled and unlike polarity charges are attracted. The strength of the electric field and thus also the force fall off with distance.

In addition to the triboelectrification, a material can be charged by induction. An uncharged, isolated conductor B is placed in the field of a positively charged insulator A (Figure 1). As a result some electrons in B will move in the field causing B to develop a negative charge on the one side and a positive charge on the opposite side where there is a deficit of electrons. The negative charges of B, bounded by the field of A, will remain in B even if the opposite side is grounded, when the free charges will flow to ground leaving a negative net charge on B.

*Table 1. An example of a triboelectric series.*



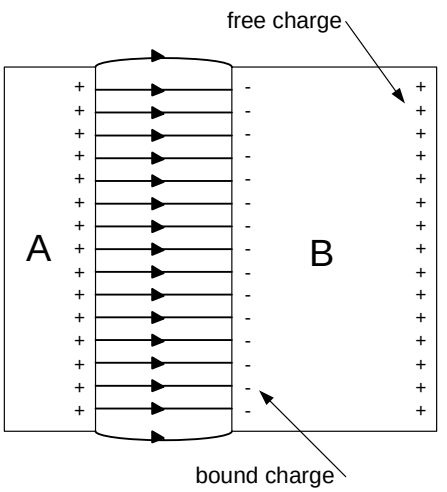




*Figure 1. Electrostatic induction.*

# 3. Electrostatic attraction

Charged surfaces attract particles, as shortly described in ch. 2. Air particles are seldom electrically completely neutral. The amount of charge per particle may be very small but, if we take into account the mass, *m*, of a particle, the relation *q/m* may be such that already a weak electrostatic force *F* can affect the flow of the particle. The electrostatic attraction (ESA) is illustrated in Figure 2. The contamination of a charged surface due to ESA and the bonding of particulate to critical surfaces depend on various factors, such as the amount of charge (or electric field strength) on the surface and the concentration, charging (amount, polarity and distribution), mass and speed of the particles. In strong electric fields (highly charged surfaces) the contamination can be very strong (note television screens at home). In cleanrooms where any contamination should be minimised it means that existing electrostatic charges should be minimised. Because charge will naturally be generated almost in any operation in cleanrooms, the generated charge should be safely dissipated or neutralised in order to avoid the contamination of critical surfaces by electrostatic attraction. That requires effective measures of electrostatic charge control and, increasingly, a specific program for static electricity management.

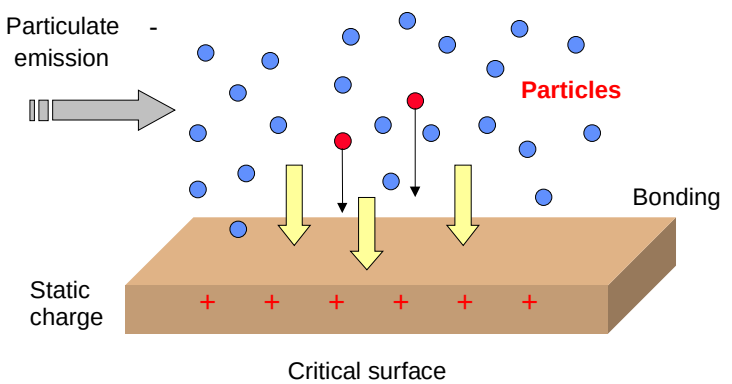



*Figure 2. Contamination of a surface due to electrostatic attraction.*

# 4. Electrostatic discharge

Electrostatic charge generated in a conducting object can be abruptly discharged if the object gets a low-ohmic connection to ground or to another conducting object. The duration of the electrostatic discharge (ESD) can be very short,

typically from nanoseconds to microseconds, but the peak current as well as the peak power and the energy density of the discharge can be high. The discharge current and the discharge energy depends on the amount of charge in the ESD, on the potential difference (voltage) of the discharge, and on the impendance of the discharge circuit. An ESD may cause failures to sensitive electronic devices. A device can be damaged due to local heating because of the discharge energy or power (depending on the discharge duration) or due to the breakdown of an insulating oxide layer because of internal overvoltage during the ESD. In addition to instant damages, an ESD may cause latent failures where a product is weakened by the ESD but still operates normally. The weakened product, however, may be very sensitive to other disturbances, such as corrosion, seriously reducing the long term reliability of the product.

There are three basic types of electrostatic discharges: 1) a discharge from a charged human body to a victim device, 2) a discharge from a charged, isolated metal object to a device, and 3) a discharge from a charged device to ground or to another conducting object. Practical need has led to the development of three standardised ESD models, respectively [1, 2]: 1) Human Body Model (HBM), which models the situation where a charged person discharges directly into a victim device. The human body is modelled as a resistor of 1.5 kΩ and a capacitor of 100 pF. 2) Machine Model (MM), which models a discharge from large charged metallic machine part, trolley or other conducting object, into the victim device. The metallic machine is modelled by a 200 pF capacitance with negligible series resistance. 3) Charged Device Model (CDM), where the main electric parameter is the capacitance of the device. CDM simulates a sensitive device becoming itself charged. An ESD event occurs when the charged part touches a metallic object. HBM and CDM cases are presented in Figure 3.

The evolution of semiconductor technology has led to devices which are more sensitive to ESD than ever. Sensitivity of electronic components to ESD is typically given as ESD withstand voltage, which is the largest ESD voltage that the device can stand without damage. Overview of device technology HBM withstand voltages (sensitivities) is given in Table 2 [3]. MM and CDM withstand levels of devices are typically much smaller than HBM withstand voltages. For example, ESD withstand voltages for MM are found to be 10–20 times less than HBM [4]. For a very sensitive device that may mean a MM withstand voltage of only a few volts. The number of very sensitive devices with HBM withstand of 100 V or below is rapidly increasing.

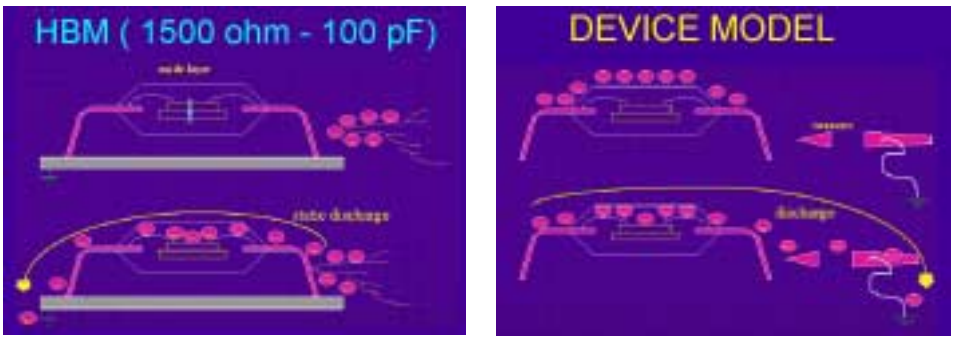



*Figure 3. Human body model and charged device model types of ESD.*

*Table 2. Overview of device technology sensitivities for the Human Body Model (HBM) type of ESD.*

| Technology | Typical HBM sensitivity (V) |
|---|---|
| MR heads, RF FETs, SAW devices | 10–100 |
| power MOSFETs, PIN diodes | 100–300 |
| laser diodes | 200–1500 |
| MMICs | 100–>2000 |
| Flat panel displays and CCDs | 50–150 |
| LEDs | 500–8000 |
| modern VLSI | 1000–3000 |
| CMOS B series | 2000–5000 |
| CMOS A series | 1000–2500 |
| MOS linear | 800–4000 |
| small geometry modern bipolar | 2000–8000 |
| power bipolar | 7000–25 000 |
| film resistor | 450–5000 |

## 5. Static electricity and ESD control methods

Grounding of products, production equipment, production environment as well as production personnel is the classical way of controlling static electricity. For

the control of ESA, there are no specific requirements for the grounding. Only the resistance of the ground path, $R$, has to be such that the charge can migrate to ground in a reasonable time. In practice, this means that $0 < R < 10^{12}$ Ω. ESD control, however, requires more specific limits for the resistance-to-ground. In practice, the surface resistance of all surfaces and materials which could be in contact with ESD sensitive electronics has to be in the range of $10^5$ Ω $< R < 10^{11}$ Ω in order to avoid ESD failures [1, 2]. For the most sensitive devices in automated production, even an narrower range of $10^6$ Ω $< R < 10^9$ Ω is preferable. The desired ground resistance is obtained by using special electrostatic dissipative materials for the control of static electricity and ESD (so called ESD protective materials). Great care, however, should be used when choosing an ESD protective material for cleanroom use because not all ESD protective materials are cleanroom compatible but can be a source of particle emission. Another fact, which should be taken into account when choosing an ESD protective material, is that their conductivity often decreases (i.e. resistivity increases) in decreasing humidity. As a result, a material which fulfils the requirements at normal humidity may become an insulator at dry conditions. Therefore, the material testing is often done at 12 % RH. Examples of the surface resistivity of different kinds of materials at different humidity are given in Figure 4 [5] (note that most of the materials in Figure 4 are not cleanroom compatible).

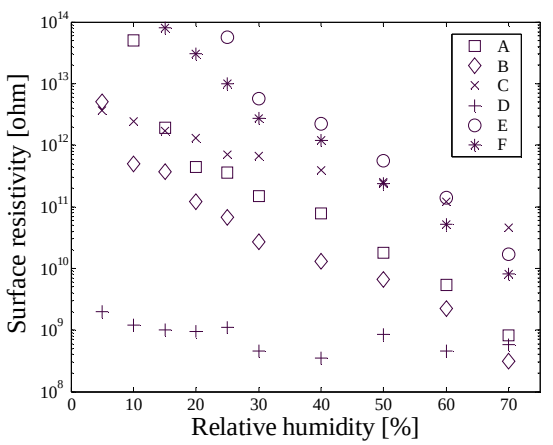



*Figure 4. Surface resistivity of various materials used for the control of static electricity as a function of relative humidity: (A) Corrugated cardboard, (B) ESD-cardboard, (C) ESD-plastic with moisture absorbing coating, (D) ESD cleanroom garment, (E) Flame retardant safety fabric, (F) Cotton.*

A study of electronic devices returned as defective after manual assembly and handling in 1998 showed that 60–90 % of such devices was damaged by electrical overstress or electrostatic discharge attributed to incorrect grounding of personnel [6]. Effective and safe grounding of standing production personnel (sometimes also worktables etc.) requires that the floor have some conductivity. Special ESD floor surfaces (ESD carpets etc.) are commonly used in cleanrooms. Also the garments, shoes etc. of the personnel should have the desired conductivity (resistance) in addition to the desired cleanroom performance. And because the problems related to static electricity are higher in dry than in normal air, humidification of the cleanroom would reduce (but not necessarily eliminate) the problems, especially in the winter time. High humidity, on the other hand, will cause other problems related to enhanced corrosion and microbial growth.

Insulating materials are very problematic from both ESA and ESD point of views. They become easily highly charged and the generated charge cannot be dissipated by grounding. Charged insulator surfaces effectively attract particles and they can be a source of direct ESD or they can charge a device giving rise to a risk of consequent CDM type of ESD. In principle, the use of insulators should be avoided in cleanrooms, but there are process stages where that is not possible. And sometimes the product manufactured in the cleanroom is inherently an insulator. Then the charge on the insulator surface should be neutralised by using ionisers. Ionisers are effective tools in static electricity and ESD management when used correctly. An effective use requires good control of ion balance and the air flow. If the ion production is unbalanced and the flow of the ionised air is not properly directed to the object, whose surface charge is to be neutralised, a false sense of security is given. An ioniser may cause little benefit and may even become a source of ESA and ESD problems.

# 6. Novel ESA and ESD control solutions for cleanrooms

More detail static electricity and ESD management related to personnel protection and automated assembly lines and present new solutions for the ESA and ESD control in cleanrooms is discussed in this chapter.

## 6.1. Cleanroom garments

Protective clothing is used in cleanroom production of electronics to prevent the products from being damaged by dust particles and ESD due to charged operators (and operator's clothing). In order to be classified as an ESD protective, the surface resistance of the garment must be in the range of $10^5\ \Omega < R < 10^{11}\ \Omega$ [2]. Cleanroom garments are the most commonly made of densely woven material in polyester filament or microfilament. Polyester is highly insulating material and, therefore, conductive threads are added into the material in order to provide a path for a charge to ground. The conductive threads are more and more frequently made by composites, that is by a mixture of conductive and insulating fibres where the conducting element is, for example, surface or core conductive carbon fibres. After use these garments are cleaned in specially equipped laundries. An example of novel cleanroom garment for electronics industry is given in Figure 5. Another class is cleanroom garments made of non-woven or disposable materials. They are limited to single use and, therefore, mainly used only by visitors. In this class of garments the desired conductivity may be obtained by using an antistatic coating.

Qualification of ESD garments used in electronics industry is nowadays based on the measurement of surface resistance of the garment. The resistive test, however, does not qualify the effectiveness of novel ESD protective garments to protect sensitive electronics from failures of electrostatic origin [3]. That is particularly true for novel cleanroom garments using core conductive threads. The resistance measurements do ignore factors which are potentially important from the ESA and ESD protection point of view, such as:

- triboelectric charging propensity of the fabric,
- the effect on performance of grounding the conductive garment elements,
- possible charge storage in insulating areas of heterogeneous materials,
- the influence of the grounded person wearing the garment on the protective performance of the garment.

Also requirements from a desired protection level are not the same in all cases. Protection of very ESD sensitive device protection in ISO class 1 or 2 environment [7] requires much more for the clothing than the protection of electronics assembly in ISO class 8. Currently that is not taken into account in the recommendations for ESD garments. There is an EU-funded research project, ESTAT-Garments [8], running where the goal is to develop proper test

method(s) and qualification criteria for novel protective clothing used in the manufacturing of electronics.

*Figure 5. A novel ESD protective cleanroom garment with shoes and a magnification of an ESD protective cleanroom garment fabric consisting of polyester with a grid of conductive threads (the magnification is not from the garment of the left picture).*

## 6.2 Automated handling equipment

The ongoing reduction of electronics device dimensions is moving the electronics assembly lines into cleaner and cleaner environment. That means, among other things, an increased challenge for static electricity and ESD control in automated handling equipment (AHE). Simple rules of ESD control [9], such as “all metallic parts must be grounded to hard ground”, “no insulating materials are allowed”, “all materials in contact with ESD sensitive devices have to be electrostatic dissipative”, cannot always be realised in complex handling equipment for various reasons. Then we have to assess risks for ESD failures by measuring the ESD-safety of the manufacturing line. If the risks for failures are

high, changes must be done into a single AHE or to the whole line. If the risks are tolerable, the operation may start or continue.

The number of potential locations of ESD (and ESA) sources or generators of electrostatic charge in an automated manufacturing line is high. That would mean a large number of measurements to be done during an ESD safety verification of AHE. The number of measurements required can be highly reduced by specifying the critical path of ESD sensitive devices [9,10]. It means that the main attention is paid to the most critical locations of an AHE and line, and those locations can be found by following the flow of ESD sensitive devices and assemblies in the line. Determining of the critical path and the functions in the path, which are important from the ESD control point of view, gives systematic for the ESD safety evaluation. There are also some process phases in the critical path which requires regularly special attention, such as conveying and device assembly. An example related to conveyors are given in Fig. 6. All these make it much easier for an equipment evaluator or auditor to find the most potential locations of ESD risks in the line, and the evaluator or auditor can concentrate on the study of these locations. The concept of critical path can be applied also to the verification of ESA (contamination) control in an AHE or line.

In automated handling equipment Machine Model (MM) and Charged Device Model (CDM) types of ESD are the most common. Accordingly, the device MM and CDM withstand voltages are the most important parameters when assessing risks for ESD failures. MM ESD can be avoided by correct grounding of all conductive equipment parts (which may not always be easy). CDM ESD can be minimised (but not avoided) by minimising triboelectric as well as induction charging of the electronic components. But perhaps more important is to provide safe dissipation or neutralisation for the charged devices by the use of electrostatic dissipative materials in contact with the electronics or by using ionisers.

It is worth to point out that an entire printed wiring board (PWB) can become easily charged, for example during conveying. A PWB can have high capacitance due to the copper layers of the board. Therefore, the discharge current and discharge energy in an ESD from a charged board may be high, leading to device failures at lower discharge voltages than what would be expected according to the withstand data of the devices. A charged PWB also

attracts particles which may lead to contact and other problems in assembly due to product contamination. A PWB charging can be minimised by proper grounding of conveyors, proper selection of conveyor belts and other materials in contact with PWB.

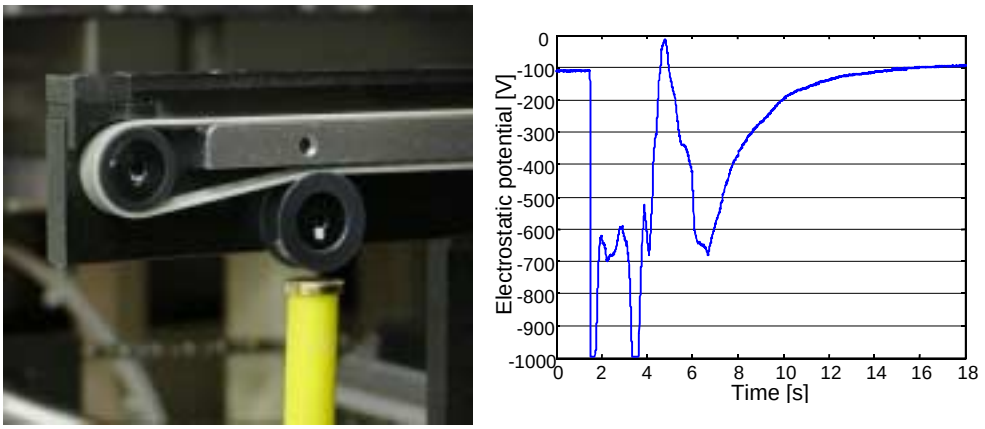



*Figure 6. Evaluation of the ESD-safety of a conveyor by the measurement of electrostatic potential (left) and an example of the potential of a running conveyor belt (right).*

## 7. Electrostatic control program

Requirements for effective and systematic control of electrostatic charge in the cleanroom production of electronics are increasing in order to minimise failures, production losses, product quality problems because of product contamination due to electrostatic attraction and damage done directly to the product by electrostatic discharge. A few companies have already established an electrostatic control program as a part of their ISO 9000/14000 quality programs in order to minimise ESA and ESD related problems. Many other companies have realised that they should have such a program as an integral part of their quality program.

Problems related to ESA or ESD appear often when implementing a new technology into a product or production equipment. It is more and more frequent that a change into new technology has resulted in serious, completely unexpected problems although product quality and failure rates with the old

technology have been in control. If the control of static electricity has been completely neglected in the past and the new technology requires effective charge control, consequences can be catastrophic. The purpose of electrostatic control program is to minimise risks related to the implementation of new product or production technology and, once in control, to maintain the desired level of ESA and ESD control in the production.

