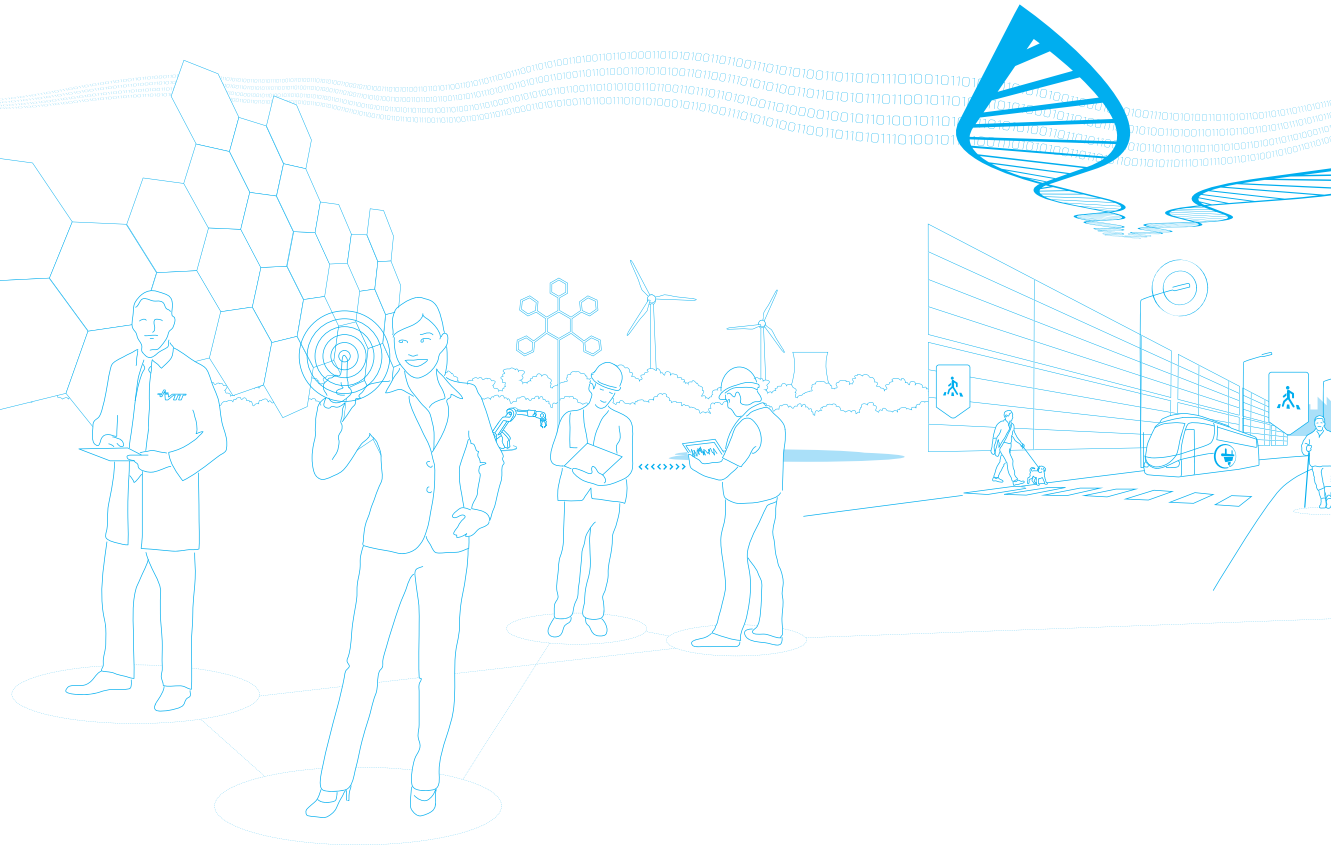
TECHNOLOGY • RESEARCH HIGHLIGHTS • VISIONS • SCIENCE •

168

45th R³Nordic Symposium

Cleanroom technology, contamination control and cleaning

VTT



VTT TECHNOLOGY 168

Keywords:
Contamination control, clean room, cleanroom, air, water, hygiene, cleaning, disinfection, cleanroom clothing, body-box test, airborne contamination, airborne particles, particle measuring techniques, hospital infections, microbiological methods, hygienic design, pharmaceutical industry, hospital, hospital pharmacy

45th R3Nordic Symposium

Cleanroom Technology, Contamination Control and Cleaning

Naantali Spa, Naantali, Finland, May 19–20, 2014

Edited by
Gun Wirtanen & Satu Salo

Program committee:
Satu Salo, Kari Leonsaari, Leila Kakko, Sirkka Malmioja, Antti Mikkola, Raimo Pärssinen & Gun Wirtanen

Organised by
R3Nordic – Nordiska föreningen för Renhetsteknik och Rena Rum

ISBN 978-951-38-8142-9 (USB flash drive)
ISBN 978-951-38-8253-2 (URL: http://www.vtt.fi/publications/index.jsp)

VTT Technology 168

ISSN-L 2242-1211
ISSN 2242-122X (Online)

JULKAISIJA – UTGIVARE – PUBLISHER

VTT
PL 1000 (Tekniikantie 4 A, Espoo)
02044 VTT
Puh. 020 722 111, faksi 020 722 7001

VTT
PB 1000 (Teknikvägen 4 A, Esbo)
FI-02044 VTT
Tfn +358 20 722 111, telefax +358 20 722 7001

VTT Technical Research Centre of Finland
P.O. Box 1000 (Tekniikantie 4 A, Espoo)
FI-02044 VTT, Finland
Tel. +358 20 722 111, fax + 358 20 722 7001

Cover photos: Naantali Tourist Information. Cover logo: Antti Huovinen.

Preface

R^3Nordic, the Nordic Society of Cleanroom Technology, is a non-profit, independent association for the promotion of new technologies in cleanroom technology and contamination control in the Nordic countries. The aim of the annual R^3Nordic Symposium is to provide knowledge within the pharmaceutical, food and electronic industries as well as hospitals and hospital pharmacies. This year the sessions at the 45th R^3Nordic Symposium are Pharma, Hospital and General Sessions and the presentations deal with construction and design, planning, auditing, contamination control, cleanroom technology and management, sterilization techniques, cleaning of clean rooms, protective clothing, monitoring techniques, rapid test methods and regulations in clean and controlled rooms. The venue of the annual symposium 2014 is Naantali Spa in Naantali.

The Programme Committee 2014 (PK14) is lead by Satu Salo and other persons involved are Kari Leonsaari, Sirkka Malmioja, Antti Mikkola, Leila Kakko, Raimo Pärssinen and Gun Wirtanen. The editors of the proceedings would like to express their gratitude to the speakers for preparing the abstracts published in the journal Renhetsteknik 1/14 as well as the extended abstracts or full papers published in these electronic proceedings. We wish that this event will be fruitful in giving new ideas to all participants and exhibitors.

Contents

PLENARY SESSIONS

Risk Analysis: Myths, Confusions and Real Sense

Alexander Fedotov

Invar Project, Moscow, Russia

Abstract

Methods of risk analysis are been actively propagated for pharmaceutical manufacturing for more than ten years and plants are pressed to apply them. What is the sense of these methods, their area of application and real value? Risk means the possibility of dangerous or unwanted event to occur. There may be financial, insurance, traffic risks as well as concerned with faults of equipment, safety of nuclear power stations, etc. People estimate or analyse risk for ages and every day, protecting themselves against unwanted events. The purpose of risk analysis is to understand Reasons – Consequences chain to find proper protection. Any new method should be supported with demonstration of its correctness and practicality with explaining what new it gives. Healthcare has specific feature that can shortly be stated as: No risk for medicinal products is permitted!

What is risk analysis?

Methods of risk analysis are been actively propagated for pharmaceutical manufacturing for more than ten years and plants are pressed to apply them. What is the sense of these methods, their area of application and real value?

Risk means the possibility of dangerous or unwanted event to occur. There may be financial, insurance, traffic risks as well as concerned with faults of equipment, safety of nuclear power stations, etc. People estimate or analyze risk for ages and every day, protecting themselves against unwanted events.

The purpose of risk analysis is to understand the Reasons – Consequences chain to find proper protection. Any new method should be supported with demonstration of its correctness and practicality with explaining what new it gives. Healthcare has specific feature that can shortly be stated as: No risk for medical products is permitted!

EU GMP and ICH Q9 promises

In 2010 ICH Q9 “Quality Risk Management” document was included in Part III of EU GMP Guideline. In the introduction to the EU GMP it says: *“The aim of Part III is to clarify regulatory expectations and it should be viewed as a source of information on best current practices”. The ICH Q9 Guide states: “Quality risk management supports a scientific and practical approach to decision-making”*. These are responsible and promising statements. But are they true or just cover misleading myths?

Methods of risk managements are separated into general and others. The general methods include Flow charts, Check sheets, Cause and Effect diagram (Fishbone or Ishikawa diagram) and others. The Fishbone diagram has the same sense as any other Reason – Consequence chain but it is presented as a fish bone (Figure 1).

Why a fish bone structure? Probably because sea food products are the most part of Japanese menu. Science is not included in this at all. Fish bone picture is only one example from numerous variants e.g. tables, schemes and graphs that we all use for ages. This is a simplest logic analysis that can be carried by anyone.

Other methods include e.g. Failure Mode Effect Analysis (FMEA), Failure Modes, Effects and Criticality Analysis (FMECA) and Hazard Analysis and Critical Control Points (HACCP). Here below follows a closer presentation of FMEA. Other methods have the similar sense.

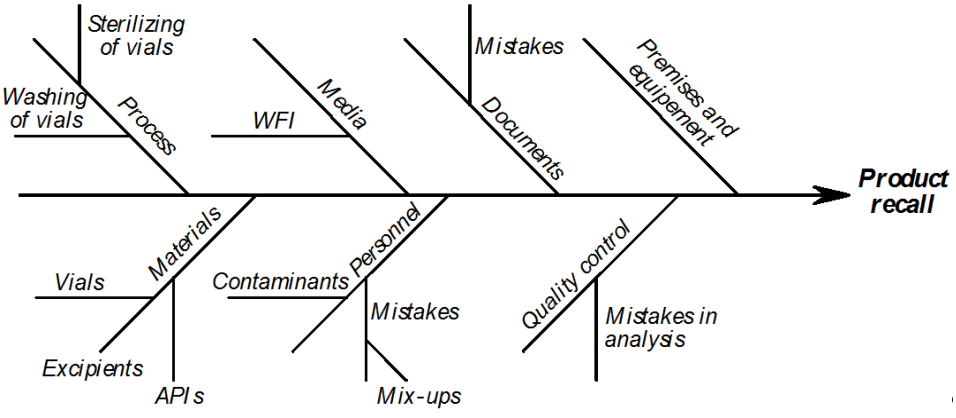


Figure 1. Example of fishbone diagram.

FMEA method: comparisons of incomparable

It is stated that FMEA gives “Quantity estimation of risk”. All risks shall be grouped on evaluation criteria: 1) Severity/Impact (I), 2) Occurrence or estimation of probability of event (O) and 3) Detectability (D). Each of criteria has numerical values e.g. numbers from 1 to 5, where 1 represents the lowest risk and 5 means the highest risk.

The Risk Priority Number (RPN) is then calculated by multiplying evaluation criteria. RPN range is from 1 (when all criteria are 1) to 125 (when all criteria are 5). RPN grows with increased risk. The acceptance level shall be specified in advance. If RPN < Acceptance level, then risk is low and no further action is needed to be implemented. In contrary correction actions are needed. This is a very dangerous approach! It has three fundamental mistakes:

I. Evaluation criteria are appointed by human subjectively. This arbitrary estimation serves further as a basis for responsible decision. What is this masking for, if one can take decision directly, say, observing poor seam welding in piping of Water for injections system?

II. Values with different sense are multiplied and the result serves as a ba-sis for conclusion. It is a huge and obvious methodical mistake. It is not possible to multiply values of different sense.

III. This arbitrary numbers are offered for estimation with high responsibility on safety, where no risk is possible! FMEA method compares incomparable issues and is not correct in principle.

Here follows two events as examples: 1) Delay of plane arrival is not dangerous (Severity is equal to 1), but can occur rather often (Occurrence is 5) and 2) Crash of plane is dangerous (Severity is 5), but very rare (Occurrence is 1).

Probability to find both events is 5 (both events shall be noticed always). Multiplying of evaluation criteria gives RPN equal to 25 for both cases, so they are equivalent in respect of risk analysis. However, it is better than discussions of medieval monks from Thomas Aquinas times on topic “How many devils can be accommodated on the tip of the needle”?

Examples for pharmaceuticals

Example of application of FMEA method for tablet press was published by Knoll (2008; p. 144). This is an example showing the sense of the method. The acceptance level is specified as 27. Results of such analysis are given in Table 1 that was published as an example to follow (not for fun). There is no result exceeding the acceptance level 27 in any line of the Table. The calculations for line 5 (patient death) gave a RPN-value equaling 10, which is less than 27. The decision was taken that no action was needed, because the risk was lower than the acceptance level. A “Scientific approach” also states that mix-up of products caused patient death (RPN = 10) was better than wrong weight (RPN = 15). This is absolute rubbish!

Death and harm cannot be compared by any numerical manipulations. Common sense has been ignored. Such situation, in which there ia a danger for human death, shall be excluded without any evaluations and numerical manipulations. Fundamental mistakes of this method was pinpointed earlier. Dangerous events shall be excluded and not averaged. In exercises we should keep in mind any danger for the society. It is not possible to com-pare severity of event and its probability of appearance.