An exact content of an electrostatic control program could vary from company to company, depending on the needs. The extent and complexity of the program should be based on the existing or anticipated future static charge sensitivity of the products to be manufactured in the cleanroom. Any program should define the scope: why there is a need for static control, what areas will be included, and what are the intended results. The program must also define methods and other technical elements to be used for ESA and ESD control. Each technical element in the program should have defined limits of acceptance, installation requirements, maintenance requirements, and a periodic verification interval. Industrial standards give good support for many methods and technical elements (but, unfortunately, not for all elements). An electrostatic control program should also include procedures for auditing. According to experience, companies tend to place considerable amounts of product at risk before the failure of the electrostatic control is noted [11]. This is particularly true for products produced in cleanrooms, whose defects may not be discovered until long after the product has left the cleanroom. Finally, employee training is essential to the success of any quality control program.

# 8. Conclusions

Electronics production will happen more and more frequently in cleanroom environment. At the same time electronic devices and products are becoming more sensitive than ever to failures of electrostatic origin: product contamination due to electrostatic attraction and direct product failures due to electrostatic discharge. Effective and systematic electrostatic control program is required in order to minimise product failures and quality problems. The program should include methods, techniques and procedures for continuous management of electrostatic charge (static electricity) and ESD in the production of electronics.

# 9. Acknowledgements

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# PROTECTIVE CLOTHING AND ESD PROTECTION IN CLEANROOMS

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## Abstract

Man is considered to be the greatest generator of contaminants in a cleanroom. Today the cleanroom environment is protected from contaminants generated by personnel with the use of special clothing – known as personnel filters or the garment system. The personnel filter is used as a barrier between the personnel and the atmosphere surrounding them. The garment system for use in cleanrooms can actually be defined as both working clothes and protective clothing. Contamination control and cleanroom technology demands protection for both the personnel and the products and processes. In order to manage the movement of the personnel and garment system in the cleanroom, more research, and guidance on measuring methods and requirements are needed. The garment system used in a cleanroom must be chosen with great care in order to obtain the correct protection level.

## 1. Introduction

Cleanrooms exist because particles on critical product surfaces cause defects. Most particles are generated within the cleanroom and clean manufacturing environment by personnel, equipment and production processes. Personnel and their activities may be one of the major sources of contamination. Present-day cleanroom garment systems act as personnel filters and prevent particles passing into the room around. They can be both working clothes and protective clothing. Clean and in some cases sterile garments are required for work in working environments in order to meet the required standards of cleanliness.

ESD (ElectroStatic Discharge) damage to electronic devices is well documented. Electrostatic charge and ESD are also known to interfere with the operation of

production equipment and to increase levels of particle contamination on semiconductor wafer surfaces. Similar problems exist in other industries requiring cleanroom and clean manufacturing environments such as disk drive (recording heads and disk media), medical, optical materials and devices, commercial / military aerospace technology, thin film / coating applications and media manufacturing.

The design phase of any contamination control program to prevent contamination from reducing product quality or yield must take into consideration the effects of contamination from all sources. Specification and use of an appropriate qowing system is essential to control product or process contamination.

## 2. Man as generator of contaminants

People continuously release small particles from the outer layer of the skin into the surrounding environment. This process occurs because cells from surface layers of skin are continually replaced by new cells from the layers below.The number of particles released from the outer layer of the body will increase as a result of the abrasive action of clothes and jewellery for instance. The more active and effectively personnel is working the more numerous particles will be released.

*Table 1. Contaminants generated from a body.*

| Activity | Number of particles generated ($\geq$ 0.5 μm / min) |
|---|---|
| Sitting or standing still | 100 000 |
| Sitting, small movement of arms or head | 500 000 |
| Sitting, moving arms, legs or head | 1 000 000 |
| Standing up | 2 500 000 |
| Walking slowely | 5 000 000 |
| Walking normally | 7 500 000 |
| Walking with speed (2.5 m/s) | 10 000 000 |
| Performing work-out | 15 000 000 – 30 000 000 |

## 3. Types of contamination from personnel

Basic processes to control and manage contaminants from personnel are: containment, filtration and retention. Various types of contamination releases and generated by personnel are:

- personnel and their personal clothing
- garment system
- garment system accessories
- contamination that bypasses or go through filtration
- contamination released from or generated as the result of interaction between any of the above types of contamination and the surroundings.

## 4. Cleanroom garment system

The uniform garment system or pieces of garment should effectively cover the operator with a filter material to reduce the possibilities of contaminants leaving the body, being dispersed in the air and later coming into contact with critical parts of the process or the product. Garment system comprise usually a combination of the following parts:

- Trousers, jacket or coat
- Coverall
- Cap and cap with integrated hair protection, hair protection covering the entire head
- Face mask and beard protection
- Eye protection
- Helmet that allows no particles from the face to be dispersed into the air
- Hood that covers the entire head
- Gloves
- Specially designed underwear
- Shoes, shoe coverings, long legged boots.

# 5. Characteristics and parametres of cleanroom clothing

Characteristics and accessories of garments may influence on the usefulness of a clothing system in certain clean areas or clean zones. Such characteristics include:

- design and construction
- cleanliness
- biological properties
- electrostatic properties
- durability
- comfort.

# 6. General design and construction items of cleanroom clothing

The design of a clothing system should take into account which cleanliness control standards have been specified. Sewing thread should be of the man-made continuous multifilament types and be compatible with the fabric. Stitching and seam construction should comply with general requirements or current bonded seaming methods, provided that seams are not more permeable than the surrounding fabric. All cut edges should be pre-serged, burred or sealed.

Cleanroom garments should be free from pockets, belts, pleats, folded or trough cuffs, logos and pen tabs. Hook-and-pile fasteners and zippers that do not provide a permanent closure are not recommended.

Coveralls should be closed by a full length oneslider to the base of the neck opening. Coverall sleeves should be designed with either adjustable snap closures at the wrists, elastic in the wrist hem or knit cuffs. The ankle closure should also consist of either adjustable snaps or elastic in the hem. The coverall leg should fit comfortably into the legging of the cleanroom boot. The frock should in length at least meet the knees of each wearer. The cap should be designed to cover the forehead and be adjustable by a tie string or an elastic band. Hoods should fully cover the head in order to contain all hair and

particulate, and be adjustable and conform with wearer´s head motions. In addition a light weight hair cover can be worn separately or under hood to cantain loose hairs.

## 7. Electrostatic charge generation in cleanrooms

The nature of operations in the cleanrooms encourages the generation of static charge. The movement of personnel is often a major cause of electrostatic discharge. Cleanrooms, cleanliness, mechanical, electrical and chemical process requirements require many kinds of insulating materials, such as glass, Teflon and other polymer products. Personnel are encloused in cleanroom garments, boots, gloves and other protective devices. Insulating surfaces generate high charges, furthermore in motion larger amounts. Wrist straps, conductive shoes and floors, dissipative or conductive constructions and furniture materials can prevent or dissipate static charge if the path to ground is existed.

It is recommended to use in cleanrooms conductive or static dissipative materials as much as possible but catering for contamination control. A conductive path should be provided between static dissipative garment and the ground to complete system. The most important is that cleanroom materials have to pass all the facility requirements for contamination control. When insulators cannot be eliminated or isolated from static sensitive products, the most common methods of static control are high humidity chemical coatings and air ionisation.

## 8. Conductive cleanroom garment

Cleanroom garments are used to completely enclose personnel to prevent personnel generated particles from entering the cleanroom work area. The materials used in these cleanroom outer garments, including shoes, boots, gloves and other protective devices, are often insulating materials capable of generating high levels of static charge when they contact other garment materials worn by personnel. Charge is generated on both the inside and outside of the garments as well as on personnel wearing the garments.

Static dissipative materials are generally created by adding conductive materials or chemicals to insulative polymers and elastomers. These may be carbon or

metallic fibres, powders, chemicals that form on the surface a conductive path or hygroscopic chemicals that attract water to the surface of a material. In all of these cases the method of creating the static dissipative property may be a potential cleanroom contaminant. Continuous cleaning of cleanroom materials, particularly the washing of garments, may cause them to lose their static control properties over time. All cleanroom materials need to be monitored periodically for effectiveness and replaced as needed.

Static dissipative garment fabrics have to demonstrate cleanroom compatibility. Fabrics are not allowed to loose carbon or metallic particles or fibres when flexed or abraded. The loosed particles of polymers tend to be a cleanroom contaminant, although they are generally chemically inert. In this case the particle size is the principal concern. The carbon or metallic additives used to create conductive and static dissipative materials are chemically active, so that particles of any size loosed from these materials may be a contamination issue.

When protecting ESD-susceptible devices from dammage or destruction, it is recommended for static dissipative garment that the observed initial triboelectric potential should not exceed 100 volts at 50% relative humidity and a temperature of 20 ˚C.

# 9. Recommended practices for clean areas and cleanroom clothing

## 9.1 GMP

EC Good manufacturing practices of medicinal products for human and veterinary use covers garment systems with regard to different hygiene classifications.

Manufacturing operations are divided into two categories; firstly those where the product is terminally sterilised and secondly those which are conducted aseptically at some or all stages.

The guidance gives for the maximum permitted number of particles in the ”at rest” condition corresponds approximately to the ISO classifications as follows:

- A and B correspond with class ISO 5
- C corresponds with class ISO 7
- D corresponds with class ISO 8.

Masks and gloves should be changed at least at every working session. Clean area clothing should be cleaned and handled in such a way that it does not gather additional contaminants which can later be shed.

*Table 2. The airborne particulate classification of different clean areas.*

| | Number of particles/$m^3$ equal to or above | | | |
|---|---|---|---|---|
| Grade | AT REST | | IN OPERATION | |
| | 0.5 µm | 5 µm | 0.5 µm | 5 µm |
| A | 3 500 | 0 | 3 500 | 0 |
| B (a) | 3 500 | 0 | 350 000 | 2 000 |
| C (a) | 350 000 | 2 000 | 3 500 000 | 20 000 |
| D (a) | 3 500 000 | 20 000 | not defined © | not defined © |

*Table 3. Clothing required for each grade.*

| Grade A/B | Grade C | Grade D |
|---|---|---|
| • Headgear should totally enclose hair and where relevant, beard and moustache.<br>• Face mask should be worn to prevent the shedding of droplets.<br>• Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn.<br>• Trouser legs should be tucked inside the footwear and garment sleeves into the gloves.<br>• The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.<br>• Clean sterile (sterilised or adequately sanitised) protective garments should be provided at each work session or at least once a day. | • Hair and where relevant beard and moustache should be covered.<br>• A single or two piece trouser suit, gathered at the wrists and with high neck.<br>• Appropriate shoes or overshoes should be worn.<br>• Garments and shoes should shed virtually no fibres or particulate matter. | • Hair and where relevant beard and moustache should be covered.<br>• A general protective suit and appropriate shoes or overshoes should be worn.<br>• Appropriate measures should be taken to avoid any contamination coming from outside the clean area. |

*Table 4. Examples of operations for terminally sterilised products in the various grades.*

| | |
|---|---|
| **A** | Filling of products when unusually at risk. |
| **C** | Preparation of solutions when unusually at risk. Filling of products. |
| **D** | Preparation of solutions and components for subsequent filling. |

*Table 5. Examples of operations for aseptic preparations in the various grades.*

| | |
|---|---|
| **A** | Aseptic preparation and filling. |
| **C** | Preparation of solutions to be filled. |
| **D** | Handling of components after washing. |

Where aseptic operations are performed monitoring should be frequent using methods such as settle plates, volumetric air and surface sampling. Surfaces and personnel should be monitored after critical operations.

*Table 6. Recommended limits for microbiological monitoring of clean areas during operation.*

| Grade | Air sample cfu / $m^3$ | Settle plates (Ø 90 mm) cfu / 4 hours | Contact plates (Ø 55 mm) cfu / plate | Glove print 5 fingers cfu / glove |
|---|---|---|---|---|
| **A** | < 1 | < 1 | < 1 | < 1 |
| **B** | 10 | 5 | 5 | 5 |
| **C** | 100 | 50 | 25 | - |
| **D** | 200 | 100 | 50 | - |

## 9.2 IES recommended practice

Recommended practice IES-RP-CC003.2 gives recommended garment configurations by cleanroom types.

*Table 7. Recommendations for cleanroom garments according IEST-RP-CC-003.2 for classes US Fed St 100 000, 10 000 and 1 000.*

| US Fed St | 100 000 | 100 000 | 10 000 | 10 000 | 1 000 | 1 000 |
|---|---|---|---|---|---|---|
| Cleanroom type | Normal cleanroom | Mixed | Normal cleanroom | Aseptic | Normal cleanroom | Aseptic |
| Frock | **R** | **R** | **R** | NR | AS | NR |
| Two piece suit | AS | AS | AS | NR | AS | NR |
| Coverall | AS | AS | AS | R | R | R |
| Shoe cover | **R** | **R** | **R** | NR | AS | NR |
| Boots | AS | AS | AS | **R** | **R** | **R** |
| Special footwear | AS | AS | AS | AS | AS | AS |
| Hair cover | **R** | **R** | **R** | **R** | **R** | **R** |
| Hood | AS | AS | AS | AS | AS | R |
| Facial mask | AS | AS | AS | R ** | AS | R** |
| Powered headgear | AS | AS | AS | AS | AS | AS |
| Woven gloves | AS | AS | AS | NR | AS | NR |
| Barrier gloves | AS | AS | AS | **R** | AS | **R** |
| Inner suite | AS | AS | AS | AS | AS | AS |
| Changes | 2 / week | 2 / week | 2 / week | Every entry | 2–3 / week | Every entry |

R recommended
NR not recommended
AS application specific
** Surgical mask recommended.

*Table 8. Recommendations for cleanroom garments according IEST-RP-CC-003.2 for classes US Fed St 100, 10 and 1.*

| US Fed St | 100 | 100 | 10 | 10 | 1 | 1 |
|---|---|---|---|---|---|---|
| Cleanroom type | Normal cleanroom | Aseptic | Normal cleanroom | Aseptic | Normal cleanroom | Aseptic |
| Frock | AS | NR | NR | NR | NR | NR |
| Two piece suit | AS | NR | AS | NR | AS | NR |
| Coverall | **R** | **R** | **R** | **R** | **R** | **R** |
| Shoe cover | AS | NR | NR | NR | NR | NR |
| Boots | **R** | **R** | **R** | **R** | **R** | **R** |
| Special footwear | AS | AS | AS | AS | AS | AS |
| Hair cover | **R** | **R** | **R** | **R** | **R** | **R** |
| Hood | **R** | **R** | **R** | **R** | **R** | **R** |
| Facial mask | **R** | **R**** | **R** | **R**** | **R** | **R**** |
| Powered headgear | AS | AS | AS | AS | AS | AS |
| Woven gloves | AS | NR | NR | NR | NR | NR |
| Barrier gloves | AS | **R** | **R** | **R** | **R** | **R** |
| Inner suite | AS | AS | **R** | **R** | **R** | **R** |
| Changes | 1 / day | Every entry | Every entry – 2 / day | Every entry | Every entry | Every entry |

R recommended
NR not recommended
AS application specific
** Surgical mask recommended.

# 10. Conclusions

Contamination control is becoming more and more important in industrial areas where there are increasing requirements for both cleanliness and hygiene in production. Contamination control and cleanroom technology demands protection for both the personnel and the products and processes. In order to manage the movement of the personnel and garment system in the cleanroom, more research, and guidance on measuring methods and requirements are needed.

# 11. Acknowledgements

I would like to thank my colleages at VTT Industrial Systems for their contribution to the work.

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# AIR QUALITY IN SEMICONDUCTOR CLEANROOMS

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# INTERACTION BETWEEN AIR MOVEMENTS AND THE DISPERSION OF CONTAMINANTS: CLEAN ZONES WITH UNIDIRECTIONAL AIR FLOW

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*RESEARCH ARTICLE*

# Interaction Between Air Movements and the Dispersion of Contaminants: Clean Zones with Unidirectional Air Flow

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*ABSTRACT: The purpose of this presentation is to describe the theoretical relations for the dispersal of airborne contaminants and to illustrate the validity of these equations occuring during factual situations, where a number of observations on air movements in open unidirectional air flow units supplied with HEPA-filters are described. In factual situations the aerodynamic system which governs the dispersion of contaminants in reality is always very complicated that risk situations must be mapped and assessed empirically. The presence of a person can give risk to wakes that may be stable or unstable. The unstable situations are in most cases caused by the influence of arms and hands. As part of the microbiological assessment of aseptic processes carried out in clean zones, it is important to investigate that such vortices do not occur in the clean working areas.*

*As the level of airborne contaminants in the operational environment may have an effect on the level of product contamination, the microbiological assessment of aseptic processes is important.*

*A system is described for microbiological assessment in unidirectional air flow units by using visual illustrative methods and particle challenge tests (measured by particle counter) for the dispersion and/or induction of particles.*

## Introduction

The air may move in two different ways. One of these is characterized by a smooth flow, free of any disturbances such as, small and temporary vortices, eddies. This is known as laminar flow. The other type of flow is characterized by small and temporary fluctuations caused by instabilities. The flow velocity is no longer constant but more or less fluctuating around an average value. This is known as turbulent flow and the disturbances are often interpreted as being small, temporary eddies.

In order to estimate the problems associated with the transport of contaminants by air, we must understand how this occurs. We must assume that, with the traditional ventilation processes and the rules we apply, the air in the rooms is more or less turbulent.

The aim is to arrange ventilation in such a way that there is a certain basic flow of air. An organized basic flow implies that the flow can be characterized by means of stream lines, i.e., the paths taken by weightless particles in the room as they follow the air stream, if the turbulent fluctuations are ignored. The transport of contaminants due to streamline flow is often described as "convective transport."

The simpliest system for an analysis of the transport of contaminants by ventilation is, therefore, convective transport along the streamlines. The disturbances caused by turbulence, the turbulent diffusion, are superimposed by this. Obviously if there is no turbulence, turbulent diffusion is replaced by molecular diffusion or Brownian motion.

It can generally be assumed that the settling velocity of contaminants is negligible, which implies that gravitation plays an inferior role.

With the assumption of a constant value of the diffusion coefficient the diffusion equation in a velocity field in rectangular coordinates becomes:

$$\frac{\partial c}{\partial t} + v_x \frac{\partial c}{\partial x} + v_y \frac{\partial c}{\partial y} + v_z \frac{\partial c}{\partial z} = D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}\right) \quad (1)$$

where

$c$ = concentration
$v_x, v_y, v_z$ = velocities in $x$-, $y$- and $z$-direction
$D$ = diffusion coefficient

This gives the simpliest possible mathematical model which describes a system with regard to transport of contaminants emitted in a source of an arbitary position.

## Unidirectional Air Flow

Dispersion from a fixed source in a uniform parallel flow is described theoretically and experimentally, inter alia, by Bird et al. (1), Fuchs (2), Hinze (3) and Ljungqvist (4). For a continous point of source in a parallel flow with constant velocity, $v_o$, in the $x$-direction, the solution of the simplified diffusion equation in the

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velocity field mentioned above becomes:

$$c = \frac{\dot{q}}{4\pi D r} e^{-\frac{v_o}{2D}(r-x)} \simeq \frac{\dot{q}}{4\pi D x_1} e^{-\frac{v_o y_1^2}{4D x_1}} \qquad (2)$$

where

$\dot{q}$ = outward flow at the point source
$v_o$ = constant velocity in the $x$-direction
$x_1$ = least distance in the $x$-direction to the point source ($=/x/$)
$y_1$ = perpendicular distance from the streamline which passes through the source $y_1 = (y^2 + z^2)^{1/2}$
$r = (x^2 + y_1^2)^{1/2} = (x^2 + y^2 + z^2)^{1/2}$

The case of parallel air flow has been investigated experimentally by Ljungqvist (4) for a room with a cross section of 1.7 × 1.7 m². It was found that, with a turbulence-free inlet, there was a complete absence of eddies for air velocities up to approx 0.3 m/s.

If the inlet opening of a room was equipped with a turbulence generating grid, the degree of turbulence obtained at even relatively low air velocities (0.2 m/s), was such that the diffusion, due to the turbulence, determined the development of dispersal for both gases and particles.

Values of the turbulent diffusion coefficient were determined by Ljungqvist (4) with the aid of Equation (2) from concentration curves measured at air velocities of 0.20 m/s and 0.45 m/s. The diffusion coefficients obtained for the two velocities were approximately 1.4 cm²/s and 2.4 cm²/s, respectively.

Ljungqvist, Reinmüller (5) have estimated the "critical contamination region" in undisturbed and disturbed parallel flow fields with dispersion from a fixed contamination source. A qualitative solution in an undisturbed parallel flow is given in Figure 1, when the velocity is 45 cm/s and the distance between the working surface and the point source is 30 cm.

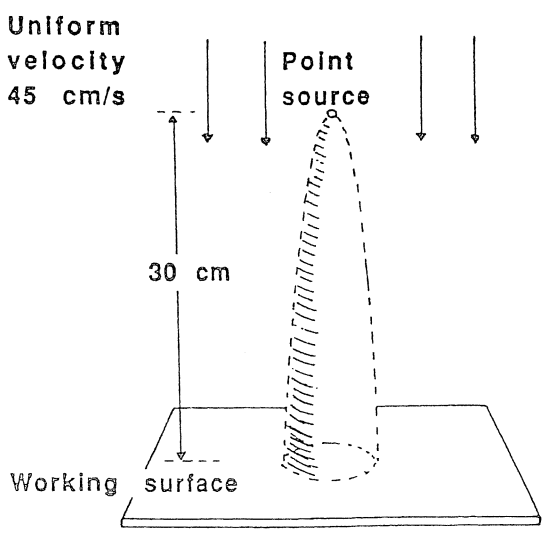


Figure 1—Critical contamination region in a uniform parallel flow field. Qualitative solution of diffusion equation in a velocity field with the velocity 45 cm/s, diffusion coefficient 2.4 cm²/s and the distance between the working surface and the contamination source 30 cm.

## Vortex

*General*

A vortex is characterized by the fact that the stream lines are closed within a region which in the following is referred to as the vortex region. According to the laws of aerodynamics, tangential velocity in the vortex region should increase as the center of the vortex is approached. However, systematic investigations by Ljungqvist (4) show that this is not always the case in vortices formed in ventilated rooms. Everything indicates that the air mass within the vortex region moves as a rigid body under the influence of powerful turbulence. A certain amount of energy is, therefore, needed to maintain a vortex, and in most cases this energy is obtained from the kinetic energy of the air on its entry into the room. The greater the kinetic energy of the air in the room, the greater are the chances of vortices occuring with closed streamlines.

Owing to the fact that the streamlines are closed, there is no convective removal of contaminants emitted within the vortex region. It is only turbulent diffusion within the vortex that causes removal of the contaminants. In a room where contaminants are emitted within a vortex region, the average concentration of contaminants inside the vortex region may, for instance, be ten times as high as in the air extracted by ventilation. Transport due to turbulent diffusion does not increase in proportion to the vortex velocity but decrease at a slower rate.

If, therefore, for a certain ventilation rate of flow we have a vortex region within which a contaminant is emitted and we double this rate of flow, the concentration of the contaminant in the vortex region is not halved but drops to somewhere between the original value and half this value.

*Mathematical Treatment*

The mathematical treatment can be formulated by the problem to solve the diffusion equation for a gas which rotates as a rigid body as there is a continual release of the diffusing substance at a source situated at a certain distance from the axis of rotation.

If the rotation takes place about the $z$-axis with the angular velocity $\omega$ and that the source is located at the point (a, 0, 0) with the outward flow $\dot{q}$, the solution is given by Ljungqvist (4) in cylindric coordinates ($x = \rho \cos\varphi, y = \rho \sin\varphi, z$) the expression of the concentration becomes:

$$c = \frac{\dot{q}}{8 \cdot \pi^{3/2}} \int_0^\infty e^{-(\rho^2 + a^2 - 2a\rho\cos(\varphi - \omega t) + z^2)\frac{1}{4Dt}} \frac{dt}{(Dt)^{3/2}} \qquad (3)$$

where

a = distance between the point source and the centre of vortex (origin)
$\omega$ = angular velocity

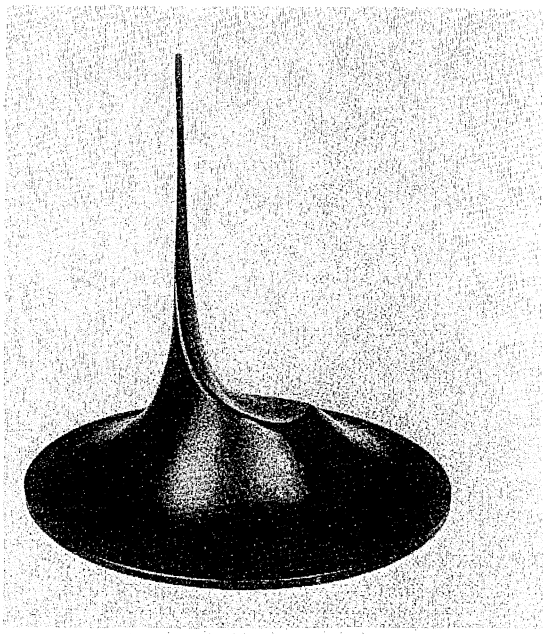

Figure 2—Model of the non-dimensional factor $F$

It is natural that a certain preference should be given to the $x$, $y$-plane, as it is in this plane that the source of emission is located.

With $z = 0$ and appropriate substitutions† in equation (3) the expression of the concentration can be interpreted as the product of the concentration at the origin and a non-dimensional factor. The solution of equation (3) for this case gives the expression

$$c = \frac{\dot{q}}{4\pi D a} \cdot F \qquad (4)$$

where

$F$ = non-dimensional factor

The factor $F$ is, apart from the positional coordinates, a function only of the angular velocity when the diffusion coefficient is constant, i.e., the turbulence is isotrop. For a certain angular velocity the factor $F$ can be plotted as the vertical coordinate perpendicular to the $x$, $y$-plane. ($\rho$, $\varphi$ -plane). In this way a surface is obtained, the general appearance of which is shown in Figure 2.

Figure 2 shows that the mean value of the concentration over the center region beside the point of emmission, is considerably higher than that outside. The above, therefore, allows us to use the concept of contamination accumulation in the context of vortices, especially as the diffusion coefficient does not increase linearly with the air change rate. In this way it has also been shown that concentration of a contamination in the extract air cannot be used for assessment of the hazards in the system dealt with here.

## Wakes, Factual Situations

Stable vortices, with their need for energy demanding turbulence, are unusual. When they do occur, it is mostly in the form of wakes, which are set up behind obstacles in a high energy, more or less parallel, air flow. Vortices are generally unstable, i.e., they have limited duration. Such vortices are often periodic, i.e., they are formed, decay, die out, and are formed again, and so on. The frequency need not be uniform but may vary. This is obviously the case when it is the movements of a person that gives rise to a vortex.

With visual illustative tests (Ljungqvist (4, 6)) where the emitted contaminants are replaced by isothermal smoke and the dispersion is recorded by means of stills and films, it has been shown that the presence of a person can give rise to wakes that may be stable or unstable.

The unstable situation is in most cases caused by the influence of arms and hands. Figure 3 shows a parallel flow field in which a dummy is placed to the side of a tube from which smoke is continuously released. It is obvious from the photograph that the dummy does not disturb the dispersion of the smoke. However, as soon as the hand of the dummy is raised, there is a dramatic change in the dispersion configuration, as can be seen from Figure 4.

It must be emphasized that this situation is extremely

Figure 3—Dummy placed at the side of the point of smoke emission in a parallel flow field. Hand in the lower position.

† with the substitutions

$$\Omega = \frac{a^2 \omega}{4D} \text{ and } \xi = \frac{\rho}{a}$$

the factor $F$ becomes

$$F = \frac{1}{\sqrt{\pi}} \int_0^{\infty} e^{-(1+\xi^2-2\xi\cos(\varphi-\Omega\tau))/\tau} \frac{d\tau}{\tau^{3/2}}$$

Total derivation, see Ljungqvist (5)

instable. If, however, a person is placed in front of the point of release of the smoke, a stable wake region is formed. This is shown in Figure 5. We can see that the smoke follows the contour of the test person's body and reaches his respiration zone. It is evident from these photographs that the release of contaminants in the lee of a person in the vortex region results in contaminants being transported to the breathing zone.

Kim and Flynn (7) describe experimental results, using hot-film anemometry and flow visualization techniques, and suggests that air flow around a person immersed in uniform freestream, has a three-dimensional nature. Above the chest level, the down-wash effect is important, from chest to elbows a combination of the down-wash and vortex shedding exists, and from waist to hip the vortex shedding appears dominant. In the region subject to the vortex shedding, each vortex is shed downstream at a dimensionless frequency (Strouhal number) of 0.19. The average area of the vortex is 0.7 times the area of a circle with a diameter equal to the width of the mannequin.

A coherent vertical flow is present in the proximity of the body; the upper section (above hip level) where the mean flow is directed upwards, and the lower section where the mean flow is directed downwards. Additionally, the end of the reverse flow zone reaches at least two widths downstream of the worker and implies that a hand held contaminant source cannot escape the influence of the recirculating flow. Figures 6 and 7 show the region of reverse flow and the air flow structure downstream of the mannequin.

The results by Kim and Flynn (7) threw new light on the airflow around a person in a unidirectional air flow. The difference between the smoke configuration in Figure 5 and the region of reverse flow in Figure 6 might be explained in terms of convective flow.

Figure 4—Dummy placed at the side of the point of smoke emission in a parallel flow field. Hand in the upper position.

Figure 5—Test person placed in front of the point of smoke emission in a parallel flow field.

According to Clark and Edholm (8) the maximum air velocities found over the face on a person in standing position are 0.3–0.5 m/s compared with some 0.05 m/s when lying. For a standing person the flow over the face is that which has been developing in velocity and thickness over the entire height of the body. In contrast, when a person is lying down the flows are generally slower and thinner than when standing. Sitting position produces flows which are intermediate between standing and lying.

## Open Unidirectional Air Flow Benches

*Air Movements in Empty Benches/Units*

Within pharmaceutical production, aseptic sterile processes are required to be carried out in a class 100 environment (Fed Std 209). To avoid particle contamination in critical process area (clean air zones), unidirectional air flow with HEPA-filtered air is used. The aim is to have sterile, particle free air flow over the open drug product and potentially emitted particles swept away from the exposed drug product.

In vertical unidirectional air flow benches the vertical sidewalls in front of the operator are usually entirely or partly open. When the other sidewalls are covering down to the working surface in the bench/unit a stagnation

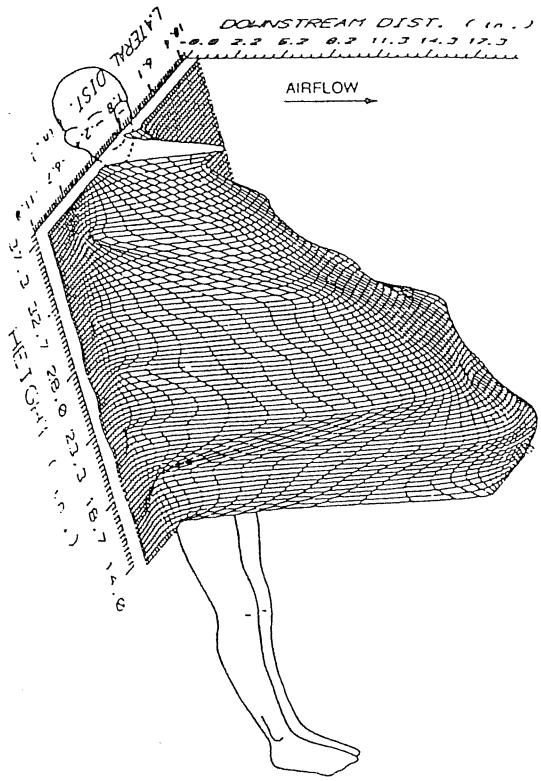


Figure 6—Region of reverse flow averaged for three different freestream velocities (from Kim, Flynn (7)).

flow with stationary vortices is usually created as shown in Figure 8.

This vortex region can be demonstrated by using smoke photography technique, see Figure 9, and in this case when the flow can be considered as two-dimensional, it can easily be predicted by using modeling software for PC see, e.g., Busnaina et al. (9, 10).

To avoid such vortices the sidewalls have to be designed with openings. At a long bench designed with equally large openings on longitudinal and opposite wall, the flow can be considered as two dimensional. If it is further assumed that the flow may be regarded as turbulence free, an exact solution of Navier-Stokes equations is possible to find. The solution was first given in a thesis by Hiemenz (11) in 1911.

This plane flow leads to a stagnation point in the middle of the bench on the working surface. Furthermore, it is worth noting that the boundary-layer is proportional to the square root of the kinematic viscosity, and that the thickness of the boundary layer does not vary along the working surface.

In reality, a stagnation region often arises where the height and appearance can vary as shown in Figure 10.

In the unidirectional air flow of an open bench a vortex street is easily created behind small obstacles. Such an obstacle can be a small lamp or a fixture connecting HEPA-filters.

Ljungqvist et al. (12) have with help of isotherm smoke, visualized the air movements behind such a horizontal 30 mm wide fixture at an air velocity of 0.45 m/s. The observed flow pattern is schematically shown in Figure 11.

The flow pattern in Figure 11 has a violent turbulent region, characterized by a vortex street and two free vortices rotating in opposite directions close to the working surface. This type of observed vortices is known as an irrotational or free vortex and sometimes as a potential vortex. This vortex is characterized by the fact that the velocity varies only with the radius and increases towards the center.

This free (irrotational) vortex which is often described in literature, differs conspicuously from the vortex earlier described (Ljungqvist (4)), where the velocity decreases linearly to the center. This vortex is known as forced or rotational vortex, and because the fluid rotates like a solid body the term "rigid body rotation" has also been used.

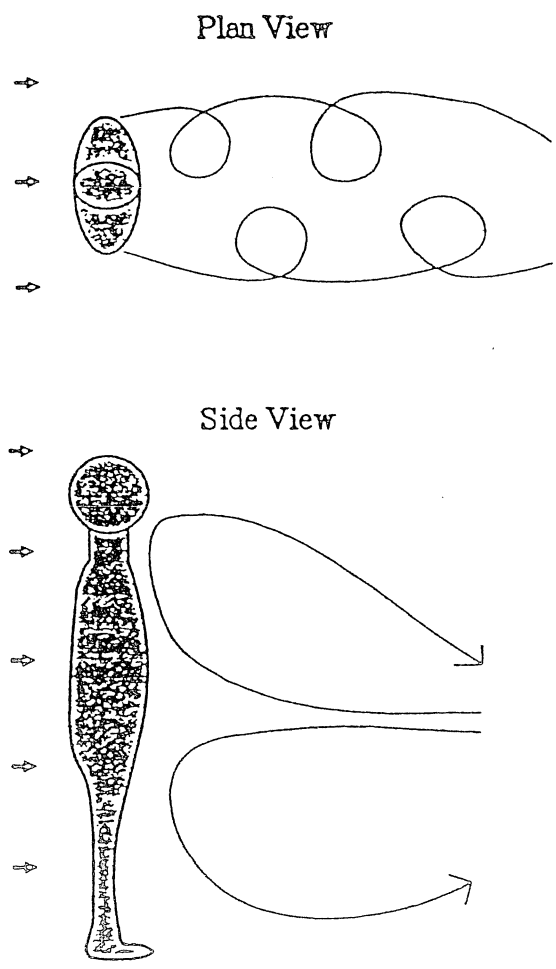


Figure 7—Airflow structure downstream of the mannequin (from Kim, Flynn (7)).

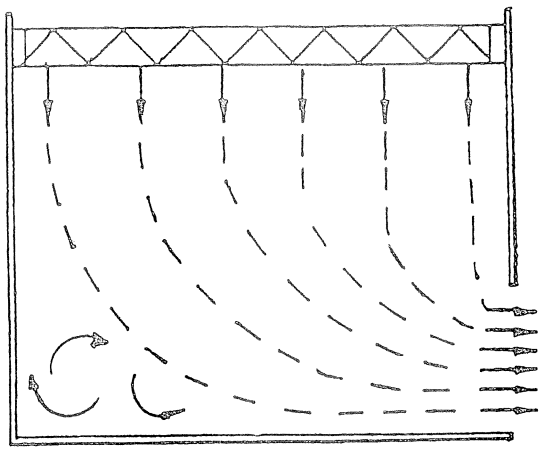

Figure 8—Vortices in a unidirectional air flow bench with covered sidewalls.

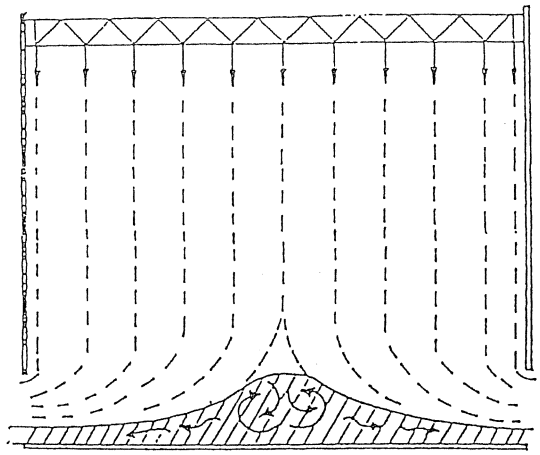

Figure 10—An example of observed stagnation region in a bench with vertical unidirectional air flow.

From the standpoint of contamination both these two types of vortex will accumulate contaminants.

*Air Movements in Benches/Units with Obstacles*

The air movements outside the vortex region in the bench shown in Figures 8 and 9 are visualized with smoke at an air velocity of 0.45 m/s in Figure 12.

If a bottle is placed in the bench a wake is easily created in the region with horizontal flow. This is visualized with smoke in Figure 13.

If the bottle is situated close to the opening of the unit, ambient air will be entrained into the clean zone in the bench. The length of the reversed region can be estimated to 2–3 times the diameter of the bottle, and can reach twice this length when the bottle is situated just beside the sidewall.

The Reynolds number, which is directly proportional to the air velocity and the size of the obstacle, is a critical quantity. According to photographs presented, inter alia by Batchelor (13) and Schlichting (14), a regular Karman vortex street in the wake of a circular cylinder is observed only in the range of Reynolds number from about 60 to 5,000. At lower Reynolds numbers the wake is laminar and at higher Reynolds numbers there is a complete turbulent mixing.

In the case shown in Figure 13 the Reynolds number is around 2,700. The situation in Figure 11, where the arrangement between the two filter modules forms a flow obstacle, Reynolds number becomes 900.