It is never appropriate to use an arbitrary chosen number for responsible decisions. Why was this number chosen as 27, not 7 nor 80? It is better to make final decision in an arbitrary honest way, than by using pseudoscientific methods? It would be worth asking the advocates, how they imagine themselves as victims in dangerous events and whether they agree in such approaches. The author of this paper has asked such question several times and the answers were always the same (after puzzled pause): your question is not scientific and not correct. But why is it not correct? Is a guesswork on coffee ground with mathematical masquerade correct?

Deliberate clouding and pseudoscientific methods are the same – but in a simpler way – as misleading or cheating people. It would be the same as to place people suffering with cold, dysentery and plague in the same room and then to give everybody evaluation criteria, calculate the RPN, specify the Acceptance level and after all these draw conclusions on whether the room ventilation is necessary or not. It would be the same as to multiple person's height and weight and to judge on the body temperature, which is absolute nonsense. This type of sinecure shall be taken away from initiators on risk analysis, because juggling safety is not appropriate. Manufacturing of medicinal products is a much too responsible matter, which should be entrusted only to professionals understanding the responsibility of their jobs.

Inspections and Mine of delayed action

ICH Q9 (Part III of EU GMP) states that it is helping the manufacture and the inspectors. How does it help manufacture? Does it help in constructing process flow charts, in finding critical points, in drawing HVAC (heating, ventilation, and air conditioning), water treatment or other schemes? All above mentioned shall be in the design, because the production cannot work without them. The arrangement of routine testing/control and writing documents is already in the GMP.

It is necessary to request to execute GMP norms and not artificially created methods. Everything in the manufacturing of medical products is linked with safety. No risk is allowed!

How about the statement "Risk analysis helps inspectors". How does it help? Völler (2008), who is an inspector, gave answer in an article. He wrote that an inspector has not enough time and cannot see all details. Pa-pers on risk analysis prepared by manufacturers make the inspector's task easier to understand the work in the plant. This is a very important opinion: Inspector observes not primary documents (water treatment schemes, batch records, laboratory records, etc.), but secondary ones, i.e. papers that reflect primary sources only partly. A fundamental danger is hidden in this approach. The inspector draws conclusion on compliance of manufacture to GMP requirements based on the manufacturer's own interpretations, not on primary documents. But in GMP the requirements are set towards primary documents, not towards interpretation.

Table 1. Example of risk assessment [1].

№	Process step or equipment	Possible failure/risk	Consequence of failure	Cause of failure	Planned measure (to minimize risk of failure)	Occurrence 1–5	Severity 1–5	Detection 1–5	RPN	Further action Yes/No
1	Machine preparation	Cleaning not sufficient	Cross contamination/microbiological contamination	Human	Optical control before assembly of machine parts	1	5	2	10	No
2	Machine preparation	Recalibration interval violated	No GMP conformity	Organization	SOP	1	4	2	8	No
3	Machine preparation	Punches installed not correctly	Tablets contaminated (metal) machine defect, loss of production	Human	Control of correct installation (manually)	1	3	1	3	No
4	Loading	Not enough loading goods	No delivery of granules for the compression process	Sensor defect	None	2	2	1	4	No
5	Automatic loading	Wrong granules	**Patient dead**	Mix up of product	•Correct labeling •Electronic check of label – identity	1	5	2	10	No
6	Machine adjustment	Wrong Adjustment	Tablet content too high, patient harm	Human	Double check Control of mass	1	5	1	5	No
7	IPC	Balance displays wrong positive values	Wrong weight, patient harm	Technical defect	Daily balance Calibration	1	5	3	15	No
8	Etc.									

It would be interesting to look how tax inspector will check the company on free interpretations of financial documents made by people who are inspected, and not on the very documents. The customer buys medicinal product at drug store that must comply with primary documents, not with exercises. Thus it cannot be allowed to evaluate manufacture by extracts from documents or comments, especially made by manufacture under control. This is a mine with delay action! If three days are not enough, let the inspector spend two weeks for study. The consumers need only reports of high quality inspectors.

History of risk analysis and some remarks

Risk analysis appeared in late 1940s. It was the time of technical explosion, when absolutely new electronic micro-mechanical systems were introduced. They were different from systems known before: density of elements in volume unit of equipment and number of elements were growing very quickly, by orders of magnitude in a short period of time. It was not understood at once that micro-miniaturization requests new approaches to provide high reliability of systems. As a result thereof technical systems had poor reliability and low availability.

The first Electronic Computer Machine in the USA could work without failure only for some hours. In 1949 about 70% navy radio-electronic equipment of US armed forces were inoperable because of malfunctions, and 50% of equipment and spare-parts failed during storage in ware-houses. It was impossible to rely on defense systems that were deemed to be effective. New principally methods to provide reliability were needed. But this was not understood at once.

It was the time of Cold War. The world was under strong fear of global disaster.

It is not a surprise that those who offered methods of solving all problems got immediate support. They simply found themselves in the right place on the right time. Just a promise to solve problem ensured budgeting on trust only and risk analysis was such an application. The FMECA method was approved by US Ministry of Defense in 1947 and one started to work on risk analysis methods. Nobody noticed the fundamental mistake in this method that compares incomparable. Of course, somebody had a dream to develop general method of solving all specific problems, not knowing their details. This idea failed. Only very common advices can be given. We all know them from own experience. It is not possible to obtain valuable in-formation without knowing the subject in-depth. But if one has knowledge on every screw and joint, then the artificial advices give no added value.

In the 1950s-70s it was widely discussed in special literature dealing with cybernetics and reliability mathematical models. As a result thereof formal methods were rejected by practitioners, who were involved in design and operation of high responsible technical systems, because non-correct principles and useless methods. It is necessary to understand in-depth technical system to prevent malfunction. Professionals have such knowledge and do not need facile or wrong advices. In contrary general ideologists are useless because of lack of competence.

US Ministry of Defense cancelled FMECA in 1998. It is hardly believable that such agency could withdraw useful document. Time was passing and risk analysis businessmen understood that they have nothing to do in technical industries, where many professionals are competent in cybernetics and similar sciences. And they turned to food industry in 1980–1990s. It was a great success for them. It occurred to be possible to exploit trust of

people in scientific-like methods with great profit. Pharmaceutical industry was the next. We thus observe very well conducted companies to occupy rich markets.

Rational sense of risk analysis

The discussion above concerns present requirements, but is there any useful sense in risk analysis? Yes, there is. But it shall be professional and useful. In existing manufactures the following actions should be undertaken:

1. To estimate manufacture compliance with GMP requirements.
2. To review documentation to bring to compliance with GMP and to execute all plans with arrangements according to GMP.
3. To organize distinct analysis system of Out-of-Specification (OOS) deviations. It is the most important element of risk analysis for existing facilities. It allows to prevent many dangerous situations, complaints and product recalls.
4. To arrange analysis of complaints, returns and product recalls.
5. To develop and undertake necessary measures on results of OOS analysis and to define whether they were effective.
6. To continue process analysis (OOS), analysis of reliability (failures) and removal of disadvantages.
7. To do all this job continuously with arranging self-inspections regularly.
8. To make trend analysis, to conduct data base for deviations and failures systematically, sorting out by reasons. To make annual report on all finding and actions undertaken. To develop program of improvements for the next year.

This job shall be properly arranged. An example performed at Nutricia-company follow. The experience of company Nutricia (the Netherlands) is a good example of clever and effective risk analysis. This company is well known for manufacturing nutrients for children. In 1993 the batch of product contained residues of disinfectants was recalled from the market. This accident pressed the company to implement the risk analysis system. The company applied an intellectual, not formal, thinking approach that was focused on sense of matter to achieve effective results. The system had the following elements:

- 36 risk analysis groups were arranged at the head factory; 20% of factory's personnel were members of these groups,
- mean number of persons in the group was 5,
- each group was gathered weekly for short working meetings,
- internal audits (self-inspections) were arranged,
- analysis of customer's complaints was organized,
- inspections of suppliers was thereafter conducted systematically and
- data base on contamination was arranged and revised with continuous support.

The problematic places were soon revealed as: 1) personnel, 2) contamination, 3) defects in raw materials and 4) deviations out-of-standards. These issues are very close to problems in pharmaceutical factories.

The scheme for work on risk analysis is simple (Figure 2):

- to conduct register of out-of-standards deviations (complaints, etc.);
- to find narrow spots (problems in critical control points);
- to eliminate weak spots;
- to analyze factory's work;
- to find narrow spots again, etc.

Typical risks for pharmaceutical manufacturing are:

- wrong content of product;
- contamination;
- mixings of products;
- wrong labels, etc.

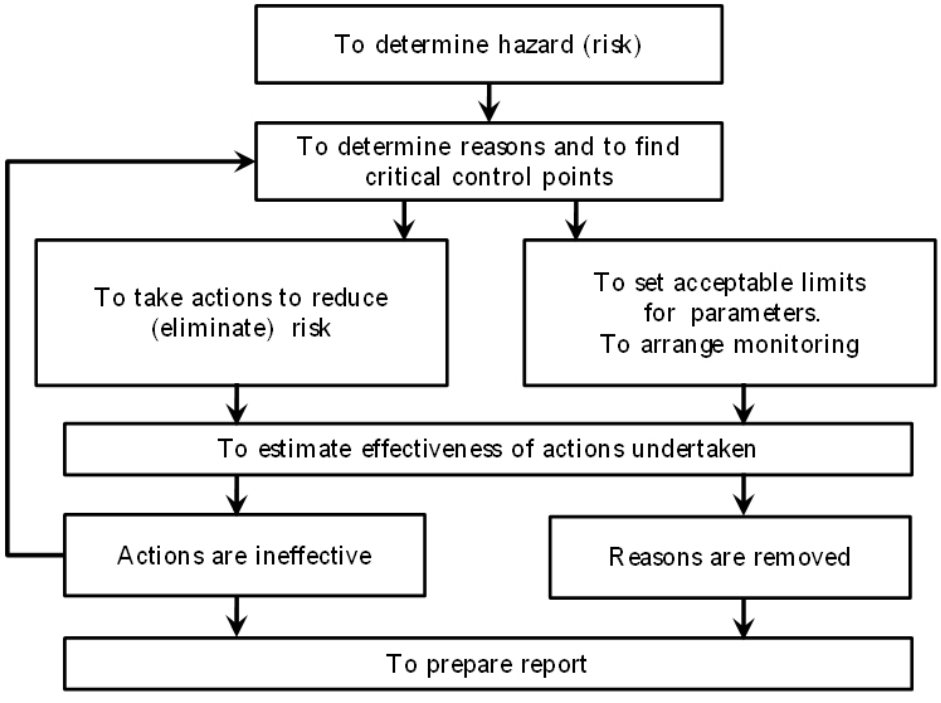


Figure 2. Scheme of work on risk analysis for existing facilities.

There can be following reasons for risks:

- poor equipment, processes and premises,
- bad materials,
- mistakes of personnel,
- poor design,
- poor procedures for surfaces cleaning with contamination of next product by residues of previous product or detergents, as a consequence,
- poor sterilization process and
- badly arranged operation and maintenance etc.

The stages of work in new facilities are:

1. To design the facilities, which is the fundamental step. Many risks can be excluded at this stage simply by following GMP Guide,
2. To construct facility according to design, then to make start up and testing,
3. To develop documentation and to follow GMP requirements in operation,
4. To select suppliers so as to exclude risk of defect because of materials,
5. To train personnel to decrease or to exclude risk and
6. To make OOS analysis, to estimate own work, etc.

Conclusion

Let us think on myths, confusions and wide acceptance of risk analysis methods described above and make a pause. We all belong to creatures named “Homo sapience” that means “Wise man”. If so, why do we accept exercises like those in the FMEA method and what is the driving force for all this? The answer is simple. Global turnover of validation companies amounts to billions and billions of dollars. Much of them have pseudo-GMP sense, known as “GMP – Great Mounts of Paper”. Thus validations are very much discredited and a new fashion to withdraw money appeared under the name of “Risk analysis”.

Firstly, it came as ICH Q9 guide (recommended document), then it was transferred to EU GMP as Annex 20 (only as informational again), then it was moved to a specially formed Part III. The idea of all these movements is clear: it is to increase role of risk analysis from information only to near-ly mandatory level, keeping in mind that recommendations become mandatory for practitioners if inspectors expect that they are to be followed. And the market is ready.

Special danger of methods enforced is that they accept unacceptable events. These methods, moving from the office of consultant to manufacture can be used by somebody to justify wrong work. It is well known that a method has no right to exist in two cases i.e. if it is wrong and misleading for users or if it gives trivial result (result obtained by simpler means or is obvious). ICH Q9 methods fall under these two cases and are not suitable for

use. Risk analysis can be useful, but it is not panacea and it has limited area of application that should not be overrated.

Some people trust that there can be magic tools to solve complex problems. This trust is a ground for cheaters of all kinds. Only knowledge and thorough detailed analysis of specific problem made by professionals can give positive result.

Everybody speaks about manufactures, inspectors and consultants. But what about customers, who are the main party? They are not aware on how quality of medical products is provided. But they have right to obtain this information. Just think about what their reaction can be when above mentioned methods are described.

GMP rules appeared 50 years ago because requirements how to arrange manufacturing were needed. At first they were clear and transparent. Further editions added a lot of cloudy and confusing texts with zero use for quality. Risk analysis is the next step. It can be the last droplet that should turn minds to understand that modern GMP is moving too far aside from its primary purpose. It is time to clean GMP from husk and create high quality normative document.

References

Knoll, A. 2008. Risk assessment in pharmaceutical engineering. GMP Re-port, No 3. Risk management in the pharmaceutical industry. Pp. 139–152.

Völler, R. 2008. Risk management from the point of view of a competent authority. GMP Report, No 3. Risk management in the pharmaceutical industry. Pp. 11–21.

Safety in Health Care Business

Tom Ståhlberg

Finnish Health Technology Association, Finland

Extended abstract

Safety in healthcare business can be summarized to be the critical balance between medical benefits offered to the patient and the possible risks taken – the more severe disease the more risks can be taken and vice versa. Thus, the balance will always be dependent on the needs of the patient, the capabilities of the physicians and other healthcare professionals, and, the available technology. An equation to be differently solved depending also on e.g. ethical issues and the economic status of the society concerned. In my presentation, the roles and responsibilities of two main actors, i.e. the medical device industry and the health care providers, will be discussed in order to see how they best can work in concert in order to provide efficient and safe diagnosis and care of patients. Furthermore, the guidance to ensure safety provided by regulations and regulators will be elucidated from preventive to post-market surveillance points of views.

Medical devices (MD) and in vitro diagnostic medical devices (IVD) are essential in almost any aspect of human healthcare and are thus critical for both diagnosis and treatment of patients, but also for monitoring the outcome of treatment and for successful preventive measures. A medical device must fulfil its promised medical benefits without introducing unnecessary and unacceptable risks to the patient regardless if it is a software, instrument or chemical product. In EU, the regulatory framework for medical devices is constructed through three different Directives (Active Implantable Medical Devices 90/385/EC; Medical Devices 93/42/EC; and, In Vitro Diagnostic Medical Devices 98/79/EC) and the amended Medical Device Directive 2007/47/EC. The main driving force of the Directives is to ensure the safety, quality and performance of medical devices by ensuring that medical devices do not compromise the health and safety of patients, users, and third parties. In September, 2012, the EU Commission published proposals for EU MD and IVD regulations which eventually will be replacing these Directives. These proposals are still processed by both the EU Parliament and the EU Council, but the

current proposals and suggested changes will be discussed as far as aspects linked to safety. Similar safety aspects are also the main driving force in other global MD and IVD regulations, and, therefore, a comparison between EU MD regulations and their international counterparts will be presented from a safety point of view, with a special emphasis on the US regulations and the working documents from IMDRF (International MD Regulators Forum).

The regulations of MD and IVD can be simplified by stating that the products are regulated 1. through registration procedures and product specific demands, and, 2. through quality management system requirements regulating the entire company. The registration procedures and product specific demands are based on risk classification of the concerned MD/IVD in all specific regulations globally. The risk classification is based on the seriousness of the disease or health impact, but also taken into account if the product is to be used by professionals or laymen. The higher the risk class, the more stringent demands! Current EU classification (e.g. for IVDs: general, self-testing and Annex II List A or List B products) compared to the new proposed regulations (Risk classes A to D) and their relationship to both patient risks and global risk classification systems will be discussed. Furthermore, the relationship between risk classes and the conformity assessment of products will be elucidated.

Manufacturers developing and producing MD and IVD are in practice obliged to have a quality management system based on ISO 13485:2003 (MD – quality management systems – requirements for regulatory purposes). In EU, the corresponding version is EN ISO 134845:2012, with the only difference being that this harmonized standard also contains Z appendices describing to which extent the standard fulfils the requirements described in the Directives. The formal objection leading to this revised standard version will be described and also why the ISO 9001:2008 is by far not enough for the medical device manufacturers. A fundamental update of the ISO 13485 will be accepted and published later in 2014 and the safety related features of the forthcoming version will be discussed. Especially, the stronger emphasis on risk management, mainly linked to the implementation of ISO 14971:2007 (MD. Application of risk management to MD), will be outlined from a change management point of view.

The ISO 14971 provides manufacturers with a framework within which experience, insight and judgment are applied systematically to manage the risks associated with the use of medical devices. I.e. identifying the hazards associated with a MD, estimating and valuating the risks associated with these hazards, controlling these risks, and monitoring the effectiveness of that control. In line with e.g. the EU Directives, the standard deals with processes for managing risks primarily to the patient, but also to the operator, other persons, other equipment and the environment. The standard has been widely accepted internationally and is e.g. a harmonized standard in EU and a recognized standard in both the USA and Canada. The starting point is that the use of a medical device entails some degree of risk. The acceptability of a risk must be judged in the context of a particular clinical procedure with the residual risks to be balanced against the anticipated benefits of the procedure. Two erroneous interpretations will be discussed. Firstly, the medical benefit-risk balance judgment must be based on the intended use, performance and risks associated with the medical device, as well as the risks and benefits of the clinical procedure and the circumstances of use. However, this fact, does not exclude the need to also consider the generally accepted state of the art in addition to the medical benefit-risk

balance! Secondly, the goal of MD risk management is not limited to the actual product (and thus the use of specific tools like FMEA), but is much broader. I.e. the goal is to incorporate risk management throughout the quality management system from e.g. management to each subsystem and throughout the entire life cycle of the product from development to post-market activities.

In order to provide safe medical diagnosis, treatment and monitoring of patients, when using medical devices, it is critical that the quality management systems of the manufacturer (ISO 13485) and the health care provider (e.g. ISO 15189:2012 Medical laboratories – requirements for quality and competence) are implemented successfully both separately and so that the implementation ensures efficient and reliable connections between the two counterparts. This is critical both in normal use (installation, qualification, validation, use and maintenance of the MD) but also, and may be especially, when something goes wrong. Complaint handling possibly leading to CAPA (corrective and preventive actions) and further to possible recall actions and/or incident reporting must be well integrated in the quality management systems of both the manufacturer and the health care provider. Only a good systematic connection between the corresponding quality management systems, will guarantee that the flow of events between a complaint or an incident report from a user and procedures at the manufacturing site may ultimately lead to efficient recall actions hindering in a timely manner that a deficient product may compromise the health of a patient. Thus, the vigilance systems linking a manufacturer and a health care provider using its MD must be seamless and efficient.

But, the marriage between a manufacturer and the user is not important only in these vigilance situations, but covers everything from defining the user requirements and specifications of the product under development to the production and quality assurance of the product, and includes also transportation, storage, use of the product and disposal of the product. One important aspect is the actual interface between the user and the MD – usability is thus one aspect to be discussed. And, all aspects of this marriage between the user and the manufacturer are strictly regulated by regulators on the health care provider and manufacturer sides, respectively!

Energy Saving in Cleanrooms

Alexander Fedotov

Invar Project, Moscow, Russia

Abstract

Energy saving is one of the most important problems of modern civilization. Cleanrooms consume a lot of energy, but common practice often specifies big power without proper understanding what for. This presentation discusses ways to reduce energy consumption, especially by means that are nearly obvious and can be realized immediately e.g. reducing of air exchange rates and increasing recovery time. Examples based in real designs made by company “Invar-project” are shown.

Introduction

Cleanrooms consume a lot of energy in comparison with non-classified areas. But there was a lack of proper attention to this problem until recent. In contrary, over-specifying of requirements and over-designs can be observed. This negative picture is often based on the normative documents or a wrong interpretation of them. Existing international standards are too general and do not reflect specific features of cleanrooms. Detailed guidance how to save energy in cleanrooms can be found in two national standards: British standard BS 8568:2013 [1] and VDI 2083 standard [2]. But the problem is so complicated and not enough investigated that further efforts to reduce energy consumption are needed. Special attention should be paid to reducing of air exchange rates and considering of real particles generation in cleanrooms to determine really needed air exchange rates in operation.

Energy consumption grows quickly with increasing of cleanliness level. It is very difficult to make general estimation, but practical examples show that power required for cleanrooms can be on orders of magnitude bigger than for non-classified room, depending of cleanliness class (Table 1). The air exchange rate depends also on e.g. heat load, local exhausts and personnel present, which is different for different processes. But the

trend is clear: energy consumption grows dramatically from one cleanliness class to another. It can be different for one class depending on air exchange rate, recovery time and other factors as shown below.

Table 1. Examples of energy consumption for different facilities.

Class	Power, kW/m^2	Energy consumption growth
Semiconductor facility		
Non-classified	0,05	100% or 1,0
8 ISO	0,10	200% or 2,0
7 ISO	0,22	440% or 4,4
6 ISO	1,15	2300% or 23
4/5 ISO	2,40	4800% or 48
Non-sterile ointments production		
Non-classified	0,06	100% or 1,0
D	0,60	1000% or 10
C (laboratory)	1,20	2000% or 20
Aseptic production		
Non-classified	0,06	100% or 1,0
D	0,41	680% or 6,8
C	0,50	830% or 8,3
B	1,52	2530% or 25,3

Methods of energy saving

Energy saving methods can either be general i.e. for all kinds of premises and buildings or specific i.e. for cleanrooms.

General methods focus the attention on:

- minimizing heat gain/losses and providing heat isolation of buildings,
- heat recuperation,
- air recirculation with minimum outdoor air as possible on safety reasons,
- placing plants in climate zones where high power in winter/summer is not needed,
- use of high efficiency ventilators, AHUs and chillers,
- use of equipment (HVAC units, filters, etc.) with reserve of power, taking in mind that equipment with bigger nominal power will consume less energy,
- avoiding unnecessary narrow tolerances for temperature and humidity and / or
- removal of heat load from process equipment rather by local in-built means then by HVAC systems and so on.

Specific methods concern cleanroom features and include:

- reducing cleanroom squares to minimum,
- avoiding over-specifying cleanliness class,
- use of HEPA filters with reduced pressure drop (membrane filter 50 Pa instead of 250 Pa glass fiber filters),
- sealing of leakages,
- local protection when higher cleanliness class is maintained in a limited area as required by process,
- minimizing number of personnel or using technologies without people (closed, RABS, isolators),
- careful attention to operation, garments, hygiene, training, etc.,
- reducing of air flow rate when not in operation,
- avoiding over-specifying of air exchange rates and recovery time and /or
- determining really needed air flow rates during testing and operation and adjusting air exchange rate to minimum based on these data.

Energy consumption differs for unidirectional and non-unidirectional flows. For **unidirectional air flow** the key factor is air velocity. It .was discussed in many publications that 0.45 m/s is over-specifying and 0.3 m/s are enough. Non-GMP areas of cleanroom application follow this rule successfully.

GMP specifies 0.36–0.54 m/s (0.45 ± 20%) now. It is interesting that in 1989 GMP EC wrote *"Laminar air flow systems should provide homogeneous air speed of 0.30 m/s for vertical flow and 0.45 m/s for horizontal flow"*.

Air exchange rate is the key factor for cleanrooms with **non-unidirectional air flow**. Majority of cleanrooms belongs to this family and the picture for them is more complicated. So let us make detailed analysis for this case.

Air flow rate and air exchange rates

High air flow rates in HVAC systems that depend on air exchange rates in rooms are the main reasons for huge energy consumption. They cause great demands for air ventilation, heating, cooling, humidification and drying for cleanrooms with non-laminar (turbulent) air flow.

How does energy consumption depend on air exchange rate? Different sources of information present different numbers. BS 8568:2013 (item B.15) says that "A 50% reduction on flow gives an 88% reduction in power". Other publications give less difference. In fact, it is hardly possible to give one formula for any case, because energy consumption depends on too many factors. So it is worth to make short analysis for facility at design stage and to estimate how air exchange rate influences on power required. Table 2 shows such example for Moscow region. In a real facility halving of the air flow (50%) gives a reduction of energy consumption to a level of 25–30% i.e. 3–4 times less.

Air exchange rate depends on different factors: 1) necessary volume of outdoor air for hygiene reasons i.e. breathing, 2) compensation of local exhausts, 3) maintaining pressure difference, 4) elimination of heat loads and 5) providing necessary cleanliness i.e. depending on the cleanroom class. All these factors should be taken in consideration at the design stage and the air exchange rate should be chosen for "worst case" condition. For cleanrooms requirements air exchange rate can be critical in most cases.

Table 2. Power consumption for cleanrooms of non-sterile ointments facility.

	Energy consumer	Power, kW for air flow	
		22250 m³/h	11000 m³/h
1	Ventilators in HVAC system	30	15
2	Air heating:		
	- hot water (1st stage),	240	151
	- electric heater (2nd stage)	80	20
3	Cooler (electric power)	53	13,5
4	Humidification	133	45
5	Total consumption:		
	- winter: electric power;	243	70
	hot water	240	151
	- summer: electric power	163	48

What air exchange rate is needed? Today most normative documents do not specify exact numbers for air exchange rate, it is left to the designers. But some documents still do specify these numbers. The most known requirement is 20 h^{-1}. The development of norms for air exchange rate is based on history.

Early age. In late 1950s USA set requirements for air exchange rate in surgical suites to be a minimum of 12 h^{-1} in existing facilities and of 25 h^{-1} in new ones.