One should be cautious when comparing Reynolds number from regular Karman vortex streets with Reynolds number calculated from factual situations in clean benches, as the air flow behind an obstacle is mostly not a typically formed Karman vortex street behind an indefinitely long circular cylinder. The wake situations during actual conditions seem often to have a three-dimensional structure (compare Figures 6 and 7).

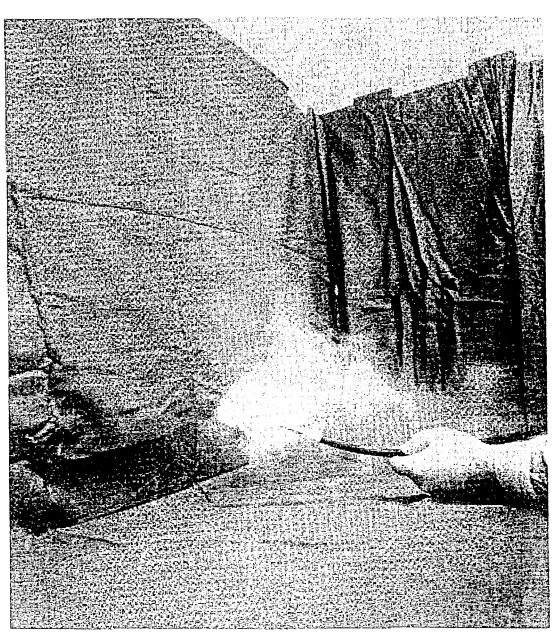

Figure 9—Vortex region in a bench with the air pattern shown in Figure 8 when air velocity is 0.45 m/s.

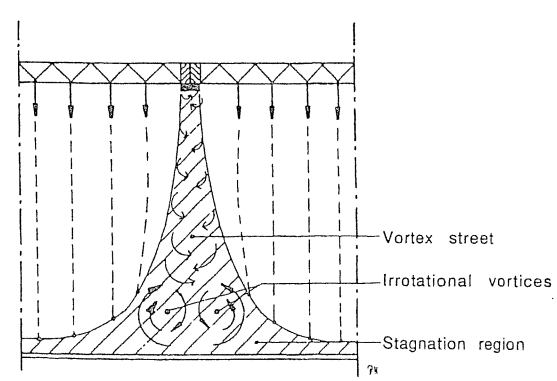


Figure 11—Observed flow pattern behind an obstacle in a vertical unidirectional air flow bench (long side).

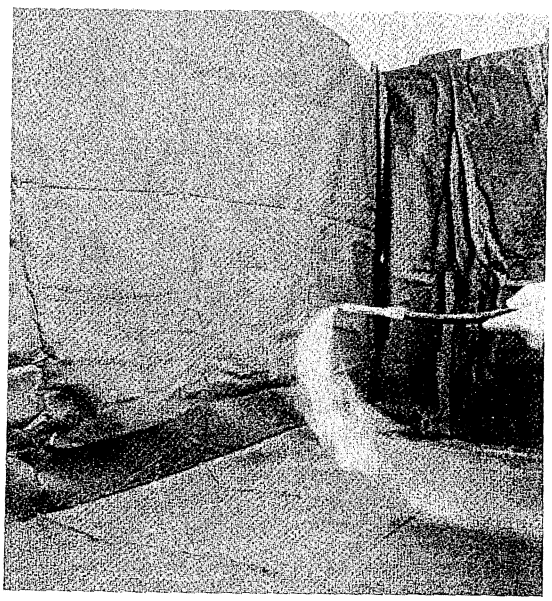

Figure 12—Undisturbed smoke dispersion in a bench shown in Figure 8 at an air velocity of 0.45 m/s.

A more complicated wake situation is shown in a production unit with a vertical flow field in Figure 14. If a hand is placed over the smoke source in Figure 12 a wake region is created in the vertical flow field as shown in Figure 15.

Essentially, personal vortices are of two kinds. The first is the relatively stable and stationary wakes created by the body. The second kind is the unstable and non-stationary vortices which arise as a consequence of the movements of the body. In this respect, it is obvious

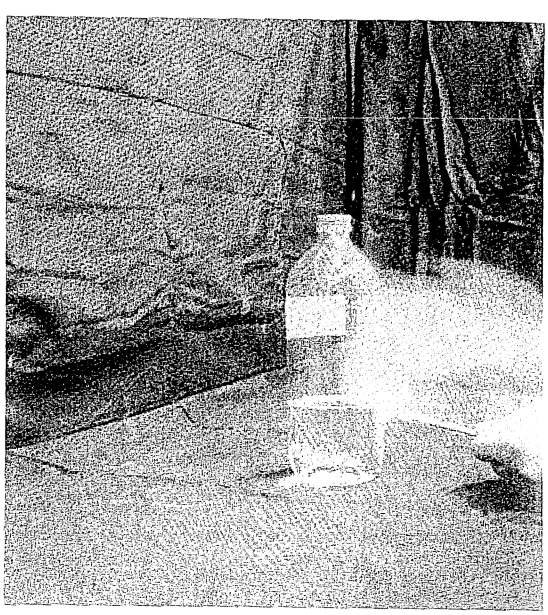

Figure 13—Dispersion of smoke behind a bottle in the bench shown in Figure 12.

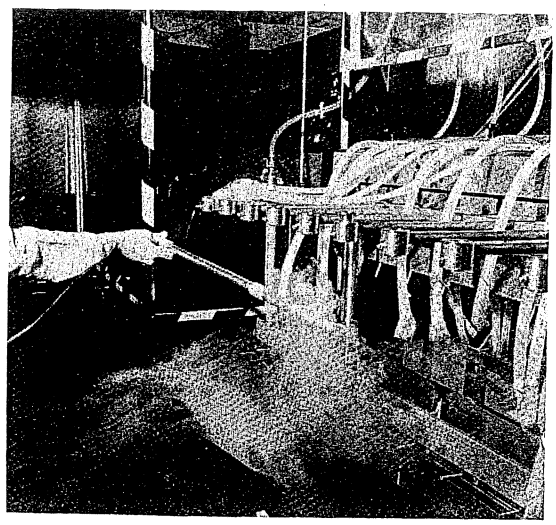

Figure 14—Dispersion of smoke in a vertical unidirectional flow bench showing a wake region behind obstacles.

that the movements of the hands and arms play a significant part. Without further comments some work situations in a clean air bench with a horizontal unidirectional air flow is demonstrated by using smoke technique, see Figures 16–18.

### RCS Air Sampler in a Unidirectional Air Flow

The Biotest Air Sampler RCS is often used for monitoring microbiological quality of air. Unidirectional air flow tests show that the dispersion region of potential contaminants around the RCS sampler is much larger than that of undisturbed parallel air flow. Tests described by Ljungqvist, Reinmüller (5) in a vertical

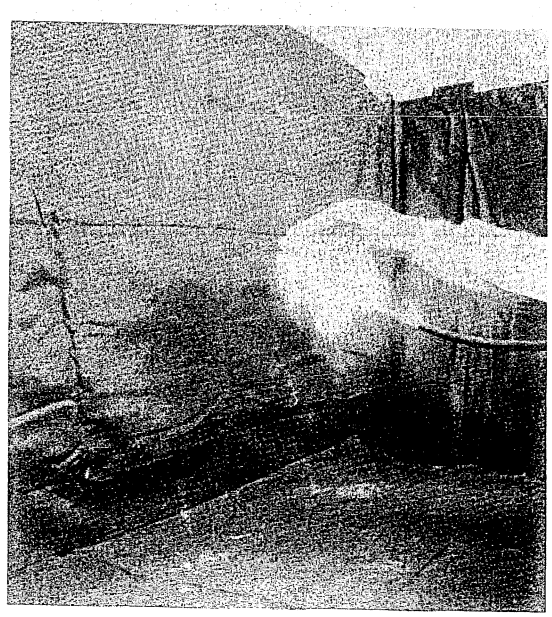

Figure 15—Dispersion of smoke behind a hand (arm) in a vertical unidirectional flow bench shown in Figure 12.

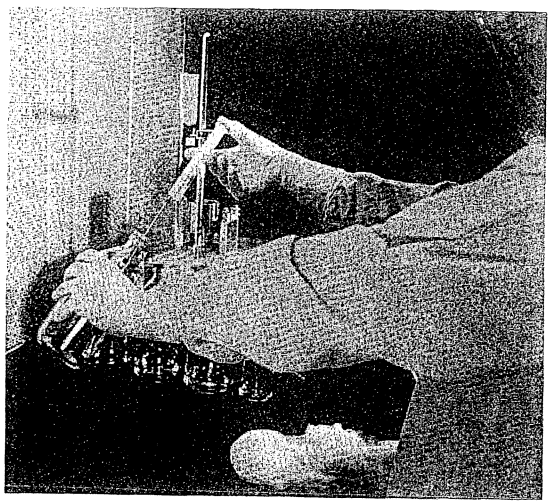

Figure 16—Dispersion of smoke in a horizontal unidirectional flow bench when operator's hands are in upper position (undisturbed smoke dispersion).

unidirectional air flow bench with an air velocity of 0.45 m/s, clearly show that the critical region (region with turbulence) around the RCS air sampler is much larger than that of undisturbed parallel air flow.

With a point source (particles) 30 cm above the working surface, an approximative calculation gives a ratio of about 20 between the contaminated area caused by the turbulence of an operating RCS, and an undisturbed parallel flow at the working surface.

Furthermore, the results indicate that when an operating RCS air sampler is located in a position close to a side wall in a clean bench with a working aperture (height 30 cm), particles from ambient air can be transported into the clean zone. Figure 19 shows such a contaminated area in a clean bench determined with the aid of a particle counter.

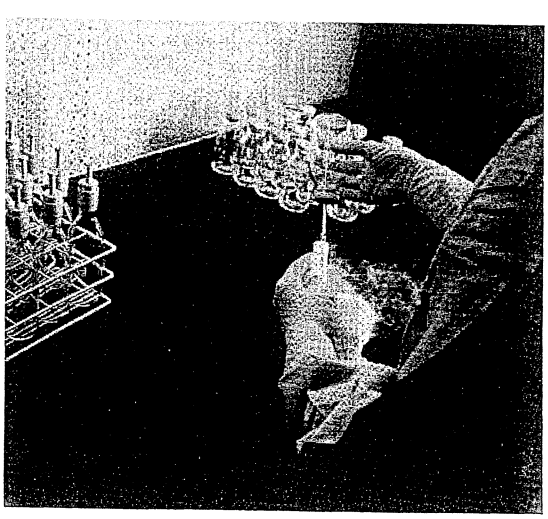

Figure 17—Dispersion of smoke in a horizontal unidirectional flow bench when operator's hands are in lower position (wake region behind hands).

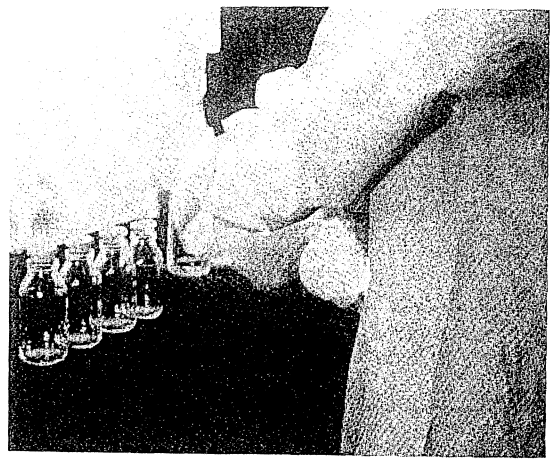

Figure 18—Dispersion of smoke in a horizontal unidirectional flow bench when operator's hands are in motion.

Due to the turbulence caused by the air sampler, the design of the side walls is important. Other disturbances, e.g., from activities in the room must also be taken into consideration. In conclusion, it must be mentioned that other types of air samplers can cause similar problems.

## Microbiological Assessment

In a clean room the microbiological burden is usually very low and it is difficult to achieve statistically significant results.

It is extremely difficult to evaluate potential microbiological hazards and too often the microbiological assessment is based on poor microbiological data and on subjective conclusions from visual observations. Because of this the assessment could be based on an unsystematic approach, where the intuition of the responsible microbiologist plays an important role.

To achieve a more objective approach to the microbiological assessment of potential hazards in clean rooms at least two ways are possible, the first by increasing the microbiological burden, and the second by increasing the particle level.

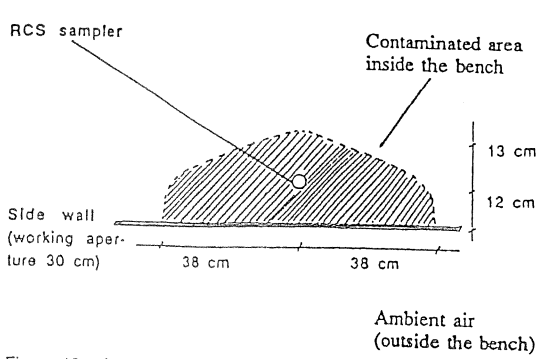


Figure 19—Area contaminated by ambient air inducted by an operating RCS air sampler placed close to front wall in a unidirectional air flow bench supplied with HEPA-filtered air (from Ljungqvist, Reinmüller (5)).

The first approach, which is direct and has been reported by Bradley et al. (15), implies a technique with a containment room where a suspension of Bacillus spores in water are blown out into the air around the tested filling equipment. The results from this investigation show that the level of airborne microorganisms in filling environment has a profound effect on the level of product contamination.

Furthermore, for the investigated filling equipment (a blow-fill-seal-machine) a direct relationship is reported between the extent of product contamination and the level of airborne microorganisms. This could allow predictions of operation conditions under which an acceptably low Sterility Assurance is attained. The disadvantage with this approach is that it cannot be used in production facilities because of the hazards of microbiological contamination on, and within, the filling machine and the increased level of microorganisms in the manufacturing area.

The second approach—"Particle Challenge Test"—is indirect and implies a technique with an increase of the particle level in ambient air around the critical region or equipment, and at the same time to measure the particle concentration in the critical region with a particle counter. The advantage with this approach is the uncomplicated, immediate registration of results. Because the critical regions only become contaminated by particles and not with microorganisms, this approach is possible to use in clean pharmaceutical production facilities.

Particle Challenge Test combined with visual illustrative methods have successfully been used by the authors for microbiological assessment in clean rooms, especially in critical regions in aseptic processing of sterile drug products.

The well known fact that the airborne microbiological contaminants are transported on particles means that the same relations in airborne dispersion are valid between particles and microorganisms. This means, that if microbiological contaminants are emitted in the region of a vortex, an accumulation can occur.

Knowledge concerning the interaction of air movements and the dispersion of contaminants plays a critical role in the microbiological assessment of clean zones with unidirectional air flow. Here the smoke photography technique gives valuable information and, combined with the results from the Particle Challenge Test, gives a fast and reliable picture of the potential contamination hazards. By defining the ratio between particle concentration in the critical region and particle concentration in ambient air, a Risk Factor is given. The possibility of entrainment of ambient air into critical areas or emission of particles (from, e.g., operators activity) with the potential accumulation can be visualized, measured and evaluated.

The use of the method should be in two steps. The first step is to visualize the main air movements and critical vortex regions by using the smoke technique. The second step is to place the probe of the particle counter in the critical area and during measurements generate particles in the ambient air, e.g., by using Air Current Test Tubes. These measurements should be performed during activity with operating equipment and personnel interferring with critical movements necessary for the process. This simulation should to some extent exaggerate the human interference during the measuring periods.

By defining the ratio between particle concentration in the critical region and particle concentration in ambient air, a Risk Factor is given. From experience, when the Risk Factor is less than $10^{-4}$ (0.01%) there should be no microbiological contamination in the process. If the Risk Factor is higher, remedial and preventive actions should to be taken, e.g., change of equipment design, change of working procedures etc, before new confirming tests are carried out.

Several different, independent complicated aseptic processes with HEPA-filtered unidirectional air (class 100) in the critical region and HEPA-filtered air in the ambient clean room with ordinary mixing ventilation have been investigated by estimating Risk Factors. The experience from these cases is that where the Risk Factor has been less than 0.01%, the final validation with media fills has shown acceptable results.

This systematic approach with Visualization of Air Movements, Particle Challenge Tests and estimating of the Risk Factor presents a method to evaluate the potential hazards on human interference in a critical zone, e.g., an aseptic filling line. It also gives valuable information about potential weak links in the process chain. The desired flow of particle free air over the exposed drug product and its components can be visualized and documented by photography or video techniques.

## Discussion

When open unidirectional air flow benches/units are being used in production sensitive to contamination, a thorough function check should with equipment in place always be carried out before start of production. Studies of air movements with visual illustrative methods give here both quick and valuable information.

In order to reduce the influence by unfavorable stagnation regions and vortex structures with its hazards for accumulation of contaminants, tests should be carried out during the design of the side walls. In connection with these tests, particle challenge tests should also be performed. Here, particles (smoke) outside the bench and a probe of a particle counter placed inside the bench for measuring the particle concentration in the critical regions and estimation of the risk factor, can give valuable information.

Investigations should also be carried out with operators during activity according to standardized movements necessary for the process.

As an example, it should be mentioned that for the production case described in Figure 11, the region that was sensitive to contamination had a critical level 10 cm above the working surface. This means, when the irrotational vortices reached about 20 cm above working

surface, that the production sensitive to contamination was in the vortex region. By studying the vortex structure and the stagnation region at different heights of the side walls and by designing the fixture connecting the two HEPA-filters more aerodynamic, it was possible to place the stagnation region below the critical height of 10 cm and in this way ensure a safe production.

In understanding the Microbiological Assessment, knowledge regarding the interaction between air movements and the dispersion of contaminants, plays a vital role. This knowledge is also important when active air sampling in critical regions is performed during production.

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# RISK ASSESSMENT WITH THE LR-METHOD

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# Risk assessment with the LR-Method

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**It is possible to identify and evaluate potential risks in the form of airborne contamination using the Method for Limitation of Risks (LR Method). This method presents a fast and reliable way of evaluating microbial safety and is a useful tool in risk assessment, e.g. HACCP. The concepts of a) Visualisation of Air Movements, b) the Particle Challenge Test and c) Calculation of Risk Factor, add up to an effective way of identifying potential hazards. It can be used for tracing dispersion routes of airborne contamination, and for the evaluation of single steps of the process.**

## Risk assessment

Commonly accepted risk assessment methods such as HACCP (Hazard Analysis Critical Control Point) can be applied to the assessment of airborne contamination of products in cleanrooms and clean zones.

In order to assess and control the microbiological hazards properly, a risk assessment system should address the following principles:

- Identification of all potential hazards associated with the process, product or individuals.
- Assessment of the risk of occurrence of a hazard and identification of preventative measures for its control.
- Designation of risk zones and, in each zone, determination of the control points, procedures, operational steps, and environmental conditions that can be controlled to eliminate a hazard or minimise its risk of occurrence.
- Establishment of the contamination control limits.
- Establishment of scheduled testing or observation to monitor the control system.
- Establishment of corrective actions to be taken when monitoring indicates that a particular procedure/ operational step/environmental condition is not under control.
- Establishment of procedures to verify that the system is working effectively.
- Establishment and maintenance of appropriate documentation.

The key to this risk assessment is to understand the processes, in this case the cleanroom performance, as well as the manufacturing process and its vulnerability to airborne contamination.