US Federal Standard 209. Probably the first mentioning of 20 h^{-1} was in early version of old Federal Std. 209B. It reflected practice of 1960s. Later this norm was withdrawn from Fed Std. 209, because it was understood that proper cleanliness level can be achieved with less air exchange rate. The task of determining air exchange rate was left for decision of designer.

FDA Aseptic Guide. FDA Aseptic Guide copied 20 h^{-1} in 1987 and nobody took care to make correction even in 2004 when this Aseptic Guide was re-edited [3]. Today FDA requirement for 20 h^{-1} for supporting rooms (8 ISO in operation) is still in force: *"For Class 100,000 (ISO 8) supporting rooms, air flow sufficient to achieve at least 20 air changes per hour is typically acceptable. Significantly higher air change rates are normally needed for Class 10,000 and Class 100 areas"*. For ISO Class 7 this Guide requested even greater air exchange rate than 20 h^{-1}.

GMP EC Guide. Early GMP EC Guide had this norm but it was cancelled in 1997. It is worth to say that this step did not give much use, because conservative requirement for recovery time 15–20 min remained (see be-low) [4].

ISPE Baseline. Many practitioners use ISPE Guide that recommends 20 h^{-1} for sterile process (both aseptic and terminal sterilization): "*Generally, air changes of at least 20 h^{-1} are expected in Grade 7 and Grade 8 rooms"* (ISO 7 and ISO 8 in operation respectively), item 5.5.3 [5].

WHO report. The recent WHO report moved towards reduction of air ex-change rate: *"4.1.6 Air exchange rates are normally determined by the following considerations (could normally vary between 6 and 20 air changes h^{-1})"* [6]. This is a step forward, but it can be neglected be-cause the same report specifies strict values for recovery time: 20 min (item 4.1.10) and even 15 min (item 8.2.14, Table 3 of this Report). WHO Guide says about 6–20 h^{-1} without explaining when and what to choose.

General. Old conservative value 20 h^{-1} is still strongly sitting in documents and em-barrasses minds (Table 3). There are no indications for non-sterile facilities, but some designers prefer to use these 20 h^{-1}, too. Sometimes 20 h^{-1} travel from "in operation" requirement ISO Class 8 of FDA to zone D where ISO Class 8 is specified for "at rest" condition only and for "in operation" no requirements exist at all!

Figure 1 shows curves of reducing particles concentration with time after starting of cleanroom operation. Calculations were made using computer program presented by Prof. J. Gustavsson.

Table 3. Requirements for air exchange rates in different guides.

	Sterile products			Non-sterile products
	Aseptic, before or after sterilizing filtration		Terminal sterilization	
	Before	After		
FDA Aseptic Guide	20	20	-	-
GMP EC	-	-	-	-
ISPE	20	20	20	-
WHO	6-20			

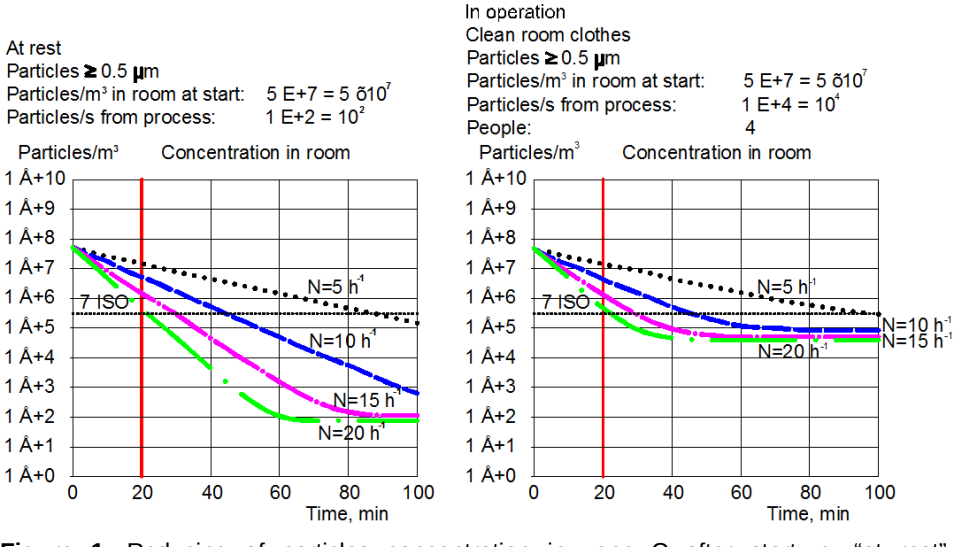


Figure 1. Reducing of particles concentration in zone C after start-up: "at rest", particles generation 100 particles/s, no personnel (to the left) and "in operation", particles from process 10000 particles/s with 4 persons (to the right).

Required cleanliness class can be maintained easily for different values of air exchange rate, even for the rate 5 h-1. All curves tend to some constant value that is quite good for this cleanroom. The difference is in recovery time. It is very important to note that the air exchange rate depends not only on cleanliness class. It greatly depends on recovery time.

Recovery time

What is recovery time? ISO 14644-3:2005 [7] says that recovery time is time required for decreasing initial concentration of particles by factor of 100 (100:1 recovery time). Some other guides specify recovery time as time required for cleanroom to reduce contamination level of “at rest” state after being “in operation” condition. Normally it is deemed that recovery time should be 15–20 min (Table 4).

Table 4. Requirements for recovery times in different guides.

	Sterile products			Non-sterile products
	Aseptic, before or after sterilizing filtration		Terminal steriliza-tion	
	Before	After		
GMP EC	15-20			-
ISPE	15-20			-
WHO	15-20			

GMP EC specifies recovery time 15–20 min for sterile production and does not make difference between **aseptic** processes and processes with **terminal sterilization** for recovery time. It does not make difference for process stages before and after sterilizing filtration, setting for both is 15–20 min. But these cases are different in principle!

It is obvious that norms for aseptic and terminal sterilization processes should not be equal. The same is for different stages of aseptic process: stages before sterilizing filtration can be weaker, than for aseptic core. The strictness of requirements shall depend on the risk of the products. This risk is dramatically different for stages before preparing stages i.e. sterilizing filtration and dry-heating of glassware, and in final operations i.e. filling and stoppering.

From the norms and their interpretation we can note interesting psycho-logical aspect. 20 h^{-1} were in Fed. Std. 209 and FDA Guide for aseptic only, then it spread everywhere. The same is for recovery time 15–20 min. This is one of the main reason of over-design, which lead to unnecessary energy consumption.

Clothes have great impact on air exchange rate. Dr. B. Reinmüller and Prof B. Ljungqvist made detailed investigations of particles generation by personal dress in different kinds of clothes. Here below rounded numbers are used to make the illustration simpler i.e. the cleanroom clothes (jacket and trousers) generated 10000 particles/s and good cleanroom overall clothes generated 1000 particles/s.

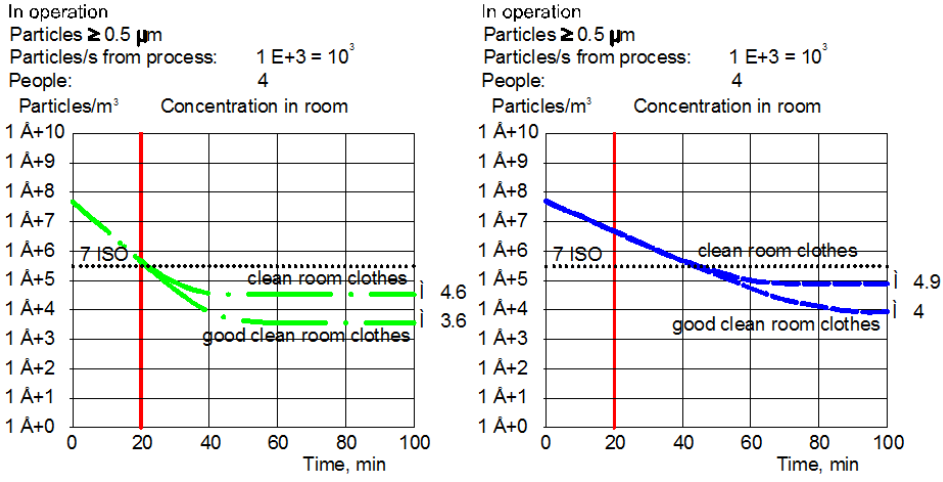


Figure 2. Reducing of particles concentration for different clothes: for air exchange rate 20 h^{-1} (to the left) and for air exchange rate 10 h^{-1} (to the right).

Figure 2 shows great improvement of air cleanliness when changing clean-room clothes to good cleanroom clothes. It is possible to reduce air ex-change rate from 20 to 10 h^{-1} by improving quality of garments, without other expenses!

Gap between cleanliness classes at design and in operation

The cleanroom class depends on many factors during design, construction and operation stages. It is not a good practice to compensate particle generation from bad clothes or poorly trained and controlled personal by exceeding air exchange rates. In contrary, it is worth to apply high quality clothes and keep good hygiene even in ISO Classes 7 and 8. The concentration of particles has a statistic nature that is difficult to consider at the design stage. The real generation of particles is normally also unknown at design stage. Therefore the cleanroom designer prefers to over-design to guarantee that the cleanroom class is kept. Thus poor operations can be compensated.

The cleanroom has normally a reserve of cleanliness. The existing practice of clean-room testing and operation does not take into account this reserve, which leads to over-consuming of energy. This paper offers a quite differ-ent approach: Cleanroom shall be well tested to determine the reserves. These reserves should also be considered at operation and the cleanroom should work at minimum capacity. Routing testing and **Good Operation Practice** should also help in this work. Now **Bad Operation Practice** exists in some cleanrooms. It means that people contaminate cleanroom as much as they can and try to clean air by increasing air exchange rate. A flexible approach in defining cleanroom parameters is needed. The esti-mation of energy consumption should be carried out at three stages:

- **in design**: to take into account necessary hygiene and safety issues, local exhausts, heat loads and to estimate air exchange rate with reasonable margin;

- **in testing**: to determine real particles generation and to possibility to keep low air exchange rate without making process worse;
- **in operation**: to check and confirm this possibility with stressing attention to proper operation procedures.

It is worth to estimate real particles generation during operation with trend analysis and monitoring in zones C and D, not only A and B. These data can be a proof of correcting of maintaining low air exchange rates.

References

1. BS 8568:2013 “Cleanroom energy – Code of practice for improving energy efficiency in cleanrooms and clean air devices”.
2. VDI 2083 Blatt 4.2 Reinraumtechnik – Energieeffizienz. Beuth Verlag, Berlin (April 2011).
3. Guidance for Industry. Sterile Drug Products Produced by Aseptic Pro-cessing – Current Good Manufacturing Practice. FDA, USA. 2004.
4. EU Guidelines to Good Manufacturing Practice. Medicinal Products for Human and Veterinary Use (GMP EC). 2010.
5. ISPE Baseline Guide: Sterile Product Manufacturing Facilities, Second Edition, September 2011.
6. World Health Organization. WHO Technical Report Series, No 961, 2011.
7. ISO 14644-3:2005 Cleanrooms and associated controlled environments – Part 3. Test methods.

PHARMA SESSION

Cleanroom Consumable Selection: Making the Right Choice

Rob Tomlinson

Nitritex Ltd, UK

Introduction

Nitritex Ltd is a cleanroom consumables company specialising in the manufacture and processing of high quality products for use in cleanroom and sterile applications. Rob has spent the last 10 years in the supply and development of cleanroom consumables products within the Pharmaceutical, Life Science and Medical Device sectors. Prior to this, worked in the area of cleanroom laundry supplying garments and laundry services to manufacturers based in the UK. Big part of role is a consultative one, helping customers to solve problems and select the most appropriate products for current manufacturing practices.

The agenda will be covering a number of topics, starting off with the definition of a cleanroom and associated concerns. This will lead onto what you should look out for when choosing the manufacturing of your cleanroom gloves. Talking about what information and accreditation's you should look for. The importance and goal of this presentation is to inform wearers that there is more to a cleanroom glove than meets the eye. We then move onto glove selection, because we all have a choice but it's about making the right choice. The major differences in Cleanroom and medical gloves will be explained, finally some interesting extracts that will demonstrate the consequences of making the wrong choice.

A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimise the introduction, generation and retention of particles inside the room and in which other relevant parameters, e.g. temperature, humidity, and pressure, are controlled as necessary. To give perspective, the ambient air outside in a typical urban environment contains 35,200,000 particles per cubic meter in the size range 0.5 µm and larger in diameter, corresponding to an ISO 9 cleanroom, while an ISO 4 cleanroom allows only 352 particles per cubic meter sized 0.5 µm. Slide five is a

simple illustration of the cleanroom airflow in action. Airflow is generated by “Blowers” located in the ceiling above the filters. The Ultra pure filtered air is forced down through the cleanroom and exits through the grilled floor (bringing with it any particulate matter that may be present). The air is forced through the airchase (or walls of the Cleanroom) where it is re-circulated through the blowers once again.

Contamination can be generated by numerous contributors in the cleanroom. For example, spillages, packaging, equipment, personnel/operators, raw materials, cleaning products and disinfectants. But how is this contribution broken down? 80% attributable to personnel, 15% generated by equipment and 5% caused by the environment itself. Personnel, when sitting/at rest are liable to shed approx. 100,000 particles per minute, when walking – 1,000,000 particles per minute and when running – 10,000,000 particles per minute.

What does this mean in relation to a cleanroom?

ISO 14644-1 Cleanrooms and associated controlled environments--Part 1: Classification of air cleanliness. In the past, cleanroom certification and classification was conducted according to Federal Standard 209E. Concerning the BioClean Brand of CR Consumables, we are concerned with ISO Class 4 and 5 operating conditions. All products are washed, packed, and processed in either our ISO Class 4, or ISO Class 5 cleanrooms. The main focus for the consumables manufacturers are particles of 0.5 microns in diameter. To explain this clearly and put it in perspective; standard copy paper is 110 microns thick, human hair can be around 75 microns in diameter and a bacterium can be 1 micron, what we are concerned about is one twenty-sixth the size of a fingerprint thickness.

For the next couple of slides I want to discuss the way our cleanroom functions, and in so describing, it should give you an idea of what you should expect your consumable suppliers to replicate when claiming to provide ISO class compatible products. Here you can see in the first two photos our DI Plant, or Deionised Water plant. This machinery provides the ultra-pure water necessary to wash cleanroom gloves, and in some cases garments. At Nitritex we operate an RO UV filtered plant that operates to a purity of 18 megohms.

A brief note on what this means: As we all know, water and electricity do not mix. Water conducts electricity. However it is not the water that is doing the conduction, it is the impurities in it. So, the conductivity of water gives a very good indication of its purity. The lower the measured conductivity the higher the purity. 18.2 Megohms is considered to be the highest level of water purity. Our machines operate at a specification of between 16–17 Megohms.

The second two photos show our 100kg Cleanroom drying machines. You will note here the filters at the top of the air intake. These are ULHA filters that filter out 99.99% of impurities before being drawn into the tumble dryer where the consumables are dried. The last photo here shows the various AHU’s (Air Handling Units) that supply the cleanroom. What is important to note here is that the level of cleanliness in the manufacturers processing area (Nitritex) is just as clean as the area in which the products will be used. With BioClean products, once the bulk, or Raw product has been loaded into the Dirty side, it does not leave the cleanroom until it has been fully washed, dried, packed, printed, and cartoned.

Here you can see the operator unloading the gloves from the “Clean” side inside the Cleanroom, where it is immediately transferred to one of the dryers beside it. Here you

can see packers inside the cleanroom packing our BioClean product. Interesting to note here are the perforated holes in the CR tables. We have these tables specially made in this fashion so as not to disrupt the airflow (discussed earlier) to any great degree. In this last photo you can see our operators manning the IPA resistant ink machine where all of our products receive their Lot, batch expiry dates etc. The packaging itself comes from trusted partners who themselves manufacture the pouches in an ISO Class4 environment. These are introduced to the cleanroom in their carton liner through the pass box, ensuring full compliance at all times.

Table 1. Classification of cleanrooms according to BS EN ISO 14644-1.

Classification Number	Maximum concentration limits (particles/m³ of air) for particles equal to and larger than the considered sizes shown below					
	0.1µm	0.2µm	0.3µm	0.5µm	1µm	5µm
ISO 1	10	2	-	-	-	-
ISO 2	100	24	10	4	-	-
ISO 3	1,000	237	102	35	8	-
ISO 4	10,000	2,370	1,020	352	83	-
ISO 5	100,000	23,700	10,200	3,520	832	29
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO 7	-	-	-	352,000	83,200	2,930
ISO 8	-	-	-	3,520,000	832,000	29,300
ISO 9	-	-	-	35,200,000	8,320,000	293,000

Glove selection and glove material

Glove selection is an extremely emotive choice as well as a technical one. In many instances we find that people opt for less protection for a higher level of comfort. But knowing how long your glove will protect you, and against what. There are many questions to be asked of these specialised gloves to ensure that you can work safely and efficiently in your respective environments. In the next few slides these criteria to be considered when choosing the correct gloves will be discussed.

Glove materials Natural Rubber Latex gloves are static insulative – static insulative materials are considered as having a surface resistance of higher than 1 x 1012 ohms/sq. The danger here is that the charge is held to a certain point then released in an uncontrolled fashion.

Nitrile gloves are, in terms of surface resistance, considered to be on the border between the insulative and static dissipative ranges. Extensive cleaning, especially in deionised water, may reduce the glove's dissipative properties. Static dissipative means that the electric charge bleeds out in a controlled manner and does not affect the properties of an item. A glove that is static dissipative has a surface resistance of more than 1 x 105 but less than 1 x 1011 ohms/sq.

Vinyl gloves (often referred to in the past as ESD gloves) offer the best dissipative qualities, by virtue of the high level of surface contaminants.

Neoprene may exhibit similar ESD behaviour to nitrile. Furthermore, as many neoprene gloves have an inner coating (often polyurethane or silicone) to facilitate donning, it is unlikely that they will have undergone rinsing in deionised water to enhance cleanli-

ness. Therefore any favourable static dissipative properties are likely to be derived from high levels of surface contaminants.

Similarities in cleanroom vs medical gloves… include: Material composition, hand style, sterility and packaging formats are the main similarities however there are many differences that are involved in the manufacturing and cleanroom processing of the gloves which add to the cleanliness and the overall cost of the glove. Such differences include the intended purpose of the glove, the particulate requirements and the differences in compliance amongst others (regulation, international standards, environment regulation, and packaging).

Airborne particle count test method while tearing open pouches… Image: our lab technician the surgical paper peel pouch on the left, and the HDPE EasyTear™ Cleanroom compatible packaging on the right. The equipment used is a LightHouse 3013 which measures the particles suspended in the air to a diameter of 0.5 microns. All testing is carried out under our Class 10 (Class 100) laminar flow hood. There are two things of note here. 1st is the material used. Of course paper inherently particulates at a far higher rate than CR compatible plastic, but of even more significance at this stage of the process is the dramatic difference in airborne particles observed at the time of opening both pouches.

The Helmke Drum test method is used to measure the particle count while the item in the drum is in a state of agitation, or motion, as the drum rotates. The results of our Cleanroom gloves Versus Surgical packed gloves, when tested using the LPC – or Liquid particle counting method. We note that surgical gloves have almost 3 times on average the amount of particles found on Cleanroom gloves. Liquid Particle Count (according to IEST-RP-CC-005.3) is the standard by which all cleanroom gloves are tested. The results of the Cleanroom gloves Versus Surgical packed gloves, when tested using the Helmke drum method which has been outlined earlier. This test was carried out by inserting unopened CR and Surgical gloves into the Helmke drum and measuring the difference in particle counts. We note that surgical gloves have more than 3 times on average the amount of particles found on Cleanroom gloves.

Here you can see the results of our Cleanroom gloves Versus Surgical packed gloves, when tested using the APC – or Airborne particle counting method, measures as each pouch was being opened We note that surgical gloves have more than 20 times on average the amount of particles found on Cleanroom gloves. This indicates that, not only the material used (paper or plastic), but the act of opening the gloves, and the mechanism employed to perform the function (peel Vs EasyTear) could have a significant impact on the volume of particles introduced into your CR environment

The results of our Cleanroom gloves Versus Surgical packed gloves, when tested using the Helmke drum method which was outlined earlier. This test was carried out by inserting opened CR and Surgical gloves into the Helmke Drum, and measuring the difference in particle counts recorded. We note that surgical gloves have more than 135 times on average the amount of particles found on Cleanroom gloves.

This particular test is significant as it demonstrates, to a certain level, the combined impact that the glove, inner wrapper and pouch, can have on the overall particle count observed.

Case study

Cases that have been summarized in report by the FDA of full facility inspections that have shown the consequences of improper selection, donning processes and lack of good SOPs can cause issues for drug manufacturers when under inspection. These inspections are not always aimed at one specific facility, in some circumstances they can be industry wide, as you will see from the example, numerous faults were found in various facilities across the US. Here are examples of just a few. What we learn from this is; while SOP's may seem long and arduous processes, they are in place for a reason and in the end can be quite cost effective, if it means avoiding a recall.

Conclusion

These are all clear cut reasons as to why the cleanroom consumable selection is quite important and explains why sometimes the selection process can take quite a long time but is a vital component to maintaining the purity of the clean environment.

References

Standard	Description
89/686/EEC	Declaration of Conformity – PPE Cat III
EN374-2:2003	AQL- penetration from Micro Organisms & Chemicals
EN374-3:2003	Chemical permeation testing
ASTM D6978-05	Permeation testing for Chemotherapy drugs
ASTM F1671-97b	Viral Penetration test (S)
EN455-2:2009	Elongation and tensile testing
EN455-3:2006	Protein from Latex Testing
EN455-3: 2006	Endotoxin testing (S)
ISO11137:2006	Sterilisation validation for Gamma Irradiation (S)
ISO11135:2006	Sterilisation validation for Ethylene Oxide (S)
ISO10993-10:2010	Biocompatibility testing
EN1149-1:1996	ESD and surface resistivity

Above is a brief summary of the standards applied to Cleanroom gloves. Those marked with a bracketed (S) usually refer to gloves that are destined for sterile use.

Biotrak a new instrument for Real time Viable Particle Detection

Thomas Lööf & Patrik Bengtsson

Brookhaven Instruments AB, Sweden

Abstract

The BIOTRAK® Real-Time Viable Particle Counter is an instrument that detects the total number of particles in the air as well as determining which of those particles are viable in nature. Additionally, the BIOTRAK incorporates a particle collection filter so the optically analyzed particles are available for subsequent speciation analysis.

The BIOTRAK Particle Counter falls into a class of instruments known as Rapid Microbiology Methods (RMM). BIOTRAK uses Laser Induced Fluorescence (LIF) to determine whether the particles are viable or not. LIF utilizes the fact that there are certain cellular metabolites associated with cell viability that fluoresce when excited by ultraviolet light. The metabolites most commonly associated with cell viability are tryptophan, NADH, and flavins (riboflavin).

Detecting the presence of viable particles in ***real time*** is the key benefit of the BioTrak detector compared to traditional active air sampling and compendial culture-based count methods that collect periodic samples that require 2 to 4 days to obtain results.

This lecture will review the basic operating principle of real-time viable particle detection equipment in general and the BioTrak detector in detail. The initial microbial testing of TSI's BioTrak® Real-Time Viable ParticleCounter conducted during product development will be presented. The BioTrakParticle Counter viable microbe detection performance was characterized according to guidance given in USP <1223> and EP 5.1.6. Additionally, the aerosol efficiency of the BioTrak is presented.

TSI will make available the final microbiological aerosol laboratory testing results to support customer validation activities. As with all environmental microbiological monitoring equipment, pharmaceutical manufacturers are responsible for performing Installation Qualification, Operational Qualification, and Performance Qualification (IQ/OQ/PQ) vali-

dation activities in their facilities. BioTrak IQ/OQ protocols have been developed and are available for customer use. The end user is responsible to define and conduct the required comparability studies to establish whether current microbiological alert and action limits are appropriate, or if new limits are needed due to the enhanced detection capability provided by the BioTrak Particle Counter. TSI will support the customer during these activities.

New Air Purification Concept for Significant Improvement in Control of Indoor Air Particle Concentration

Hannu Salmela, Kimmo Heinonen, Tapio Kalliohaka, Matti Lehtimäki, Matti Niemeläinen & Seppo Enbom

VTT Technical Research Centre of Finland, Finland

Abstract

The ability to control the particle concentration of the indoor air is relatively limited with the existing techniques used in air conditioning systems of buildings. The present methods to reduce the particle concentration of the indoor air are mainly related to the increase of the ventilation rate and/or the use of supply air filters with high collecting efficiency. Both alternatives seem to be unrealistic if an attempt is made to reduce the particle concentration significantly, for example 90%, in an office or school environments or in "semi-clean" industry premises requiring cost-efficient indoor air purification.

The particle concentration of the indoor air can be significantly reduced with a novel air purification concept innovated by VTT Technical Research Centre of Finland. The concept can be integrated into existing air conditioning systems without increasing the ventilation rate or without major increase in energy consumption. In several cases the expected reduction of the particle concentration is at least 90%. The precondition is to use electret filters in combination with a special electric charging system for the filtration of the supply air, and air diffusers provided with effective filtration for the filtration of the indoor air.

Introduction

Nowadays, in typical buildings such as in offices, schools, hotels or apartments, the particle concentration of the indoor air can be reduced mainly by increasing the ventilation rate, improving the collecting efficiency of the supply air filtration, or with (mobile) indoor

air purifiers. In buildings, where the ventilation rate is low (such as apartments), these solutions may significantly reduce the relative particle concentration of the indoor air. However, if the ventilation rate of the building is relatively high (such as 2–3 times in an hour), it is difficult to achieve very significant reduction in the particle concentration with reasonable costs. For an example, if the particle concentration of the indoor air needs to be reduced by 50%, the ventilation rate needs to be doubled. In addition, the increase in ventilation can cause inconvenience due to the draughtiness of the indoor air.

Room air purifiers are relatively widely used to reduce particles and other impurities in the indoor air. If the air exchange of the application environment is low, the equipment may significantly reduce the particle concentration. However, the higher the air exchange the more difficult it is to reduce the particle concentration. For an example, in a class room of 40 m^2 and airflow rate of 3 $dm^3/m^2/s$, the reduction in particle concentration by 50% may be achieved with an air purifier that has an effective air flow rate (the product of the collecting efficiency and the air flow) of at least 120 dm^3/s. The reduction of particle concentration by 80% would require at least four air purifiers with potential drawbacks relating to noise level, space requirement and heat load.