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Within a cleanroom, people are the predominant source of airborne microbial contaminants. Potential risk situations created by interaction between people, air movements, and airborne contaminants are difficult to predict and to evaluate.

In unidirectional air flow, wakes and vortex streets are easily created behind obstacles, causing regions of turbulence. In front of machinery and working surfaces situated perpendicular to the main direction of flow, unidirectional air flow often leads to stagnation regions. The air movements in these cases are mostly irregular and difficult to predict. Contaminants emitted in such areas might accumulate or disperse in an unpredictable way. Factual situations are complex and must be mapped and assessed empirically. The presence of people can cause unstable wakes. In most cases, these unstable situations are caused by the movements of arms and hands. Unstable situations within the clean zone can cause ambient (less clean) air to be entrained into the zone. Contaminants can also be dispersed from moving arms and hands.

Whyte (1986) presented a model for predicting the product contamination from the concentration of airborne bacteria[1]. Bradley *et al.* (1991) used a microbiological challenge test to show that the level of airborne micro-organisms in the filling environment has a profound effect on the level of product contamination[2]. A direct relationship was reported between the extent of product contamination and the concentration of airborne micro-organisms.

Using common microbiological methods currently available (such as active sampling of air, surface sampling, and media fills) it is difficult to identify, measure and evaluate potential hazards under cleanroom conditions. The detection level of the microbiological methods and the time needed for analyses make it difficult to ascertain how e.g. the performance of single operations and interventions affect microbiological risks.

The use of challenge tests for the evaluation of cleanroom processes (such as sterilisation processes, sterile filtration methods, and HEPA-filter installations) to gain

microbiological safe processes is well established.

Microbiological challenge tests are not suitable for use in cleanrooms and manufacturing areas. As the same conditions of airborne dispersion are valid for particles with and without micro-organisms, assessment of airborne microbiological contamination can be carried out by tracing particulate contamination routes. Assessment of potential microbiological hazards from airborne contamination can thus be performed with a non-microbiological challenge method, which is part of the Method for Limitation of Risks (LR-Method). The LR-Method is a non-microbiological approach involving the visualisation of air movements, a challenge test with airborne particles and determination of a Risk Factor for the microbiological evaluation of potential hazards. Because this method does not rely upon highly variable microbial air sampling methods, it yields results that are less subjective and more reliable.

## The Method for Limitation of Risks (LR-Method)

The LR-Method provides a reliable procedure for assessing potential microbiological risks of airborne contamination in clean zones in a systematic way.

The LR-Method is performed in three steps.

- The first step is to visualise (e.g., by using smoke technique) the main air movements and identify turbulent regions and critical vortices where contaminants can be accumulated or dispersed in an unpredictable way.
- The second step – the challenge test – identifies potential risk situations. The particulate challenge test involves placing the probe of a particle counter in the critical area where, during normal operations, the product is exposed and taking continuous total particle counts while generating particles in the surrounding air (e.g., by using air current test tubes) to a challenge level of more than 300,000 particles equal to and larger than 0.5µm per cubic foot (approx. $10^7$ particles per $m^3$). These measurements must be carried out during simulated production activity.
- The third step is to evaluate the risk situation by calculating the Risk Factor, which is defined as the ratio between measured particle concentration (number/$ft^3$) in the critical region and the challenge level in the surrounding air. Because of limited measurement accuracy at high concentrations, a value of 300,000 is used as a challenge level in all Risk Factor calculations.

The illustrative technique of smoke studies provides a useful technique for visualising air movements and dispersal of contaminants. This technique requires that isothermal smoke is released continuously and almost momentum-free using a diffuser. The smoke pattern is recorded by means of still photography and video. Ljungqvist (1979, 1987) has demonstrated that smoke particles are typically so small that, under normal turbulent conditions, they are dispersed in the same way as gases[3,4].

During the challenge test the process simulation and operating conditions should preferably exaggerate the human interference and interventions in order to identify potential risk situations more rapidly. To assure the result, generally not less than three measurements of not less than one minute each should be performed for each intervention and at each representative location. The maximum concentration (number/$ft^3$) value of each intervention and location respectively forms the basis for Risk Factor calculations. The advantage with this approach is the uncomplicated, immediate recording of results using electronic discrete particle counters (DPC). The critical regions become contaminated only by non-viable particles, and this approach can be safely used in microbiological clean zones with no added risk of contamination.

The LR-Method, which relies upon visualisation of air movements, particle challenge testing and calculation of the Risk Factor, presents an effective way of limiting potential risks. It can be used for tracing the dispersion routes of airborne contamination, for identification of risk situations, for evaluating risks connected to single process steps, for immediate evaluation of changes, and for assessment of potential risks. A modification of the LR-Method can be used for evaluating the response of sampling locations in clean zones.

When the Risk Factor is less than $10^{-4}$ (0.01%) during the challenge test, there are no risks of airborne microbiological contamination during normal operational conditions, according to experimental findings from more than 30 aseptic production lines studied.

Experiences with the use of the LR-Method have been described earlier by several authors[5–10].

## Examples from safety cabinets (Class II)

Microbiological risk assessment has been performed and compared for three manual aseptic processes. The processes were sensitive to airborne contamination and had to be carried out in safety cabinets (Class II benches). The different cases were evaluated and designated A, B, and C. The process specifications for these test cases were as follows:

A. Large equipment (flasks, 3 litres) and occasional use of a gas burner, the safety cabinet is situated in a class 100,000 area (ISO Class 8 operational).
B. Large equipment (flasks, 3 litres) similar to A but without a gas burner, the safety cabinet is situated in a class 100,000 area (ISO Class 8 operational).
C. Small equipment (vials, 10 ml), no gas burner, the safety cabinet is situated in a class 10,000 area (ISO Class 7 operational).

The safety cabinets had been checked after installation and the results fulfilled the preset requirements for safety cabinets. Smoke visualisation in the empty cabinets showed that the main air movements were acceptable and similar for all three cabinets.

During the challenge test, the measuring probe of the particle counter was placed inside the cabinet, 0.15m from the front edge and 0.1 to 0.3m above the work surface, depending on where the critical region for the process was established. The height of the front opening of the safety cabinet was 0.20–0.25m, which is the normal aperture height during working conditions. Particles, ≥0.5µm, were generated (using air current test tubes) to a challenge level of more than 300,000 particles/ft$^3$ in the ambient air close to the front opening of the safety cabinet.

Results from the measurements are shown in **Table 1**. Maximum levels from samples of 1 ft$^3$ are reported in the table.

The results in **Table 1** show that the Risk Factor is satisfactory ($\leq 10^{-4}$) for case B and C, while in case A, the Risk Factor is $1.5 \cdot 10^{-2}$, which indicates a potential microbiological risk. The factual outcome in case A had shown a low frequency of microbiological contaminated products. Further investigations of sources to the potential risks were performed and these results are presented in **Table 2**.

As seen in **Table 2**, potential hazards were identified and evaluated. Within the cabinet, the use of a burner, transfer of equipment, and active air sampling represented risk situations. Opening of doors in the room and rapid passage behind the operator caused risk situations. During the investigation, the LR-Method was used simultaneously with microbiological test methods. The results from the microbiological samples taken outside and inside the cabinet did not show any difference between the various conditions.

The Biotest Reuter Centrifugal Sampler (RCS) air sampler, has often been used for the microbiological monitoring of critical aseptic environments supplied with unidirectional air flow. The results in this investigation indicated that an operating RCS air sampler, placed on the working surface close to the front edge (less than 0.2m from the edge), entrained ambient air into the cabinet and thus increased the risk of airborne contamination[11,12].

With the LR-Method it was possible to identify specific risk situations and thus avoid them or reduce their frequency or their duration. Changes in working procedures could be evaluated without delay. Detailed working instructions (SOPs) containing precise recommendations for risk reduction were written based upon the information obtained from the LR-Method. In addition, the identification of risk situations was found to facilitate the operator training.

**Table 1:** Measured maximum particle levels during the challenge tests and calculation of the Risk Factor

| Case | Condition | Particle levels (Number of particles ≥ 0.5µm/ft$^3$) | | Risk Factor |
|---|---|---|---|---|
| | | Ambient air in front of aperture | Maximum values within the cabinet | |
| A | Empty cabinet | >300,000 | <30 | $<10^{-4}$ |
| | Simulated activity | >300,000 | 400 | $1.3 \cdot 10^{-3}$ |
| | Disturbances | >300,000 | 4,500 | $1.5 \cdot 10^{-2}$ |
| B | Empty cabinet | >300,000 | <30 | $<10^{-4}$ |
| | Simulated activity and disturbances | >300,000 | 30 | $10^{-4}$ |
| C | Empty cabinet | >300,000 | <10 | $<3 \cdot 10^{-5}$ |
| | Simulated activity and disturbances | >300,000 | <10 | $<3 \cdot 10^{-5}$ |

**Table 2:** Mapping of sources of potential risks, case A (excerpts from study). Measured maximum particle levels during the challenge tests and calculation of the Risk Factor

| Condition | Particle levels (Number of particles ≥ 0.5µm/ft$^3$) | | Risk Factor |
|---|---|---|---|
| | Ambient air in front of aperture | Maximum values within the cabinet | |
| Empty cabinet | >300,000 | <30 | $<10^{-4}$ |
| Burner on and simulated activity | >300,000 | 600 | $2 \cdot 10^{-3}$ |
| Burner on and opening of door | >300,000 | 2,800 | ca $10^{-2}$ |
| Burner on and rapid passage behind operator | >300,000 | 4,500 | $1.5 \cdot 10^{-2}$ |
| Production activity. Transfer of equipment into the cabinet | >300,000 | 500 | $1.7 \cdot 10^{-3}$ |
| Burner on and critical operation | >300,000 | 200 | $7 \cdot 10^{-4}$ |
| Active air sampler located close to the front aperture. Sampler not operating | >300,000 | <10 | $<10^{-4}$ |
| Active air sampler located close to the front aperture. Sampler operating | >300,000 | 30,000 | $1 \cdot 10^{-1}$ |

## Examples from aseptic filling lines

Aseptic filling lines, located in HEPA-filtered unidirectional air flow, require a special design if wake and turbulent regions, which cause the accumulation of contaminants in critical regions, are to be avoided. Filling lines designed with little or no regard to aerodynamic requirements can cause enormous difficulties from a microbiological point of view, due to the interaction between air movements and the dispersion of contaminants.

Large surfaces perpendicular to the main air flow direction (e.g., rotating feeding tables with large horizontal surfaces) cause wake regions that entrain ambient air into the clean zones. Also, the rotation of the table surface might increase the risk.

Different types of feeding tables and stopper bowls have been investigated using the LR-Method. **Table 3** shows the calculated Risk Factor during installation qualification (IQ) and performance qualification (PQ) of rotating feeding tables, straight feeding tables and stopper bowls (excerpts from investigations).

**Table 3:** The calculated Risk Factor during installation qualification (IQ) and performance qualification (PQ). Not less than 300,000 particles ≥0.5µm per ft³ were generated in ambient air (close vicinity) during the challenge test. Media fill results are presented from filling lines in each case.

| Condition | Particle sensor location | Risk Factor IQ | Risk Factor PQ | Media fil results Contamination rate in % |
|---|---|---|---|---|
| Rotating feeding table | Above the table | $6 \cdot 10^{-1}$ | | >>0.1 |
| | Below the table | 1.6 | | |
| Straight feeding table | Above the table | $<10^{-4}$ | $<10^{-4}$ | <0.1 |
| Stopper bowl | Along the edge, slanting shape, large table | ca $10^{-3}$ | | |
| | Along the edge, slanting shape, small table | $<10^{-4}$ | $<10^{-4}$ | <0.1 |
| | Along the edge, straight shape, perforated bowl | $<10^{-4}$ | $<10^{-4}$ | <0.1 |

The results from **Table 3** show that there is less risk associated with smaller horizontal surfaces, than with those that are larger. The results from microbiological media fills are in agreement with results from the challenge test.

The LR-Method gives valuable information regarding the probability of contamination, but the Risk Factor does not take into account the influence of exposure time and frequency. For example, open bottles on a feeding table or stoppers in a stopper bowl with long exposure time (more than 10 minutes) can, even if the Risk Factor is low, have the same total risk of contamination from the air as, for example, a vial in a filling station with short exposure time (less than 10 seconds) and higher Risk Factor.

To evaluate the total potential risk for a single process step, exposure time and frequency of incident must be taken into account[13,9].

Use of the LR-Method does not replace the final evaluation of aseptic processes with the media fill tests.

## Evaluation of sampling locations

Another important application of the LR-Method and its challenge test is the evaluation of sampling locations. The response to incidents can be evaluated by placing the measuring probe of the particle counter at the inlet of the operating air sampler or particle probe and generating particles at locations where interventions take place. Due to the limitation of measurement accuracy at high concentrations, a value of 300,000 (particles ≥ 0.5µm per ft³) is also used in this case as a challenge level in the Risk Factor calculations.

An increased Risk Factor indicates correlation between monitoring location and risk situation. **Tables 4–6** show excerpts from a case study and provide examples of the evaluation of sampling locations.

As shown in **Tables 4–6**, the sampling location can be evaluated with regard to its ability to respond to risk situations. In this case, sampling locations were selected primarily so that interventions defined as "unacceptable interventions" should give response and thus be detected (minimum false negative results) and, secondly, so that interventions defined as "permitted interventions" should not give any response (minimum false positive results). When the clean zones and their processes are being monitored, it is essential to select sampling locations that indicate the presence of potential risks within the critical

**Table 4:** Evaluation of a sampling location on an aseptic filling line. Excerpt from a case study. Sampling location above feeding table.

| Challenge region | Number of particles/ft³ at the sampling location | Risk factor | Response to incident |
|---|---|---|---|
| Outside feeding table, simulating allowed intervention | 30 | $10^{-4}$ | Low |
| On feeding table, simulating unacceptable intervention | 3,700 | ca $10^{-2}$ | Medium |

**Table 5:** Evaluation of a sampling location on an aseptic filling line. Excerpt from a case study. Sampling location in filling zone.

| Challenge region | Number of particles/ft³ at the sampling location | Risk factor | Response to incident |
|---|---|---|---|
| In front of filling zone, simulating allowed intervention | <10 | $<10^{-4}$ | Low |
| In filling zone, simulating unacceptable intervention | 27,000 | ca $10^{-1}$ | High |

**Table 6:** Evaluation of a sampling location on an aseptic filling line. Excerpt from a case study. Sampling location beside stopper bowl.

| Challenge region | Number of particles/ft³ at the sampling location | Risk factor | Response to incident |
|---|---|---|---|
| Outside LAF, simulating stopper feeding | 40 | $1.3 \cdot 10^{-4}$ | Low |
| Outside feeding table, simulating allowed intervention | 10 | $<10^{-4}$ | Low |
| Beside stopper bowl, simulating unacceptable intervention | 37,000 | ca $10^{-1}$ | High |

zone. The verification of the appropriateness of the sampling location is an important issue.

## Conclusion

The LR-Method discussed here is primarily an engineering tool that provides information concerning weak links and potential risk situations with regard to airborne particulates. This information is of vital importance for risk assessment, e.g. HACCP. The method provides results for training purposes and motivates operators to act in an aseptically correct manner. The method can also be used when designing microbiologically safe filling lines with respect to airborne contamination. However, the use of the LR-Method does not replace the final evaluation of aseptic processes with media fills.

The concept of hazard identification and risk analysis is effective in the microbiological assessment of airborne contamination in cleanrooms and clean zones. This assessment should be built on an understanding of the cleanroom and its function, of air flow patterns and dispersion of contaminants as well as knowledge of the manufacturing process itself, including the flow of product, people and material. Potential risk situations created by the interaction between people, air movements, and airborne contaminants are difficult to identify and evaluate with traditional microbiological measuring methods.

As the same conditions of airborne dispersion are valid for particles with and without micro-organisms, assessment of airborne microbiological contamination can be carried out by way of tracing particulate contamination routes. Therefore, using the Method for Limitation of Risks (LR-Method), potential risks in form of airborne contamination can be identified, evaluated, and excluded or minimised. As the LR-Method does not contaminate the cleanroom with micro-organisms but rather with particles, it can be safely used in cleanrooms requiring high levels of microbiological cleanliness. The use of the LR-Method results in a more effective risk assessment of airborne contamination. Using this method, the dispersion routes of potential airborne contaminants can be identified and the contamination risks eliminated or minimised.

When the clean zones and their processes are being monitored, it is essential to select sampling locations that respond to risks within the critical zone. A verification of the appropriateness of the sampling location is an important issue. The challenge test from the LR-Method has been modified and can be of aid in this verification. When sampling locations are selected and evaluated in a rational manner, the monitoring by particulate and microbiological sampling can be improved in quality and the sampling locations could be reduced in numbers. Real-time measurement techniques using particle counters provide the ability to detect immediately any changes in the cleanroom. Particle monitoring is a valuable tool for the systematic evaluation of changes, whether those changes reduce or increase process risks.

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# CLEANROOM-DRESSED OPERATOR IN UNIDIRECTIONAL AIRFLOW; A MATHEMATICAL MODEL OF CONTAMINATION RISKS

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# Cleanroom-dressed operator in unidirectional airflow; a mathematical model of contamination risks

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**In pharmaceutical manufacturing, people often work in vertical unidirectional air flow. A mathematical model is presented, describing some factors concerning the risks of airborne contamination when a cleanroom-dressed operator is standing in vertical unidirectional flow. The results indicate that aseptic work in a region below the operator's knee always constitutes a risk situation and should be avoided.**

## Introduction

Air supplied to a cleanroom is filtered and therefore does not usually contribute to airborne contamination. The cleanroom is also pressurised to prevent contamination from adjacent areas. To maintain air quality, sufficient air flow of at least 20 air changes per hour is normally required (controlled areas). In the controlled area the concept of air mixing is used, whereby complete mixing of air is considered to be achieved.

The sources of airborne particles within the room are people and machinery. People are the main source of airborne bacteria. The fact that the airborne microbial contaminants are transported on particles means that the same conditions of airborne dispersion are valid for particles with and without micro-organisms.

To avoid particulate and microbiological contamination into critical process regions (critical areas), unidirectional air flow with HEPA-filtered air (0.45 ± 0.1 m/s) is used as protection from ambient air. In pharmaceutical manufacturing, people often work in vertical unidirectional air flow, e.g. when feeding stoppers and closures, or loading and unloading freeze-dryers.

The purpose of this paper is to present a mathematical model describing some factors concerning the risks of airborne contamination when a cleanroom-dressed operator is standing in vertical unidirectional airflow.

## Mathematical treatment

A vertically standing barrel, i.e. a cylinder with its axis in a vertical position and with a constant radius, approximates the operator. The x-axis is situated in the same position as the central axis of the cylinder and is directed downwards towards the floor. The origin is situated in the centre at the top of the cylinder (operator's head). The HEPA-filtered unidirectional air flow with constant velocity is moving downwards in the $x$-direction and particles are evenly emitted over the whole cylinder surface.

To get an expression of the particle concentration profile as a function of the distance $\rho$ from the centre of the cylinder and the distance $x$ from the origin of co-ordinates along the cylinder, the mathematical treatment consists of solving the diffusion equation with the above assumptions.

In unidirectional flow with constant velocity, assuming a constant value for the diffusion coefficient, and neglecting the diffusion in the main direction of flow, the stationary diffusion equation in cylindrical co-ordinates ([$\rho$, $x$] with symmetry, i.e. $\varphi$-independence) becomes:

$$\frac{1}{\rho}\cdot\frac{\partial}{\partial\rho}\left(\rho\frac{\partial c}{\partial\rho}\right)-\frac{v_o}{D}\cdot\frac{\partial c}{\partial x}=0 \qquad (1)$$

where $c$ = particle concentration (number/m$^3$)
$D$ = diffusion coefficient (m$^2$/s)
$v_0$ = constant velocity in the $x$-direction (m/s)

The boundary conditions are

$q_l$ = 0 when $x \le 0$
$q_l$ = constant when $x > 0$ and $\rho = \rho_l$
$\lim c(\rho,x) \rightarrow 0$ when $\rho \rightarrow \infty$ for every $x > 0$

where $q_l$ = source strength, outward particle flow per unit of length from the cylinder source (number/[s, m])
$\rho_l$ = radius of the cylinder (m)

The partial differential equation in **Equation (1)** is solved by use of the Laplace transformation in $x$ with the above boundary conditions. The solution contains the modified Bessel functions.[1] If the quantity characteristic length $v_0\rho^2/D$ is large in comparison to the studied $x$-values, the asymptotic expansions for the modified Bessel functions are used to give a fast converging series for the Laplace transformation of the solution.[2] The final solution with three terms in the expansion is obtained as:

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$$c(\rho,x)=\frac{q_l}{2\cdot\pi\cdot\sqrt{v_0\cdot D\cdot\rho\cdot\rho_1}}\cdot\left\{2\cdot\sqrt{x}\cdot\mathrm{ierfc}\left[\sqrt{\frac{v_0}{D}}\cdot(\rho-\rho_1)\cdot\frac{1}{2\cdot\sqrt{x}}\right]-\frac{1}{2\cdot\sqrt{\frac{v_0}{D}}}\cdot\left(\frac{1}{\rho}+\frac{3}{\rho_1}\right)\cdot x\cdot\mathrm{i}^2\mathrm{erfc}\left[\sqrt{\frac{v_0}{D}}\cdot(\rho-\rho_1)\cdot\frac{1}{2\cdot\sqrt{x}}\right]+\frac{3}{16\cdot\frac{v_0}{D}}\cdot\left(\frac{3}{\rho^2}+\frac{2}{\rho\cdot\rho_1}+\frac{11}{\rho_1^2}\right)\cdot x\cdot\sqrt{x}\cdot\mathrm{i}^3\mathrm{erfc}\left[\sqrt{\frac{v_0}{D}}\cdot(\rho-\rho_1)\cdot\frac{1}{2\cdot\sqrt{x}}\right]\right\} \quad (2)$$

where $\mathrm{i}^n\mathrm{erfc}$ = repeated integrals of the complementary error function

## Contamination risks

The risk of contamination depends upon two critical factors; the concentration of the contaminants and the motion of the contaminants. It can generally be assumed, in regions with distinct air flow fields, that the settling velocity of contaminants is negligible, which implies that gravitation plays an inferior role. If particulate contaminants are being considered, then it is the rate of incidence of the particles that characterises the risk. When considered mathematically, this incidence is of vector character and the term *impact vector* or *flux vector*, is used.[3,4,5]

The risk is dependent to a great extent, but not entirely, upon the impact vector **K**:

$$\boldsymbol{K}=-D\,\mathrm{grad}\,c+\boldsymbol{v}\cdot c \quad (3)$$

where $\boldsymbol{v}$ = velocity vector (m/s)

In principle, the numerical value of **K** indicates the number of particles passing a supposed unit area, placed perpendicular to the direction of particle flow, per unit time.

As above, the diffusion in the $x$-direction is neglected and the components of the impact vector are given by

$$K_x=v_0\cdot c \quad (4)$$

and

$$K_\rho=-D\frac{\partial c}{\partial\rho} \quad (5)$$

where

$$\frac{\partial c}{\partial\rho}=\frac{q_l}{2\cdot\pi\cdot\sqrt{v_0\cdot D\cdot\rho\cdot\rho_1}}\cdot\left\{-\sqrt{\frac{v_0}{D}}\cdot\mathrm{erfc}\left[\sqrt{\frac{v_0}{D}}\cdot(\rho-\rho_1)\cdot\frac{1}{2\cdot\sqrt{x}}\right]+\frac{3}{4}\left(\frac{1}{\rho_1}-\frac{1}{\rho}\right)\cdot\sqrt{x}\cdot\mathrm{ierfc}\left[\sqrt{\frac{v_0}{D}}\cdot(\rho-\rho_1)\cdot\frac{1}{2\cdot\sqrt{x}}\right]+\frac{1}{32\cdot\sqrt{\frac{v_0}{D}}}\cdot\left(\frac{29}{\rho^2}+\frac{18}{\rho\cdot\rho_1}-\frac{33}{\rho_1^2}\right)\cdot x\cdot\mathrm{i}^2\mathrm{erfc}\left[\sqrt{\frac{v_0}{D}}\cdot(\rho-\rho_1)\cdot\frac{1}{2\cdot\sqrt{x}}\right]-\frac{3}{32\cdot\frac{v_0}{D}}\cdot\left(\frac{15}{\rho^3}+\frac{6}{\rho^2\cdot\rho_1}+\frac{11}{\rho\cdot\rho_1^2}\right)\cdot x\cdot\sqrt{x}\cdot\mathrm{i}^3\mathrm{erfc}\left[\sqrt{\frac{v_0}{D}}\cdot(\rho-\rho_1)\cdot\frac{1}{2\cdot\sqrt{x}}\right]\right\} \quad (6)$$

For a more detailed description of the interaction between air movements and dispersion of contaminants and contamination risks, see Ljungqvist and Reinmüller (1997).[6]

At any value of $x$ the following impact condition must be fulfilled:

$$q_l\cdot x=\int_{\rho_1}^{\infty}K_x\cdot2\cdot\pi\cdot\rho\cdot d\rho \quad (7)$$

## Calculation procedure

The error function (and the complementary error function in the form of the 1-error function) can be found in any mathematical software on the market e.g., Mathcad. It is also included in some pocket calculators. If the error function is not available, the complementary error function can be calculated from a table of the normal distribution function $\phi$, which can be found in any mathematical statistics book. To use the table it is useful to calculate the independent variable as follows:

$$z=\sqrt{\frac{v_0}{D}}\cdot(\rho-\rho_1)\cdot\frac{1}{2\cdot\sqrt{x}} \quad (8)$$

The quantity $z\cdot\sqrt{2}$ is denoted by $y$ and the normal distribution function $\phi$ is very close to 1 for large $y$-values. The maximum $y$-value in the table is often 5, i.e. the largest z-value is around 3.5.[2] The relation between the complementary error function and the normal distribution function is

$$\mathrm{erfc}(z)=2\left[1-\phi\left(z\cdot\sqrt{2}\right)\right]\qquad z\le 3.5 \quad (9)$$

The nearest value of $y$ in the table of the normal distribution function is denoted by $y_0$. The intermediate value is

$$\phi(y)=\phi(y_0)+(y-y_0)\cdot\frac{1}{\sqrt{2\cdot\pi}}\cdot e^{-\frac{y_0^2}{2}} \quad (10)$$

The repeated integrals of the complementary error function can be calculated from recurrence relations and the result is

$$\mathrm{ierfc}(z)=-z\cdot\mathrm{erfc}(z)+\frac{1}{\sqrt{\pi}}\cdot e^{-z^2} \quad (11)$$

$$\mathrm{i}^2\mathrm{erfc}(z)=\frac{1+2\cdot z^2}{4}\cdot\mathrm{erfc}(z)-\frac{z}{2\sqrt{\pi}}\cdot e^{-z^2} \quad (12)$$

$$\mathrm{i}^3\mathrm{erfc}(z)=-\frac{z\cdot\left(2\cdot z^2+3\right)}{12}\cdot\mathrm{erfc}(z)+\frac{z^2+1}{6\cdot\sqrt{\pi}}\cdot e^{-z^2} \quad (13)$$

Finally, the concentration can be calculated using **Equation (2)**.

For large z-values, i.e., $z>3.5$, the asymptotic expansion of the repeated integrals of the complementary error function can be used[2] and the concentration can be calculated directly with a precision better than 1%, from the following:

$$c=\frac{q_l}{2\cdot\pi^{3/2}}\cdot\sqrt{\frac{x}{v_0\cdot D\cdot\rho\cdot\rho_1}}\cdot\frac{e^{-z^2}}{z^2}\cdot\left\{1-\frac{3}{2z^2}-\left(1-\frac{3}{z^2}\right)\cdot\frac{\rho-\rho_1}{32\cdot z^2}\cdot\left(\frac{1}{\rho}+\frac{3}{\rho_1}\right)+\left(1-\frac{5}{z^2}\right)\cdot\frac{3\cdot(\rho-\rho_1)^2}{512\cdot z^4}\cdot\left(\frac{3}{\rho^2}+\frac{2}{\rho\cdot\rho_1}+\frac{11}{\rho_1^2}\right)\right\} \quad (14)$$

For $z>3.5$ the $K_\rho$ value can be neglected.

## Some calculations

In order to use the derived theoretical expressions, we need to know the outward particle flow per unit length from the source and the value of the diffusion coefficient. Investigations performed by Reinmüller (2001)[5] show that the strength of the contamination source "people during activity, dressed in modern cleanroom clothing systems", for particles equal and larger than 0.5µm, is estimated to be about 500-800 particles per second and meter.

In most cases, values of the turbulent diffusion coefficient are determined experimentally. The case of isotropic-turbulent parallel flow has been investigated experimentally[4] for a room with a cross-section of $1.7 \cdot 1.7 m^2$ at air velocities of 0.20m/s and 0.45 m/s. The diffusion coefficients obtained for the two velocities were approximately $1.4 \cdot 10^{-4} m^2/s$ and $2.4 \cdot 10^{-4} m^2/s$, respectively. Therefore, in turbulent unidirectional air flow, the diffusion coefficient seems to be proportional to the air velocity to the power of 0.68.

The radius of the cylinder was obtained by measuring the circumference of the authors' stomachs and a value of 0.15 meter was chosen.

Numerical calculations with the values given above show that, for the concentration in **Equation (2)**, if only the two first terms are included the accuracy will be about 3%, and if only the first term is used the accuracy decreases to 15%. However, three terms are included in the calculations used below. Furthermore, the impact condition in **Equation (7)** holds for $x$-values of up to 3 meters with an accuracy within the range of 0.6%.

Using the above numerical values, profiles of concentration and particle impact along the air stream at a distance of 0.1 meter from the cylinder surface i.e., a distance of 0.25 meter from the centre of the cylinder ($\rho$ = 0.25m), are shown in **Figures 1-6**.

The results in **Figures 1-6** show that, at a certain distance, the concentration and particle impact increase with decreasing air flow velocity. The values of $K_\rho$ are much less than those of $K_x$, depending on the convective transport in the $x$-direction.

The concentrations at a fixed $x$-value of 1.5 meters as a function of the distance in the $\rho$-direction (perpendicular to the cylinder axis) at an air velocity of 0.45 m/s and 0.2 m/s are shown in **Figure 7** and **Figure 8**, respectively.

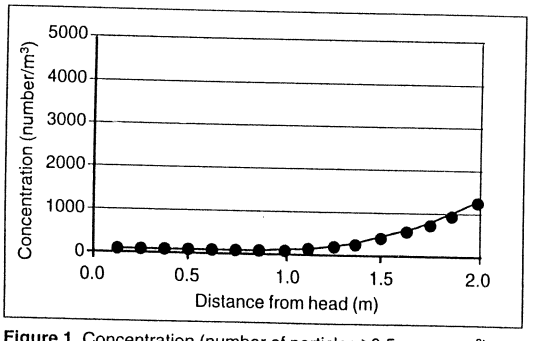


**Figure 1.** Concentration (number of particles ≥0.5 µm per $m^3$) at a distance of 0.1m ($\rho$ = 0.25 m) from the cylinder surface (with $\rho_1$ = 0.15 m) as a function of the distance ($x$-direction) from the top of the cylinder in unidirectional air flow with a velocity of 0.45 m/s and a diffusion coefficient of $2.4 \cdot 10^{-4} m^2/s$. The source strength of particles (≥0.5 µm) per second and meter was chosen as 800.

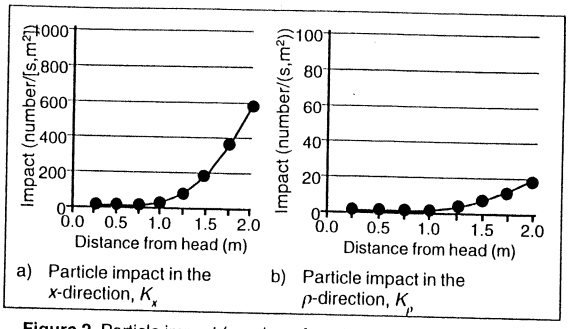


**Figure 2.** Particle impact (number of particles ≥0.5 µm per second and $m^2$) as a function of the distance ($x$-direction) from the top of the cylinder with the same data as given in **Figure 1**.

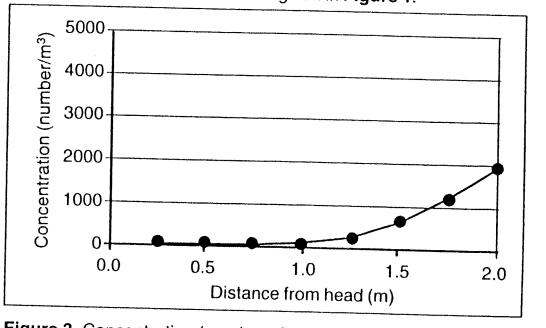


**Figure 3.** Concentration (number of particles ≥0.5 µm per $m^3$) at a distance of 0.1m ($\rho$ = 0.25m) from the cylinder surface (with $\rho_1$ = 0.15m) as a function of the distance ($x$-direction) from the top of the cylinder in unidirectional air flow with a velocity of 0.35m/s and a diffusion coefficient of $2 \cdot 10^{-4} m^2/s$. The source strength of particles (≥0.5 µm) per second and meter was chosen as 800.

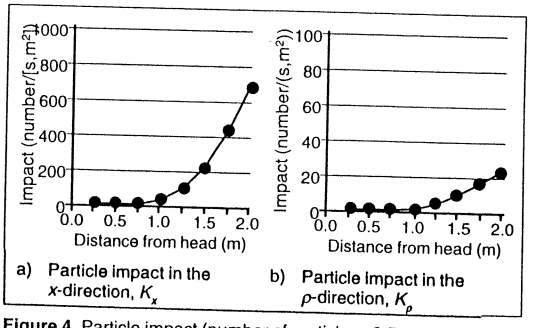


**Figure 4.** Particle impact (number of particles ≥0.5 µm per second and $m^2$) as a function of the distance ($x$-direction) from the top of the cylinder with the same data as given in **Figure 3**.

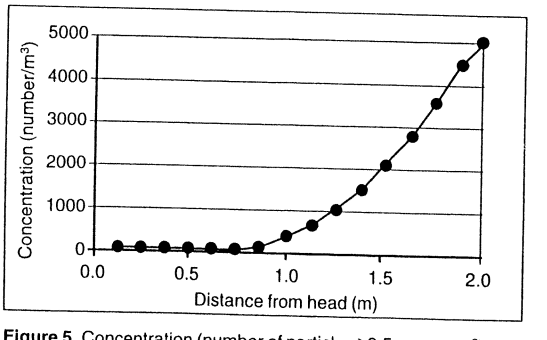


**Figure 5.** Concentration (number of particles ≥0.5 µm per $m^3$) at a distance of 0.1 m ($\rho$= 0.25m) from the cylinder surface (with $\rho_1$ = 0.15m) as a function of the distance ($x$-direction) from the top of the cylinder in unidirectional air flow with a velocity of 0.2m/s and a diffusion coefficient of $1.4 \cdot 10^{-4} m^2/s$. The source strength of particles (≥0.5 µm) per second and meter was chosen as 800.

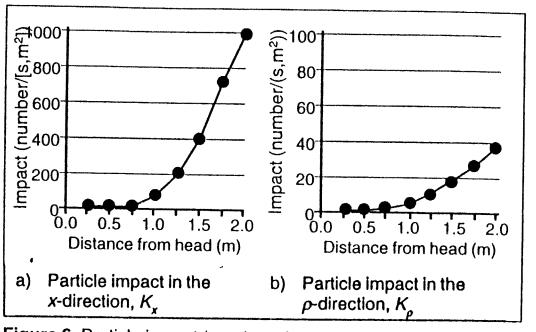


**Figure 6.** Particle impact (number of particles ≥0.5 µm per second and m²) as a function of the distance (*x*-direction) from the top of the cylinder with the same data as given in **Figure 5**.

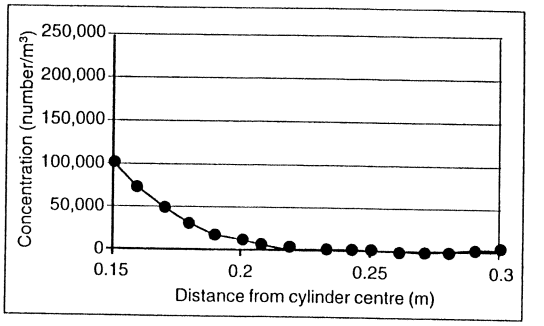


**Figure 7.** Concentration (number of particles ≥0.5µm per m³) at a distance of 1.5 m from the top of the cylinder as a function of the distance perpendicular to the central axis of the cylinder (ρ-direction) in unidirectional air flow with a velocity of 0.45m/s. All other data are as in **Figure 1**.

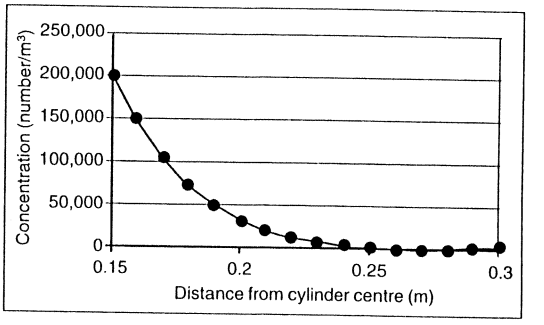


**Figure 8.** Concentration (number of particles ≥0.5µm per m³) at a distance of 1.5m from the top of the cylinder as a function of the distance perpendicular to the central axis of the cylinder (ρ-direction) in unidirectional air flow with a velocity of 0.20m/s. All other data are as in **Figure 5**.

Concentration values in **Figures 7** and **8** at distances exceeding 0.25 meter from the centre of the cylinder have reached values below the levels permitted in grade A environments.

It should be noted that the mathematical model discussed here does not take into account the dispersion caused by movements from people. Therefore, the critical distance in real-life situations is greater than 0.25 meter.

## Conclusion

When a cleanroom-dressed operator stands in vertical unidirectional airflow, the results clearly indicate that the risk of airborne contamination decreases with increasing velocity of the air flow. Furthermore, the risk increases as the distance x increases. At *x*-distances less than 0.7 meter (head and chest region), the concentration and impact values are small, but they increase rapidly at *x*-distances greater than 1.5 meter (below the knees).