Indoor air purification concept

A significant reduction of the particle concentration of the indoor air requires that 1) the particle concentration in the air stream entering into the indoor environment through the air conditioning system can be essentially reduced and 2) considerably more clean air can be produced into the indoor environment. Considering techno-economic boundary conditions, the prerequisite is that the improvement is achieved without adding the ventilation rate, the size of the air conditioning system or the energy consumption significantly.

Air handling unit solution

In an air handling unit (AHU), the increase in filtration efficiency of the supply air of a building can be carried out by electrically charging the particles and applying electret filters for the particle filtration (Figure 1). This solution can be usually implemented without major changes to the air handling unit. The electric power required for the charging of the particles is in the range of 1 $W/m^3/s$, which is practically insignificant. Because the pressure drop of the charger-filter combination is in the same range as with traditional filters, the energy consumption of fans is not increased. The filtration stage may also contain activated carbon layer for removal of ozone and odours.

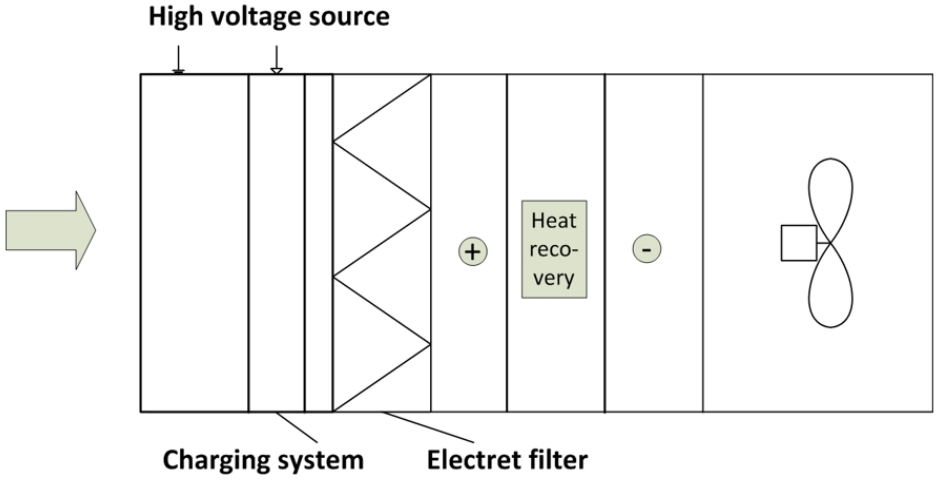


Figure 1. An electric charging system and an electret filter installed in an air handling unit.

Air diffuser solution

In an indoor environment, the stream of purified air can be multiplied by filtering also the air stream which is created due to induction when the supply air flows through the air diffuser into the room (Figure 2) [1, 2]. To achieve a cost-efficient solution, this requires that the pressure drop due to the particle filtration is very low (<<1 Pa). Although the use of mechanical filters with pressure drop below 5 Pa has been reported [3], in this case the particle filtration can be most cost-efficiently implemented using an electric filtration solution. The generation of particles and ozone due to the charging stage of the solution is negligible.

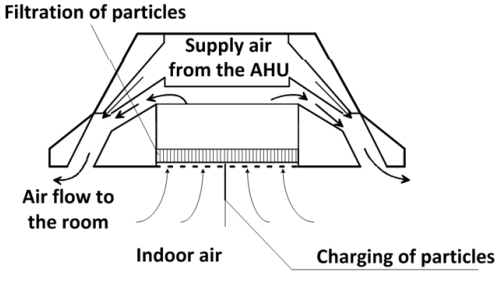


Figure 2. Operating principle (left) and a prototype (right, modified Swegon Parasol) of the air diffuser equipped with particle filtration.

Applications of the concept

Some of persons who have been exposed to different impurities (e.g. moulds) of the indoor air have become sensitive to such an extent that they are not able to stay even in indoor air renovated premises [4]. It is worth thinking whether it would be feasible to make premises (for example in office and school buildings) with considerably higher indoor air quality, but also reasonable costs compared to solutions that can be achieved with the existing technology.

The new particle filtration solutions enable to produce distinctly cleaner indoor environments only by applying the solutions into existing air conditioning systems. To achieve the best result, both the air handling units and the air diffusers should be equipped with the new solutions. Examples of clean indoor air premises where the concept has been applied are illustrated in Figures 3 and 4.

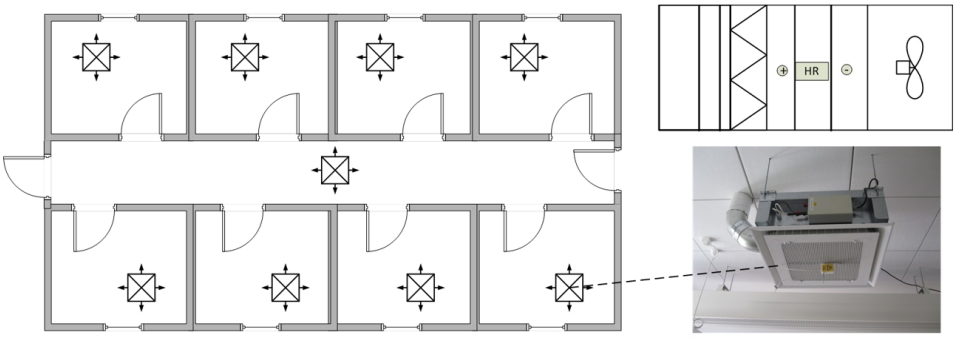


Figure 3. Application of the concept in an office unit: the air handling unit is equipped with the charger/electret filter combination and the air diffusers with the particle filtration.

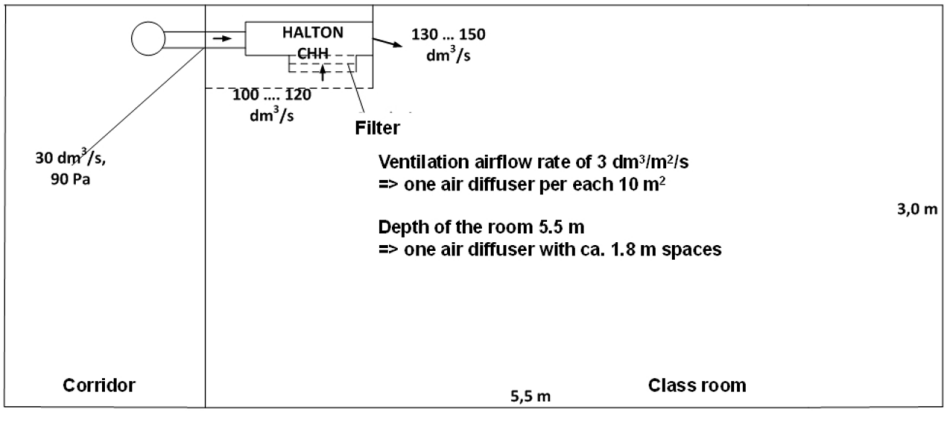


Figure 4. Application of the concept in a class room: the air diffuser (modified Halton CHH) is equipped with the particle filtration.

In the class room application the expected reduction of the particle concentration is ca. 75% when the air diffuser solution is applied. If also the air handling unit solution is applied, the expected reduction of the particle concentration is ca. 95%.

The concept has also been applied in a pilot production environment, where the ventilation rate is typically higher compared to office or school environment. In this case only the air handling unit solution in the supply air of the production premises was implemented. After the implementation, the collecting efficiency of the filtration stage of the AHU could be increased from 78% to 99.3% (0.3 µm particle size class) and the particle concentration of the indoor air could be reduced 96% (from ISO class 8 to close to ISO class 6 [5]) without making changes to the basic structure of the air conditioning system. The performance of the system in the test target has been followed for over one year.

Results

The effect of the air purification concept to particle concentration of the indoor air has been studied in VTT´s office building in Tampere, Finland. The supply air filtration solution (Figure 1) was installed to an air handling unit that provided the supply air to a small office wing consisting of three rooms. Four air diffusers provided with the particle filtration (Figure 2) were installed to the wing. In connection with the installations of the equipment the ventilation rates of the rooms were adjusted to correspond to present ventilation regulations. The increased pressure in the air duct required by the air diffuser solution was possible to reach by opening the adjustment plate of the supply air duct.

Before the installations the particle collecting efficiency of the air diffusers was measured using particle reduction method in a 233 m^3 test room in a laboratory. According to the measurements, it was assessed that with the air diffuser (modified Swegon Parasol) it is possible to achieve ca. 65–67% reduction of the particle concentration in case the air infiltration is insignificant. In this case the restricting factor was the overpressure available in the supply air duct of the test room and its effect on the induction properties of the air diffuser. Besides the modified Swegon Parasol, similar studies were conducted with a modified Halton CHH air diffuser. In this case the assessed reduction of the particle concentration was slightly higher, 74–77%. However, the size of the CHH air diffuser was not suitable for the office rooms selected for the study.

After the installations the particle concentration of a) the supply air from the air handling unit into the air diffuser, b) the supply air into the room from the air diffuser and c) the indoor air of the room was measured. The particle concentration was measured in three situations: 1) neither of the filtration solutions were in use (reference situation with F7 class filtration in the air handling unit), 2) the air diffuser solution was in use, and 3) both the air handling unit solution and the air diffuser solution were in use. The measurements were performed with a MetOne 3313 particle counter. The air samples were collected using valve machinery controlled by a computer.

The measurement results are presented in Figure 5. The particle concentrations have been normalised based on the particle concentration of the supply air from the air handling unit. When both solutions are off, the relative particle concentration in the room is 92% compared to the supply air from the AHU, 36% when the air diffuser solution is in operation and 12% when both the AHU and the air diffuser solutions are in operation, respectively.

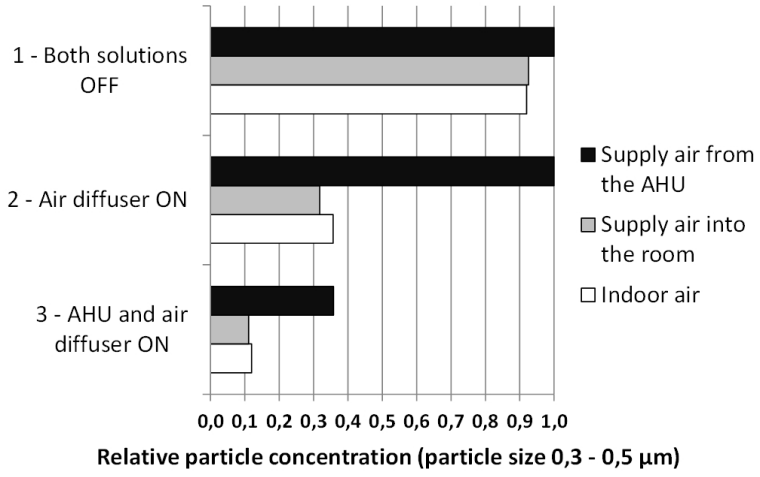


Figure 5. Effect of the air purification solutions to particle concentration in an office room.

The particle concentration as function of time is illustrated in Figure 6. The total time of the study was ca. 5 h 30 min. For the first ca. 40 min both the air purification solutions were off, after which the particle filtration in the air diffuser was turned on. The steady state condition of the test room was achieved after ca. two hours, after which the charging system in the air handling unit was turned on. After ca. 1 h 45 min the particle filtration in the air diffuser was turned off.

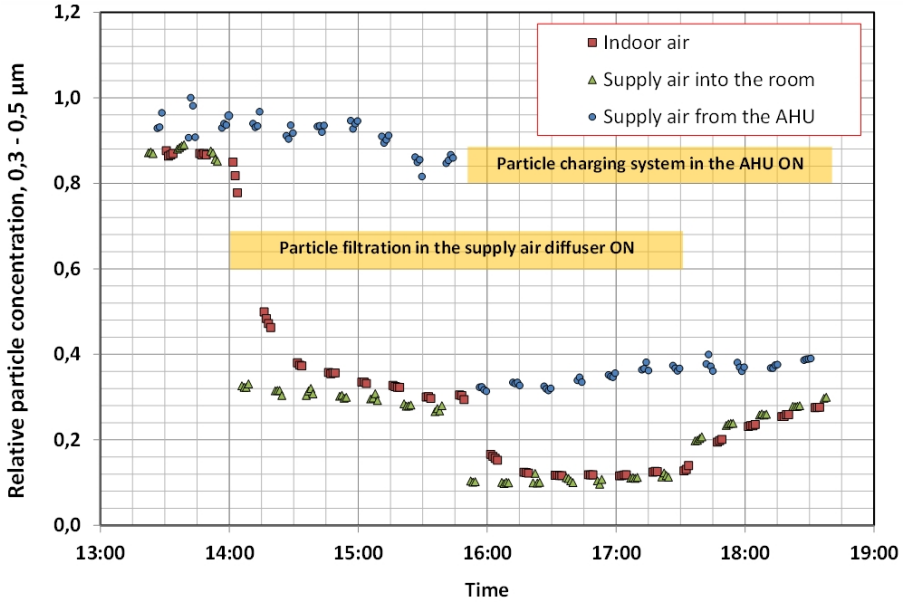


Figure 6. Effect of the air purification solutions to particle concentration in an office room during a 5 hour 30 minutes test sequence.