In real-life situations, within the region close to the floor (x ≥1.5m), the air flow is no longer unidirectional, as the air flow in most cases deviates to exhaust systems in the walls. This provides further confirmation of the observation that aseptic work within the region close to the floor always constitutes a risk situation and should be avoided.

The mathematical model of contamination risks discussed here should be taken into account when designing aseptic work-stations in Grade A (critical regions).

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# PARTICLE DISPERSION FROM A CYLINDRICAL SOURCE IN A CLEAN ZONE WITH UNIDIRECTIONAL AIRFLOW

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## Abstract

The purpose of this M.Sc. thesis performed at KTH, Building Services was to perform an experimental investigation and to illustrate the particle dispersion from an operator located in a clean zone with unidirectional airflow. In this study the airflow of particle dispersion from the operator was simulated using a cylindrical source, where particles are emitted in a diffuse way from the entire surface. The obtained results are compared to a mathematical model described in Ljunqvist et al (2003). The mathematical model uses a cylinder to imitate the human shape. The cylinder's coordinate system has a x-axis that stretches from the cylinder's top (head) down to the cylinder's bottom (feet) and a ρ-axis, where ρ is the perpendicular distance from the cylinder's centre line and outward, the cylinders radius is called $\rho_1$. The HEPA-filtered unidirectional airflow has constant velocity and is moving downwards in direction x. Particle dispersion from the operator is here resembled by a smooth dispersion of particles, $q_l$ (No of particles/m,s) from the entire surface. The mathematical model is a solution of the stationary diffusion equation in cylindrical coordinates and calculates the particle concentration as a function of the distances ρ and x.

The experiment was performed in a qualified dispersal chamber at KTH, Stockholm. The cylindrical source is basically a plastic pipe with perforated surface, the pipe is filled with particles (oil mist) that are emitted through the holes. The particle counter was sensitive to particle concentration exceeding $10^6$ particles (≥ 0.5 μm)/$ft^3$ therefore the measurements has been carried out to find the distance from the cylinder's centre (ρ) to a position where the particle concentration is close to zero. This is called contamination region edge (c(ρ,x) ≈ 1 particle (≥ 0.5 μm)/$m^3$) and the area closer to the cylinder is called contamination region (c(ρ,x) > 1 particle (≥ 0.5 μm)/$m^3$).

The following results are obtained at a air velocity of 0.45 m/s and the diffusion constant $2.4 \cdot 10^{-4}$ $m^2/s$ (Ljungqvist (1979). The cylinder height is 1.75 m and the radius is 0.055 m. The value of the source strength was difficult to estimate so different values have been used in the theoretical calculation. Figure 1 shows both measured and theoretical calculated contamination region edges for the cylindrical source.

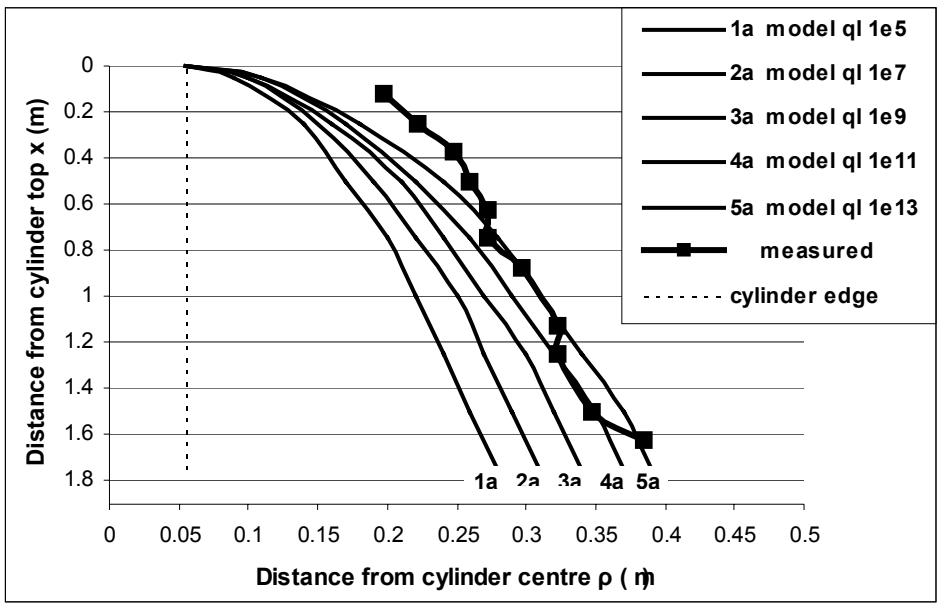


*Figure 1. Contamination region edges ($c(\rho,x) \approx 1$ particles/$m^3$) at air velocity 0.45 m/s. Thicker line represent edge for measured values. The rest, numbered 1a–5a, shows where the same edge is according to the theoretical model. The numbering represents different source strength ($q_l$), starting at $ql = 10^5$ particles/(m,s) and increasing outward.*

Figure 1 shows that there is a correlation between measurements and calculations. Note that a large increase of the source strength only expands the contamination region with a couple of centimetres.

Measurements has also been performed on an operator inside the dispersal chamber at a air velocity of 0.45 m/s. the operator has the height of 1.86 m and an estimated radius of 0.15 m. The measurements have been performed with the operator's arms moving in standardised cycles (Reinmüller 2001). The source

strength has been measured and calculated to 500 particles (≥ 0.5 µm)/(m,s), this value might according to literature be somewhat small so additional source strength (20 000 particles (≥ 0.5 µm)/(m,s)) has been used in the theoretical calculation. Figure 2 shows both measured and theoretical calculated contamination region edges for an operator. The contamination region edge is here set to ≈1 particle (≥ 0.5 µm)/ $ft^3$ (35 particles/$m^3$).

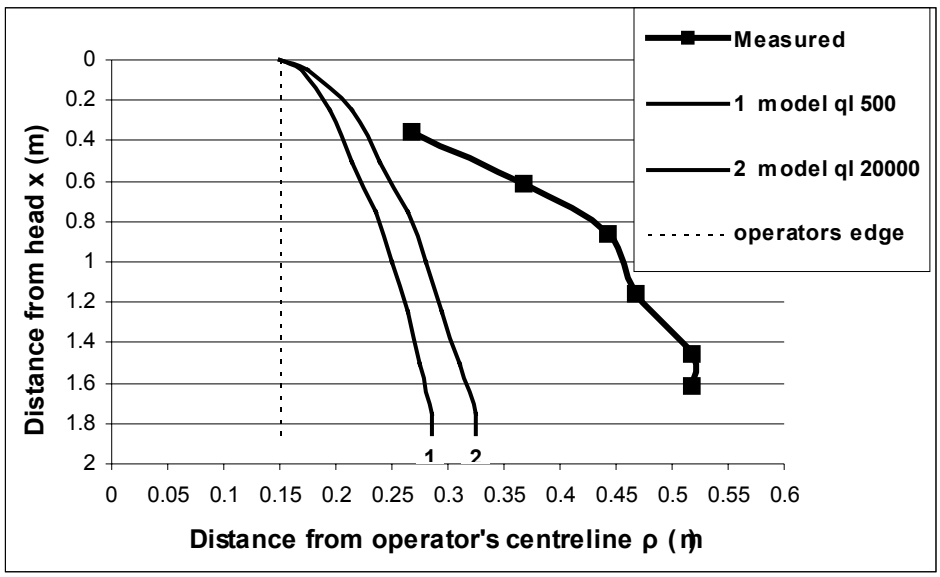


*Figure 2. Contamination region edge ($c(\rho,x) \approx 1$ particles/$ft^3$) at air velocity 0.45 m/s. Thicker line represent edge for measured values. Thinner lines shows where the same edge according to the theoretical model with source strength 500 and 20 000 particles/(m,s).*

Figure 2 shows that the measured result with operator in activity (arm movements) differs from the theoretical calculations. The distances from the cylinder's centre to the contamination region edge for the operator are about 50 percent larger than those theoretically calculated.

There are a couple of questions that needs to be considered when evaluating the results. One source of error is that the particles are emitted with a velocity perpendicular to the x-axis. This causes the particles to move a certain initial distance before the velocity slows down and the dispersion can be called diffuse (required by the mathematical model). This means that the distances ρ

(contamination region edge) should be reduced with this “initial distance”, which is around 1–2 cm. This indicates that the source strength that correlates best, $q_i=10^{13}$ particles/(m,s) is reduced to $10^9$ particles/(m,s) which are considered more realistic. Another source of error is the value of the diffusion constant, this has been investigated and is affecting the contamination region in a limited way.

Despite the difficulty to determent the source strength the conclusion is that the result from the theoretical model is in correlation with the results experimentally obtained. The mathematical model describes the particle dispersion from a standing still operator in a HEPA-filtered unidirectional airflow. It should be noted that the mathematical model discussed in this thesis does not take into account the increased dispersion caused by movements from people. Performed tests indicate that the contamination region around an operator in a zone with unidirectional airflow will increase at least 50 percent when the operator is moving hands and arms.

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# QUALIFICATION OF A PHARMACEUTICAL CLEANROOM FACILITY ACCORDING TO ISO 14644-1 AND 14644-2

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## 1. Approval stages and test programmes

The qualification of pharmaceutical cleanroom facilities includes different approval stages and test programmes. The approval stages are intended to demonstrate that the installation complies with specific requirements for construction, start-up and operation. The test programmes are designed to prove that requirements for airborne contamination control and other related parameters are met. Qualification programmes have to make sure that:

- a documented set of cleanroom user requirement specifications has been defined together with the design of the sterile concept (design qualification / risk analysis)
- the required equipment has been installed (installation qualification)
- the operational requirements have been met (operational / performance qualification) and
- a programme for safeguarding continuous compliance throughout the production process exists.

The qualification programmes require:

- documented procedures for all stages of qualification
- validated test procedures
- calibrated test equipment
- a documentation system linking qualification certificates and production reports.

Initial qualification activities are planned and carried out prior to the start-up of a new facility to demonstrate compliance with a set of target data laid down in a supplier's contract. The supplier or another service provider may be responsible

for demonstrating compliance, giving instructions on carrying out the measuring procedures and making the relevant target data available.

The performance of ongoing qualification activities is an essential responsibility of pharmaceutical manufacturers. They have to initiate and evaluate such activities but the actual work may be carried out by a service provider. Ongoing qualification activities are typically performed at a fixed time interval under 'at rest' conditions. Initial and ongoing qualification programmes are typically related to:

i. cleanroom classification
ii. airflow (including airflow visualization/smoke studies)
iii. air pressure difference
iv. installed filter leakage
v. recovery
vi. containment leakage.

The test programmes for selected approval stages, e.g. "Operational Qualification" (OQ), "Performance Qualification" (PQ), "Ongoing/Routine Qualification", and monitoring are shown in Table 1 and referenced to the relevant ISO 14644 parts.

## 2. International cleanroom standardization

The new standard ISO 14644-1 was published in 1999 to meet increased technological requirements for cleanliness classification. ISO 14644-2, which contains specifications for testing and monitoring to prove continued compliance with ISO 14644-1, was published in 2000. Both are intended to provide globally accepted rules for cleanroom qualification procedures. After a period in which the use of local/national standards has predominated, the current trend for global production and marketing strategies is leading to a steadily growing interest in globally accepted rules for the design, construction and qualification of pharmaceutical cleanrooms. A convincing indication of this development was the withdrawal of the widely used United States Federal Standard 209 E [4] in 2001 and its rapid replacement at national and international level by the new ISO Standard.

*Table 1. Qualification and monitoring test programmes for pharmaceutical facilities according to ISO 14644 [1–3].*

| Test methods | ISO 14644 part | OQ | PQ | Ongoing/ Routine Qualifi-cation | Moni-toring |
|---|---|---|---|---|---|
| **Demonstrating compliance with particle concentration limits** | | | | | |
| **Cleanroom classification** | -1, -2 (-3: B.1) | x | x | x | (x) |
| **Additional tests for all classes** | | | | | |
| **Air flow** | -2, (-3: B.4) | x | - | x | (x) |
| **Air pressure difference** | -2, (-3: B.5) | x | x | x | x |
| **Optional tests** | | | | | |
| **Installed HEPA filter leakage** | -2, (-3: B.6) | x | - | x | - |
| **Airflow visualization** | -2, (-3: B.7) | x | - | (x) | - |
| **Recovery** | -2, (-3: B.13) | x | - | (x) | - |
| **Containment leakage** | -2, (-3: B.14) | x | - | (x) | - |

# 3. Test programmes

## 3.1 Cleanroom classification

The measuring procedures for the verification of airborne particulate cleanliness classification (using a discrete-particle-counting, light-scattering instrument)

have been defined by ISO 14644-1 and –2 /1, 2/. These definitions include requirements for measuring equipment, calibration, sampling procedures, recording and statistical treatment of particle concentration data. Annex F of ISO 14644-1 deals with a sequential sampling procedure for environments in which the air being sampled is significantly more or less contaminated than the specified class concentration limit for the specified particle size.

The number of sampling point locations is derived from the square root of the cleanroom / clean zone area in square meters.

When particle measurement is being carried out for initial or ongoing qualification purposes, it is convenient to use the classification limits and follow the recommended test procedures of ISO 14644-1 exactly. For monitoring purposes, however, i.e. under operational conditions, it may be appropriate to define individual limits that take into account the specific needs of individual processing locations (e.g. sterile powder processing, aerosol and/or vapour release).

## 3.2 Airflow

During the initial qualification process, it is appropriate to use a higher number of sampling points for air flow measurement than during subsequent ongoing/routine qualification activities. Within initial qualification, target levels for compliant air flow have to be defined, while routine qualification has to refer to these target levels.

## 3.3 Air pressure difference

Air pressure difference measurement is required for all stages of qualification. It is suitable for both initial demonstration of basic airborne contamination control requirements and real-time monitoring of compliant cleanroom operation.

## 3.4 Optional tests: Installed HEPA filter leakage

A detailed procedure for installed HEPA filter leak testing is contained in ISO/DIS 14644-3 [3], which specifies the traditional aerosol photometer (AP) method and the more modern discrete particle counter (DPC) method. Although the equivalence between these methods and between different aerosol substances has been demonstrated for a given range [5], in certain applications preference should be given to the DPC method, owing to its better resolution [6].

HEPA filter leak detection normally requires the use of an aerosol challenge. Suitable aerosol substances as well as requirements for measuring equipment (particle counter, aerosol generator, dilution system) are specified by ISO 14644-3. This test method is typically used within initial and routine qualification.

## 3.5 Airflow visualization

Airflow visualization is typically performed to demonstrate that cleanroom installations comply with the basic requirements for unidirectional airflow in the critical areas and to establish target parameters for airflow monitoring in subsequent qualification stages.

## 3.6 Recovery

As long as an installation remains unmodified in terms of equipment, flow rates, pressures etc., the recovery characteristics are unlikely to change. This test is therefore typically conducted during the initial qualification process.

The time required to reduce an initial, artificially added aerosol concentration by a factor of 100 (the 100:1 recovery time) is the main result of this method.

## 3.7 Containment leakage

Containment leakage testing is preferably used for initial qualification purposes. Depending on the need to demonstrate product and/or personnel protection, a limited application in routine qualification has been reported.

# 4. Conclusion

ISO 14644-1 and –2 define a suitable series of measuring procedures for qualification of pharmaceutical cleanroom facilities. Including the specifications of the ISO draft 14644-3, they are the first internationally approved regulations covering the whole range of pharmaceutical cleanroom qualification, with detailed specifications on measuring techniques and equipment.

# References


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2. ISO 14644-2:2000. Cleanrooms and associated controlled environments – Part 2: Specifications for testing and monitoring to prove continued. compliance with ISO 14644-1. ISO, Geneva.

3. ISO/DIS 14644-3:2002. Cleanrooms and associated controlled environments. – Part 3: Metrology and test methods. ISO, Geneva.

4. U.S. Federal Standard 209E. "Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones". September 11, 1992; General Services Administration, Washington D.C., USA.

5. Gail, L. & Ripplinger, F. Correlation of alternative aerosols and test methods for HEPA filter leak testing. Proc. Int. Symp. on Contamination Control, Institute of Environmental Sciences and Technology, Phoenix, Arizona, 1998.

6. IES-RP-CC006.2:1997. Testing Cleanrooms, clause 6.2: HEPA and ULPA filter installation leak tests. IEST Institute of Environmental Sciences and Technology, Mount Prospect, Illinois.

# RELIABLE AIR VELOCITY MONITORING IN CRITICAL AREAS

Andreas Fäh
Luwa Ltd., Uster, Switzerland

## 1. Introduction

Air velocity monitoring is known as a crucial parameter for the production in critical areas (GMP Zone A/B). Currently most of the determinations are made using hot wire anemometers which continuously checking the air flow.

Based on the URS, which was forwarded to us by the customer, we were facing an unknown situation. The area were the device should be installed was exposed to rather aggressive disinfecting agents which would with a high degree of certainty, harm the sensor after a short period of time leading to incorrect readings and further problems related to the daily production. This mainly due to the change in the properties of the fragile hot wire as a fact to oxidation processes induced by the disinfecting agents. Hereafter the evaluation process and some details of the installed equipment should be highlighted and described.

## 2. Problem Benchmarking

The problem was to find a solution in order to guarantee a reliable and proper determination of the air velocity on a continuous base. Based on the URS provided by Pharmacia AB, the problem as well as the responsibilities were clearly named and defined.

In fact the layout of the installations to be checked was not uniform leading to the problem that different determination techniques had to be applied. Most of the areas to be controlled were equipped with a CG-distribution panel thus guaranteeing a uniform air flow pattern but some of the areas were only fitted with final HEPA filters leading to turbulent air flow.

Based on this situation alternative solutions were checked and a suitable system was evaluated using a inert flow cell and a conventional diaphragm cell for the determination of the velocity values in the different scenarios.

Problematic was also the different requirements, which had to be handled. On one hand the differences had to be reported and visualized within a short notice on the other hand changes provoked by differences in pressure derived from opening of doors etc. as well as irregularities in fan speed and delay times had to be eliminated. Incorporation of the signals into the existing facility monitoring system (Fix) was an additional obligation.

A PLC having the ability to provide the superordinated monitoring system the required signals and receiving set-point- or alarm limit values from the Fix system and at the same time guaranteeing an independent use of the air velocity determination was desired. The customer requested both an alarm visualization and monitoring of the actual values at the point of use.