Discussion and conclusions

According to the results, the ability of air conditioning systems to control the particle concentration of the indoor air can be considerably improved without increasing the ventilation rate or without major increase in the energy consumption. The most significant need of the additional energy is caused by the higher pressure drop of the air diffusers that purify the indoor air. If the additional pressure of the air diffuser can be produced by opening the adjustment plate of the supply air duct, the increase of the pressure will not, however, add the energy consumption.

In the laboratory studies, it was assessed that with the air diffuser solution it is possible to achieve ca. 65–67% reduction of the particle concentration. In practice, the particle reduction in an office room was slightly lower, 63%. The most significant reason is probably air infiltration (air leaks) in the practical test environment. However, on the basis of the laboratory tests it can be assessed with a moderate accuracy what kind of results can be achieved with the solution in practice.

The subsystems of the modular concept can be used together or separately depending on the application and the target level of the particle concentration. To achieve the best result, both the air handling units and the air diffusers should be equipped with the novel filtration solutions. The air purification solutions presented in this paper can be used to implement sections in buildings which are considerably cleaner compared to the present practices, for instance in office or school buildings, hotels, hospitals or in premises used in demanding industrial production.

Acknowledgements

The development work of the air diffuser solution started with a small project in 2008 funded by TEKES, the Finnish Funding Agency for Technology and Innovation. The development work continued in 2012–2013 funded by VTT. Since 2013 the development work has continued in a research project funded by the Finnish Work Environment Fund, VTT and other cooperating organisations.

The electric filtration technique of the air handling unit has been studied in 2012–2013 funded by VTT.

References

1. Enbom, S. & Lehtimäki, M. 2009. Sisäilman puhdistaminen ilmanjakolaitteessa – ratkaisu sisäilmaongelmiin. Sisäilmaseminaari 2009. 6 p. In Finnish.

2. Taipale, A., Enbom, S., Lehtimäki, M. & Säämänen, A. 2009. ISAC: a novel air cleaning technique for controlling indoor air quality – Utilization of the induced air flow in the supply air diffuser. In: Proceedings of Healhty Buildings: Paper 461.

3. Ardkapan, S.R., Afshari, A., Bergsøe, N.C., Gunner, A. & Afshari, J. 2012. Combining active chilled beams and air cleaning technologies to improve indoor climate in offices: Testing a low pressure mechanical filter in a laboratory environment, Healthy Buildings 2012: 10th International Conference. Brisbane, Australia, Queensland University of Technology.

4. Reijula, K., Ahonen, G., Alenius, H., Holopainen, R., Lappalainen, S., Palomäki, E. & Reiman, M. 2012. Rakennusten kosteus- ja homeongelmat. Eduskunnan tarkastusvaliokunnan julkaisu 1/2012. 205 p. In Finnish.

5. Anon. 1999. Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness (ISO 14644-1:1999). CEN. 37 p.

Safety Cabinets in Cleanroom Environment

Jyri-Pekka Multanen

Labo Line Oy, Finland

Abstract

Five types of safety cabinets used inside cleanrooms can be distinguished:

1. Fume Hoods (FH / BSC I)
2. Laminar flow clean benches for product protection only (LCB)
3. Class II Safety Cabinets (BSC II / LFC)
4. Class III Safety Cabinets / Isolators / Glove boxes (BSC III)
5. Isolators / Glove boxes with less than ISO 5 cleanliness of air.

These safety cabinet types can be subdivided to several subgroups according to specific features or applications, for example “PCR cabinet”, “Biosafety cabinet”, “BSC II Type B2 cabinet”, “Cytotoxic cabinet”. The first target of the presentation is to clarify the terminology and differences of the types of safety cabinets from the perspective of users and designers of clean rooms. The presentation focuses on details regarding terminology, standardization, structure and function of the safety cabinets.

The presentation then focuses on Class II Type A2 Safety Cabinets (standard EN 12469). The pre-installation requirements set by EN 12469 are discussed from the perspective of design of ventilation, automation and layout of clean rooms. It is also discussed, how insufficient installation location may affect the validation results. From the perspective of users of safety cabinets, it is discussed how the structure and function – and the work routines of users – affect the safety and comfortability of work.

Introduction

Clean rooms feature a range of safety cabinets. From the perspectives of choosing appropriate cabinets and designing clean room lay-out and ventilation, it is important to

understand the range of safety cabinet types available and what is required from the installation environment. From writer's personal perspective as a distributor of safety cabinets, there is still much confusion and misunderstandings, mostly due to the wide range of different types of cabinets and also because of the variation in terminology.

The newest important European standards (by CEN), EN 12469 for Class II Microbiological Safety Cabinets (MSC II) and EN 14175 for Fume Hoods (FH) bring about some clarity, but still many important issues are not covered by these two standards.

The purpose of this article and presentation is to bring the viewpoint of a safety cabinet distributor to support the discussion of clean rooms. On one hand, cabinet types and their terminology – and especially the standard EN 12469 – are reviewed. On the other hand, a more practical view based on real life situations is present. Designing a well functioning clean room usually requires cooperation between several contractors, including clean room main contractor, ventilation, automation con-tractors, equipment and cabinet distributors, even architects etc. Hopefully the dialogue between different contractors in a clean room project is facilitated after this Symposium. In this context, the term "product" refers to the substance that is used inside the work area of a safety cabinet.

Safety cabinet types

Safety cabinets can be divided to subgroups in several ways. One good way is based on [5]. Laminar flow cabinet is a term that causes confusion. Laminar flow itself means an area of uniformly flowing HEPA filtered air. Laminar flow can be incorporated into several kinds of cabinets. The first of them is referred to as Laminar Flow Clean Bench (LCB). The other is termed Microbiological Safety Cabinet (MSC) and biological safety cabinet (BSC).

Fume hoods (FH) or ductless fume hoods (DFH), with active charcoal filter replacing the duct, provide operator protection only, or operator and environment protection if a filter adsorbs or blocks the material to be exhausted from the cabinet. European standard EN 14175 covers fume hoods, and is not reviewed in more detail in this presentation. Synonyms for fume hoods include "fume cupboards". A ductless fume hood provided with a HEPA filter is also defined as Class I Microbiologi-cal Safety Cabinet (MSC I). It is important to note, that fume hood or MSC I is a cabinet that does not at all provide product protection, sterile air in work area. In a view of a clean room designer, all ventilated cabinets may first seem the same, and is thus easier to put them under the same term (Fume hoods).

The LCB does not provide personnel protection. It is actually not connected to the ventilation system, but its airflow may affect the ventilation of clean room. There are several application specific subtypes of LCB, including "PCR cabinets". The main point to be taken about these cabinets is that the air flows out directly from the cabinet work area [5], and thus does not provide operator or environment protection.

The MSCs are divided to subgroups Class I, II and III. Class II is further divided to Class II Type A2 and Type B2 (types A1 and B1 have practically become obsolete). Class II Type A2 recirculates ca. 70% of air, whereas Type B2 does not recirculate [4, Table 2]. Recirculation should be avoided, in case evaporating, hazardous and HEPA filter penetrable substances are handled. Class III MSC is an isolator, a "glove box". A clear and more profound re-view of different types of MSC is presented in [2 and 4].

Biosafety levels 1, 2, 3 and 4, must not be confused with Classes I, II and III of Microbiological Safety Cabinets. A good review of biosafety levels is presented in [4, Table 1].

Microbiological hazards of safety levels 2 and 3 require a Class I and or II MSC, and level 4 requires Class III MSC.

Also the air cleanliness classes (ISO 14644) 1 to 9 should not be confused with either biosafety levels or classes of MSCs. In addition to selection of the type of MSC, it must be assessed, whether or not the MSC should be exhaust ventilated or not. A basic rule of thumb in a clean room, however, is to always ventilate. Retention of particles and aerosols by HEPA is an important topic that is not discussed in this article.

Table 3 of [4] reviews a good guideline for selecting the most appropriate type of MSC, and whether it should be ventilated or not. Based on writer's personal experience, the MSC II are the most important safety cabinets to be discussed, and the presentation thus emphasizes mostly on them.

It is shortly reminded, that there are also other than MSC type glove boxes and isolators sometimes in clean rooms. The applications are either handling of hazardous material e.g. radioactive substances or nanopowders or handling of materials susceptible of airbourne oxygen and/or moisture.

Standard EN 12469 for MSC II

The harmonized European standard EN 12469 was published in 2000 [1]. A good review of Class II MSCs and some national preceding standards were reviewed by Christiansen and Niemeläinen [2]. However, the standard [1] does not (yet) distinct types A2 and B2 of MSC II. Therefore, when EN 12469 is mentioned with MSC II, one is referring to MSC II type A2, specifically, not type B2.

The testing of any product to conform to a relevant standard can be divided to at least the following test phases: i) type testing (preferably by an independent organization such as TÜV), II) factory testing prior to shipment, iii) field certification before first use, iv) field certification after certain period of time, relocation etc.

In principle level, the most important aspects covered by the EN 12469, are: i) protection of opera-tor, ii) protection of product from airbourne decontamination sources, iii) protection of product from cross contamination.

EN 12469 defines to some extent the methods, how the targets of safety, mentioned above, can be confirmed. Table 5 of [1] presents, which methods are compulsory in the field recertification phase, which thus must be done in the clean room environment. Annex D covers the aerosol challenge method to test the HEPA filters for leaks, Annex G the volumetric airflow measurement, Annex H the airflow velocities requirements.

MSC II installations in cleanroom environment

In the reports [2, 4 and 6] specific distances to doors, ventilation canals, personnel movement, adjacent cabinets, walls etc. required around MSC II cabinets are described. Christiansen and Niemeläinen [2] also adviced that the exhaust lines from MSC II must not be used for adjusting pressure between clean room's departments, the MSC II must have own exhaust canals, and that the ventilation and its blowers must be appropriately dimensioned. Omitting these recommendations will cause a risk that the MSC II cannot conform to EN 12469 in field certification phase. Practice has shown [2], that this risk had

been remarkably omitted at least back in 1998. A further note from the writer is, that not every possible case in a real life situation can be covered by any standard or guideline. The writer strongly supports a stronger cooperation firstly between companies and authorities, and secondly between companies with different task in the same project, to increase mutual understanding of theory and practice.

According to Christiansen and Niemeläinen [2], there are two optional ways of connecting an MSC II to exhaust ventilation: either with a thimble exhaust collar or an anti-flowback valve. A thimble exhaust collar allows air to pass from outside the MSC II in case of a sudden over-suction, and allows a flow back to direct to outside of MSC II and not through HEPA filter in the reverse direction, causing danger of air flowing from inside the cabinet to user. Anti-flowback valve in turn prevents a flowback from the canal.

Annex J of [1], [3] and [6] discuss, when and how the MSC II must be decontaminated. A normal decontamination interval, based on writer's experience is ca. 8–10 years, when HEPA filters need change. The most common method of formalin evaporization is the cheapest but most dangerous, and requires many safety precautions, and may cause the installation environment to be shut down for one work day. Hydrogen peroxide method is an optional method but is some 10-fold more ex-pensive than the formalin method.

Literature

1. Anon. 2000. EN 12469: Biotechnology – Performance criteria for microbiological safety cabinets, European Committee for Standardization.

2. Christiansen, V. & Niemeläinen, M. 1998. Luokan II suojakaappien erityisominaisuudet. Report from VTT Valmistustekniikka, April 1998.

3. Xiang Qian, L. 2002. Biosafety cabinet decontamination. Esco Micro Pte Ltd.

4. Anon. 2013. A guide to biosafety & biological safety cabinets, Esco Micro Pte Ltd publication.

5. Anon. 2014. Comparison of lab ventilated enclosures. http://www.escoglobal.com/resource.php?id=153 (February 2014).

6. Anon. 2013. User and service manual. Nordic Safe, Class II Microbiological Safety Cabinet. Esco Micro Pte Ltd., Singapore.

How to Get New Micro Method Started!

Fredrik Alexandersson

Miclev AB, Sweden

Introduction

Rapid microbiological methods have begun to appear in pharmaceutical and other industries for applications in aseptic operations. An introduction of real time monitoring methods for water and air and principle of operations will be presented.

Several studies for water monitoring will presented to show the capability of this technology as a process verification tool for process optimization and real time feedback process control analyzer for trouble-shooting and suggestion how to set new alarm limits for monitoring online water quality.

Background

Several new instruments based on new technology, i.e. real time microbial sensor using optics for particle detections, are available:

- Instantaneous Microbial Detection (IMD) of Azbil BioVigilant, Inc. for air applications (Figure 1),
- BioLaz Real-Time Microbial Monitor (BioLaz) of Particle Measuring Systems, Inc. for air applications and
- Real-Time Microbial Monitoring Systems (RMS) of Instant BioScan for water applications (Figure 2).

These instruments detects microbes by using a Mie scattering sensor for measuring the size of particles. The microbes are differentiated from inert particles using detection of intrinsic fluorescence of metabolites (NADH and riboflavin) inside the microbial cells. The microbial detection is carried out in real time, and no reagents nor sample preparation is needed.