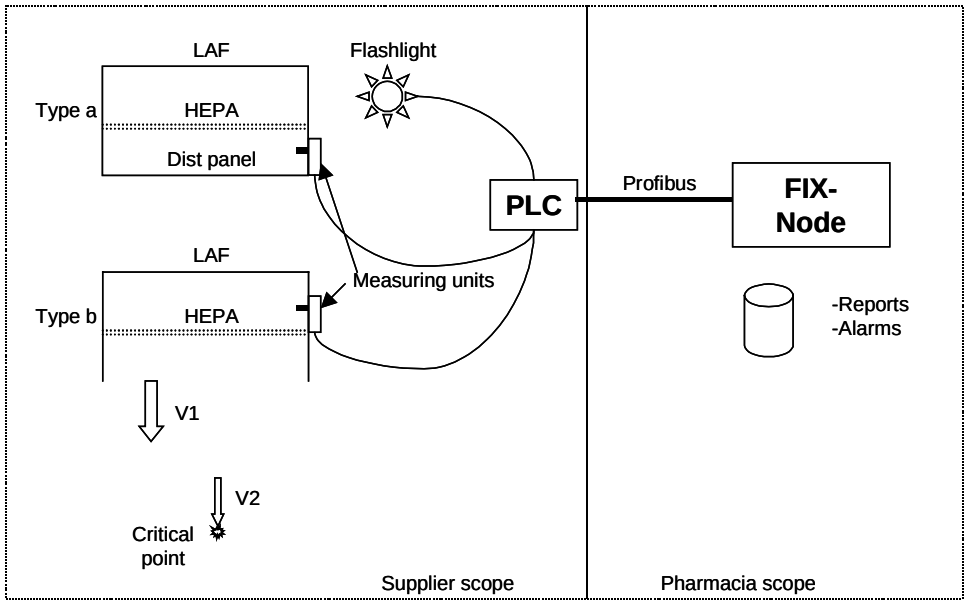


*Figure 1. Picture of the two different systems and the cut of the responsibilities.*

# 3. Potential solutions

Based on the URS and the given data the following possibilities had been evaluated:

a.) Using the classic hot wire anemometers including an adequate protection during the disinfecting cycles.
b.) Determination of the life cycle for a classic hot wire, thus leading to a predefined exchange plan.
c.) Using different technique for the determination by eliminating the influence of the aggressive disinfecting agents. Alternative determination techniques such as flow meters with inert cells, diaphragm meters, differential pressure transmitters at the filters.
d.) Using a independent PLC for the sensors and only providing a signal for the superordinated system (Fix) by a profibus to the interface.
e.) Local setting of the sensor parameters by a wireless device. Calibration and setup of small changes to the sensor. Local read out of the actual values by a hand held palm.
f.) Visualization of alarms at the point of use by a conventional flash light.

# 4. Chosen solutions

## 4.1 Flow meter

The system installed was a flow meter having an inert cell with a determination range between 0–100Pa. The measuring cell used is made of silicon oxide, silicon nitrite and pure silicon and determines the air velocity by the difference of heating loss between the measurement cells. The silicon parts are protecting the measurement elements and allowing the determination of the values under any condition or chemical behavior of the air. Due to the used technique the flow direction can also be determined thus preventing wrong readings in case of negative flow.

This system was used for installation which were fitted with a CG-distribution panel with a uniform distribution of the air velocity. With the delivered palm-software PascalTool it was possible to calibrate the sensor and set environmental conditions locally.

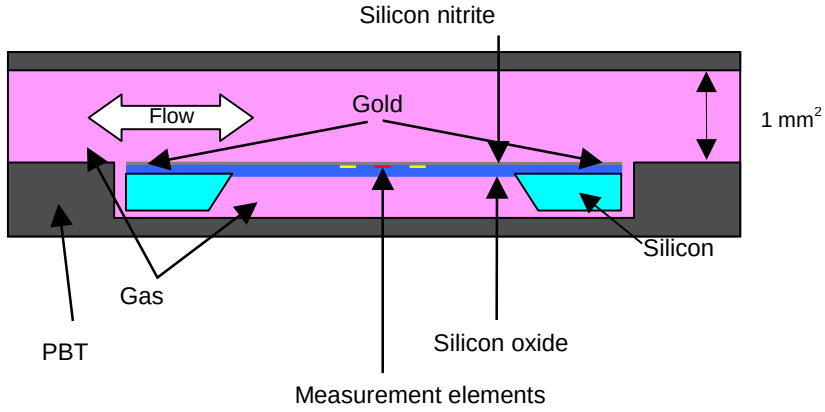


*Figure 2. Measuring principle of the flow cell.*

Technical Details:
Manufacturer / Type: Novasina, CH-Pfäffikon / PascalDat 20/100
Range: -20 – +20 Pa / -100 – +100 Pa
Sensitivity: K0.05 Pa or 1.5% mV / K0.25 Pa or 1.5% mV
Output signal: 4–20mA

Strengths: Reliable system, easy calibration, actual values can be checked with palm, semiautomatic adjustment of tubing parameters and other critical values can be made by a palmδ and the corresponding software delivered by the company

Weakness: Sensitive to changes in material, diameter, form and geometry of tubing

## 4.2 Diaphragm meter

In case the air distribution was not uniform (final filtration without distribution mesh) the air velocity had to be determined by measuring the difference of pressure before and after the filter.The closed cell was a must in order to prevent the contamination of the Zone A/B by air, which was taken before the final filter. This was only possible with a diaphragm cell using a copper-beryllium membrane being capable of measuring differences between 0–250Pa. This type of cell can guarantee the desired accuracy for the use in the critical area.

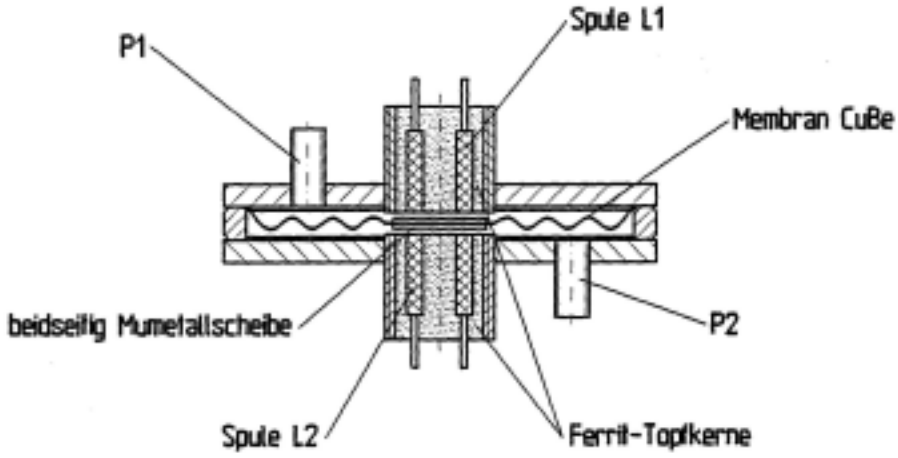


*Figure 3. Measuring principle of the diaphragm cell.*

The measuring device has a unique system to self-adjust the zero value on regular interval thus being capable to compensate drifts during the use and giving reliable readings.

Technical Details:
Manufacturer / Type: Halstrup-Walcher, D-Kirchzarten / DPT P92K
Range: 0–250 Pa
Sensitivity: K1%
Output signal: 4–20 mA

Strengths: High accuracy and long term stability, Automatic adjusting of zero value on a continuous base, prevention of contamination of clean rooms by unfiltered air due to the closed system

Weakness: Mechanical signal has to be transformed into electric signal, equipment does not realize slow blocking of the filter over time thus there is still a potential of providing wrong results.

## 4.3 PLC

A conventional PLC, which is used as the link from analogue to digital signals, was chosen. A central touch panel for the supervisor showing actual alarms was installed and receipt of alarms could be handled in order to keep the production running. The PLC was providing the signals to the superordinated Fix system by a profibus interface.

Technical details:

Manufaturer / Type: Siemens AG, CH-Zürich / Siematic S7-300, TP 170B

Description of the system: The system used was a conventional Siemens Siematic S7-300 unit capable of handling the 44 analog inputs from the different sensors. Alarms are handled by cross-checking the preset limits from the iFixδ system with the inputs from the PLC and in case of a deviation disengage an alarm on the defective installation and at the same time generate a message on the touch panel and creating an alarm log on the Fix system.

Strengths: Reliable system, easy configuration, extension easily possible

Weakness: The PLC itself does not generate alarms thus if used as a stand alone system the use is limited or needs to be reprogrammed as not set points are available.

## 4.4 Visualization/Controls by the operator

Visualization was made using a simple clean room compatible flash light which was indicating an actual alarm. The signal for the alarm itself is provided by the superordinated Fix system, which also decides when an alarm is released. Adjustments for the flow meters are made by a protected palm using a PascalTool software were the set points and local circumstances can be controlled and if necessary be adjusted. Actual air velocity values can also be checked using the palm.

# 5. Execution / Qualification

The installation as well as the IQ had to be completed during the annual shut down of three weeks. Unfortunately a clear pre-check of the situation was due to the production schedule not possible and the material and spare parts had to be ordered according to information given by the drawings and the technical personnel of the site. OQ and PQ had to be completed during the requalification phase of the clean rooms following the shut down. The problems, which occurred during the installation, were mainly due to unforeseen situations regarding the wiring of the equipment in the false ceiling of the clean rooms. This was connected with a substantial increase of manpower as the delays in wiring had to be compensated.

Material, which was missing e.g. cable channels, had to be organized locally leading to certain delays in the process. Regarding timelines we were able to complete the planned work in time and the regular re-qualification activities could be started as planned. Due to the clear and well documented qualification scope by Pharmacia there were no further delays in the OQ phase. The PQ was afterwards completed by Pharmacia's QS department and the new system could be used as of December 2002. Since then no major problems occurred and further installations in other facilities are planned.

# 6. Summary

A reliable system covering the needs from the customer was found using different techniques to determine the air velocity in the critical production areas by eliminating the impact of wrong readings due to defective measuring units. Based on a extended URS provided by the customer there were no main shortfalls in the project.

Unforeseen items could, with the exception of improper wiring plans in the false ceiling, be eliminated due to a detailed project planning in collaboration with the customer. Preparation of the material for the installation could be improved by upfront evaluation of potential local suppliers for additional materials needed.

IQ and OQ didn’t cause any main problems only communication of the internal QA/QS requirements of the customer should be taken into account at an earlier stage. The IQ part could be easy handled during the installation phase. It’s highly recommended that all OQ activities are completed during the planned requalification phase and in close cooperation with the customer and his QS/QA department. PQ was clearly the task of the customer and only supported by the supplier of the installation.

# SYMPOSIUM PROGRAMME

# Programme on Monday June 2nd, 2003

| Time | Pharma session | Food session | Electronic session |
|---|---|---|---|
| 8.00 – 10.00 | **Registration, Exhibition and Coffee** | | |
| 10.00 – 10.30 | Opening ceremony, Raimo Pärssinen, *Chairman of the Programme Committee & Lennart Hultberg, Chairman of the R$^3$-board* | | |
| 10.30 – 11.15 | Activities in Turku Science Park, *Director J Mäkinen, Projektikonsutit Oy* | | |
| 11.15 – 12.00 | Food and feed safety in Europe, *Pirkko Raunemaa, Member of the EFSA Management Board* | | |
| 12.00 – 12.45 | Aseptic processing and sterility assurance – Re-examination of familiar terms and their relevance in process control & product safety *Dr. James E. Akers, Akers Kennedy and Associates* | | |
| 12.45 – 14.00 | **Lunch and Exhibition** | | |
| 14.00 – 14.30 | Finally a new test method for air filters – EN 779, *Tech. Dir. Jan Gustavsson, Camfil* | Nordic dairy hygiene co-operation – DairyNET, *Ass. Prof. Gun Wirtanen, VTT Biotechnology* | Production environment for new interconnection technologies, *Jarmo Määttänen, Elcoteq Network Corporation* |
| 14.30 – 15.00 | Air cleaning device for destruction of microbes based on electroporation effect *Dr. Alexander Fedotov, ASENMCO* | An ultrasonic washing system and its applicability in cleaning of returnable plastic crates, *Irma Klemetti, Valio Ltd.* | Cleanleness demands for production environments of miniature electronics, Jouko Vähäkangas, University of Oulu, |
| 15.00 – 15.30 | **Coffee break and Exibition** | | |
| 15.30 – 16.00 | Fabrics of cleanroom garments *Salme Nurmi, VTT Industrial Systems* | Evaluating hygiene in closed process systems – State of the art *Prof. Alan Friis, BioCentrum-DTU* | New Microelectronics cleanroom facility in Otaniemi, *Ulrika Gyllenberg, VTT Information Technology* |
| 16.00 – 16.30 | Biological indicator D-values – Their application to the sterility assurance levels *Dr. John R. Gillis, SGM Biotech, Inc.* **45 min** | The virtual cleaning test – is it possible? *Dr. Bo B.B. Jensen, BioCentrum-DTU* | R&D environment for micromodule technology, *T. Majamaa, VTT Electronics* |
| 16.30 – 17.00 | | Tank cleaning studies using CFD – A preliminary case study *Satu Salo VTT Biotechnology* | NanoFab – A flexible production system in cleanroom environments *Bo Bängtsson, Bäcken Industrifysik AB* |
| 17.00 – 18.30 | **Free time, bus transportation to Caribia Spa and from Caribia Spa via Hamburger Börs to the City Reception** | | |
| 18.30 – 20.00 | **City Reception in the Forum Marinum area** | | |
| 20.00 – 23.00 | **Evening program** | | |

# Programme on Tuesday June 3rd, 2003

| Time | Pharma session | Food & Biotech sessions | Electronic session |
|---|---|---|---|
| 8.30 – 9.15 | Hygienic equipment and systems – regulations, issues, standards and GMP, *Gordon Farquharson, Bovis Lend Lease* | Towards riskless food? *Director General Matti Aho, Ministry of Agriculture and Forestry* | Management of static electricity and ESD in cleanroom production of electronics *Jaakko Paasi, VTT Industrial Systems* |
| 9.15 – 10.00 | Risk assessment in the pharmaceutical industry, *Dr. Gerry Prout, ICN Pharmaceuticals Inc.* | Safety and hygiene management in manufacturing packaging materials *Dr. Laura Raaska, VTT Biotechnology* | Protective clothing and ESD protection in cleanrooms, *S.Nurmi, VTT Ind. Systems* |
| 10.00 – 10.30 | **Coffee break and Exibition** | | Air quality in semiconductor cleanrooms, *Björn Hellström, Sentera Sensor Systems* |
| | | | **10.20 – 11.00 Coffee break and Exibition** |
| 10.30 – 11.00 | Regulatory aspects of biomedicines *Paula Korhola, NAM* | Comparison of 3 air sampling methods *Hanna Miettinen, VTT Biotechnolgy* | |
| 11.00 – 11.30 | GMP in vaccine production *Maija Hietava, Turku Vocational Institute* | Tools in analyzing the process hygiene, *Tuomas Virtalaine, Net-FoodLab Ltd.* | Routes for *Listeria monocytogenes* in dairy industry, *Dr. Jóhann Örlygsson, IFL* |
| 11.30 – 12.00 | Process development & manufacturing of biopharmaceuticals, *K. Ringbom Biovian* | Process hygiene in food production *Vesa Mäntynen, Atria Oy* | *L. monocytogenes* contamination routes in food industry, *Tiina Autio, Uni. Helsinki* |
| 12.00 – 12.30 | Medipolis GMP, pilot for Biotech companies *CEO Sirkka Aho, Medipolis GMP Ltd* | Functional foods – Are they safe ? *Laura Jalkanen, Functional Food Forum* | Reasons for persistent *L. monocytogenes* contamination, *Janne Lundén, University of Helsinki* |
| 12.30 – 14.00 | **Lunch and Exhibition** | | |
| 14.00 – 14.30 | Validation of a novel rapid sterile transfer port system used in barrier filling lines, *Dr. Suraj Baloda, Millipore Corp.* **50 min** | Hygiene aspects of food process equipment, *Kaarina Aarnisalo, VTT Biotechnology* | |
| 14.30 – 15.00 | | Hygiene requirements in fermentation, *Dr. Ilkka Virkajärvi, VTT Biotechnology* | |
| 15.00 – 15.30 | **Coffee break and Exibition** | | |
| 15.30 – 16.00 | Disposable systems in aseptic manufacturing, *P. Sivertsson, Pharmacia* **30 min** | Resistance phenomena in dairies due to disinfection, Dr. Solveig Langsrud, Matforsk | |
| 16.00 – 16.25 | | Chemical residue testing with photobacteria, *Dr. Juha Lappalainen, Aboatox* | |
| 16.25 – 16.50 | Validation of a novel aseptic connection device, *Dr. P.T Blosse, Pall* **50 min** | Validation of $H_2O_2$ method for sanitation of cleanrooms, *J. Niittymäki, Steris Corp.* | |
| 17.00 – 18.00 | **The Annual meeting of the R3 Nordic Association (for members only)** | | |
| 19.30 – 24.00 | **Banquet at Hotel Caribia Spa** | | |

# Programme on Wednesday June 4th, 2003

| Time | Session on $R^3$ technology | Food session |
|---|---|---|
| 9.00 – 9.30 | Interaction between air movements and the dispersion of contaminants: clean zones with unidirectional air flow *B. Ljungqvist & B. Reinmüller, Royal Institute of Technology* | |
| 9.30 – 10.00 | Risk assessment with the LR-method *B. Ljungqvist & B. Reinmüller, Royal Institute of Technology* | Automatic milking system research in Finland *A. Suokannas, University of Helsinki* |
| 10.00 – 10.30 | **Coffee break and Exibition** | |
| 10.30 – 11.00 | Cleanroom-dressed operator in unidirectional airflow; a mathematical model of contamination risks *B. Ljungqvist & B. Reinmüller, Royal Institute of Technology* | Milk quality on the Finnish automatic milking systems, *Heidi Palomaa, University of Helsinki* |
| 11.00 – 11.30 | Particle dispersion from a cylindrical source in a clean zone with unidirectional airflow, *Rickhard Jonsson, Energo AB* | Milking hygiene in automatic milking *DVM Mari Hovinen, University of Helsinki* |
| 11.30 – 12.00 | Qualification of a pharmaceutical cleanroom facility according to iso 14644-1 and 14644-2, *Lothar Gail, Siemens Axiva* | Cow behaviour in automatic milking system *Satu Raussi, MTT Agrifood Research Finland* |
| 12.00 – 12.30 | Reliable air velocity monitoring in critical areas Andreas Fäh, Luwa Ltd. | Production of immune milk *Reijo Kunelius, Novatreat Oy* |
| 12.30 – 13.00 | **Closing remarks and Invitation to the 35th $R^3$ Symposium in Denmark in May 2004** | |
| 13.00 – 14.00 | **Lunch and Exhibition** | |
| 14.00 – 16.30 | Demonstrations to Turku Polytechnic, Novatreat or Focus Inhalation | |

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# 34th R3-Nordic Contamination Control Symposium

Abstract

R3-Nordic, the Nordic Association for Contamination Control, is a non-profit, independent association for the promotion of new technologies in contamination control in the Nordic countries. The venue of the annual symposium is Turku Polytechnic in the BioTurku region. The aim of the annual R3-Nordic Symposium is to provide knowledge of contamination control and clean room technology dealing with topics in the pharmaceutical, food and microelectronic industries. The topics at the 34th R3-Nordic Contamination Control Symposium are contamination control, clean room technology and management, regulations and standards in clean rooms, clean room clothing, isolation applications, R3 technology and air handling, environmental monitoring in production, process design, production hygiene, cleanability, cleaning and disinfection, risk assessment, risk management in packaging material production, quality systems, contamination control, occupational safety as well as production of pharmaceuticals, biopharmaceuticals, biomedicines and vaccines. We wish that this event will be fruitful in giving background information and new ideas to all participating in the symposium and people reading the proceedings.

Keywords
contamination control, cleanroom, clean room, air handling, hygiene, cleanability, cleaning, disinfection, isolators, clothing, process design, risk assessment, process management, food industry, biomedicines, biopharmaceuticals, pharmaceutical industry, microelectronic industry

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