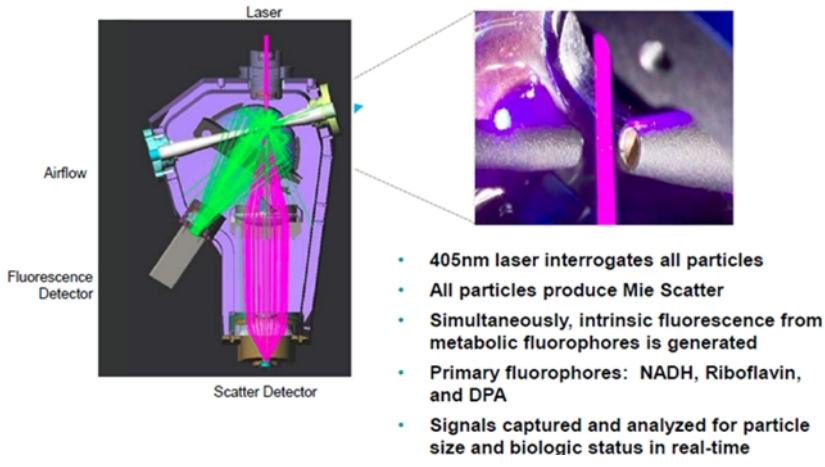


Figure 1. The picture shows the principle of the detection system in the Instantaneous Microbial Detection (IMD).

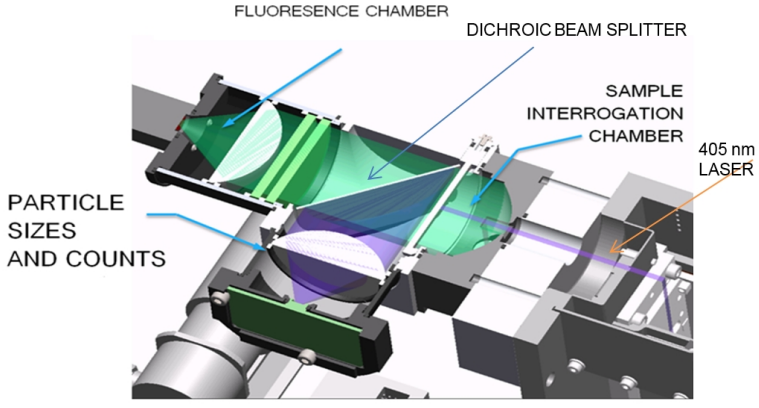
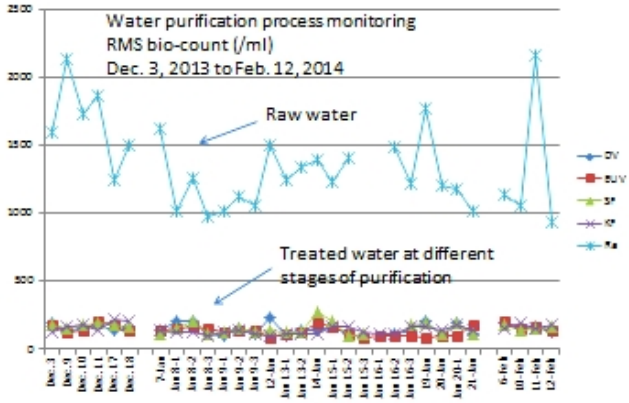


Figure 2. The picture shows the principle of the detection system in the Real-Time Microbial Monitoring Systems (RSM).

Applications

The real time microbial measuring capability of RMS can be useful as a process verification tool for process optimization and as a real time feedback process control analyzer for trouble-shooting (Figure 3). The sampling of surface and cleaning validation are important parts in aseptic operations and a sensitive, real-time analyzer e.g. RMS can be beneficial in visualizing sampling results and timely validation. In Figure 4 aseptic swabbing was used as sampling method. The samples were analyzed using RMS for sampling swab rinses. The RMS is screening the results in less than one minute.

In the case study is showed how the RMS method can be used e.g. in setting alarm limits for RMS-W online monitoring of water line. The procedure is based on Statistical Process Control methodology and this procedure of new limit setting is applicable for both pharmaceutical water lines and municipal water treatment.
The RMS bio-count data for "Water source B" (time period: 01:51 to 07:18, April 17) was used to calculate statistical parameters (Figure 5). The average RMS bio-count in this case was (μ) = 707 and the standard deviation was (σ) = 575. These statistical parameters were used to set the following alarm and action limits (Alert limit: μ + 3σ = 2432 & Action limit: μ + 4σ = 3007).



Explanatory note:
(1) Large variation in raw water represents natural seasonal fluctuation of raw water source
(2) Treated water displays stable trend→ indication of stable control of the purification process

Figure 3. A trending graph for RMS bio-counts per litre of raw and treated water.

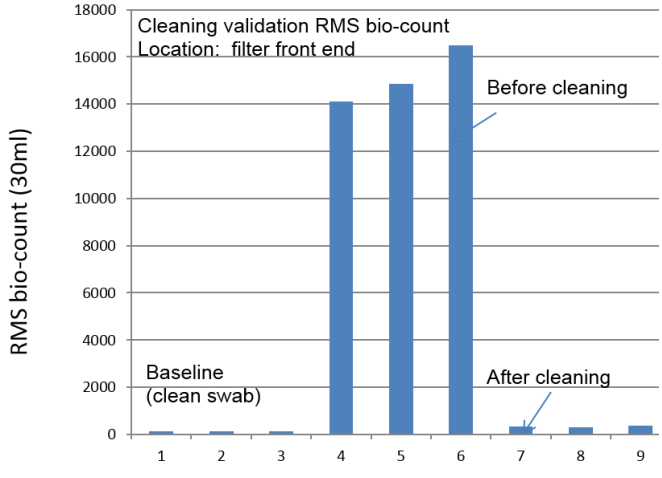


Figure 4. Cleaning validation results based on RMS bio-counts.

Summary

Microbial analysers e.g. IMD, BioLaz and RMS based on new types of optics are now commercially available. Real time and continuous monitoring capabilities of these analysers can be of benefit in aseptic operations in pharmaceutical and other industries.

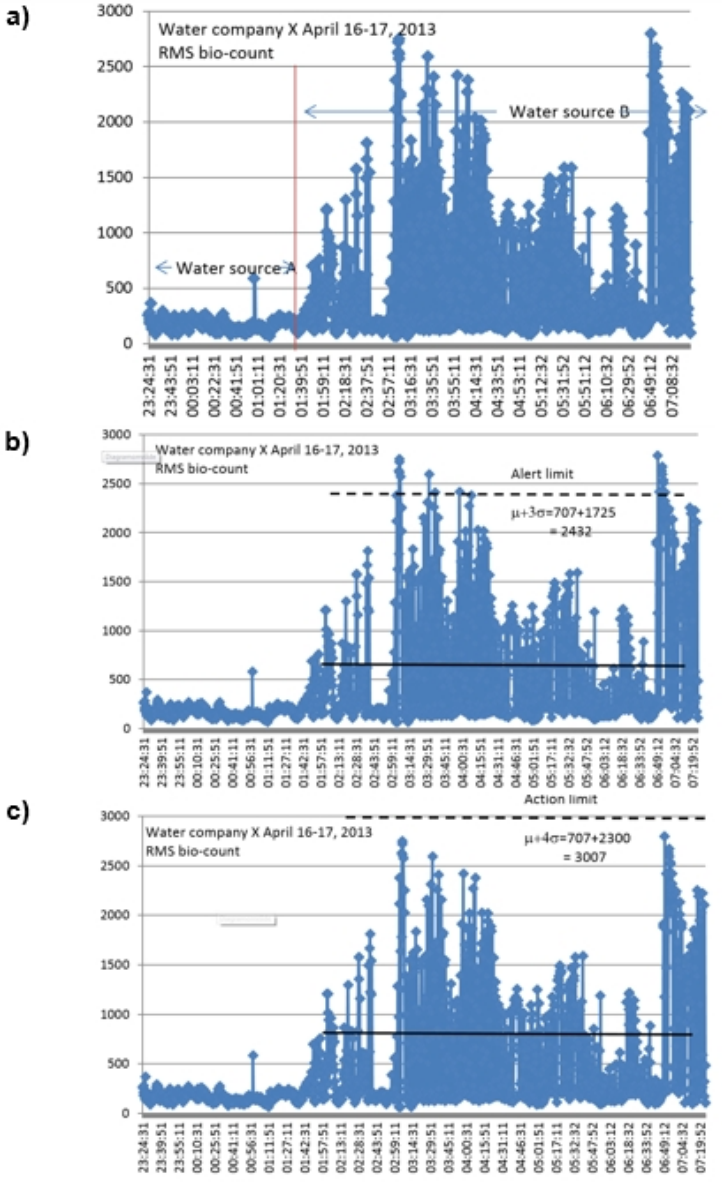


Figure 5. RMS bio-count data for a) calculating statistical parameters, b) setting alert limit parameters and c) setting action limit parameters.

References

http://www.biovigilant.com/products/, April 15, 2014.

http://www.ibioscan.com/applications.html, April 15, 2014.

http://www.pmeasuring.com/particleCounter/microbialAirSamplers/BioLaz, April 15, 2014.

The i.v. Room of the Future

Bastiaan Moulart

Health Robotics Srl, the Netherlands

Abstract

Health Robotics is a leading supplier of life-critical sterile compounding Robots with 80% total IV Robots global market share including 90% the oncology robots. Health Robotics provides more than 500 hospital installations in 5 continents with the only fully integrated, robotics – based technology, IV Workflow, and manual compounding software automation. Health Robotics' 2nd generation platform products (i.v.STATION®, i.v.SOFT®, i.v.STATION® ONCO, and i.v.STATION®) have been found (through scientific and peer-reviewed studies) to greatly contribute to ease hospitals' growing pressures to improve patient safety, increase throughput, and contain costs. Through the effective and efficient production of sterile, accurate, tamper-evident, and ready-to-administer IVs and TPNs, Health Robotics' medical devices and integrated workflow solutions help hospitals eliminate life-threatening drug and diluent exchange errors, improve drug potency, decrease other medical mistakes and sterility risks, work more efficiently, reduce waste and controlled substances' diversion, decrease pharmacy technician upper-limb injuries, and diminish the gap between rising patient volume and scarce nursing and pharmacy staff.

i.v.STATION® – robot compounding non-hazardous i.v. preparations

i.v.STATION® represents a revolutionary approach in the quest for safe, accurate, efficient, cost effective, and ready-to-administer IV Admixtures. Constructed around a scalable, distributed, and fail-safe architecture, it offers unprecedented final container flexibility, life-critical patient safety, robotic precision and performance. It may be deployed in a variety of locations e.g. central and satellite pharmacies and direct patient care areas, due to its self-contained form, ISO Class 5 environment, and small "foot print".

i.v.STATION® ONCO – The 2nd generation robot for compounding oncology sterile preparations

i.v.STATION® ONCO represents a complementary offering to other Health Robotics' solutions for sterile compounding of Intravenous Cancer Therapy: CytoCare and i.v.SOFT Assist. Engineered as an evolution and blending of some of the fully-tested and globally-embraced technologies embedded within i.v.STATION and CytoCare, i.v.STATION ONCO automatically compounds, caps, and labels commercially-available soft plastic bags and syringes from 8 different brands and 10 different sizes within an ISO-5 environment on negative pressure. i.v.STATION ONCO however does not support sterile compounding with ampoules, bottles, CSTDs, unusually small vials, and elastomeric infusors, which Health Robotics' customers will need to continue to compound with market-leading CytoCare and/or i.v.SOFT Assist solutions. i.v.STATION ONCO's dimensions are: 2m * 1m * 1.5m, weight 410 kg, power [220V or 110V], Class H14 HEPA filters.

i.v.SOFT ASSIST® – The workflow engine for the future i.v. room

Closed-Loop I.V. Therapy and Parenteral Nutrition Management. i.v.SOFT® helps Pharmacy Technicians, I.V. Room Supervisors, and Pharmacy Directors to compound and manage the safe and efficient manual production of I.V. Admixtures according to hospital-defined sterile compounding guidelines, and to help bring the Health System Pharmacy into compliance with USP 797 regulations in the US and GMP guidelines elsewhere.

i.v.STATION 2® – The totally automated solution for compounding sterile parenteral nutrition and non-hazardous medications

i.v.STATION 2 is Health Robotics' dual-use IV Robot to deliver "Totally Automated Parenteral Nutrition" in addition to non-hazardous IVs. This 2014 project leapfrogs the status-quo of TPN Automation (6, 9, 10, 12, or 24-channel partially-automated devices from companies such as Baxter, Neocare, Fresenius, B|Braun, and Baxa) to a new level of TPN CPOE software-machine integration (including the multi-language features lacking in today's global market). In addition to all the patient safety features existing on i.v.STATION, i.v.STATION 2 supports up to 34 ingredients/medications (so Pharmacy Technicians do not need to manually add ingredients); automatic priming of the IV lines; automatic labeling of the I.V. Bags (to reduce manual errors and labor costs in the labeling process); gravimetric control of every ingredient; automated powder reconstitution; Bar-Code recognition of source-ingredient containers (to eliminate source-ingredient error); automatic waste system; an ISO-Class 5 sealed chamber [including overnight UV-lamps sterilization] that can enable Health-System Pharmacists to compound safe TPNs with greater sterility than traditional TPN pumps in LAFs; simultaneously filling 20 I.V. Bags without operator assistance.

Barrier Technology

James Drinkwater

Pharmaceutical & Healthcare Sciences Society, UK &
Head of Aseptic Processing Technologies and GMP Compliance F Ziel Gmbh

Abstract

There have been developments in barrier separation technology, Isolators and RABS – Restricted Access Barrier Systems to support new biological and therapeutic product developments where both bio-contamination and cross contamination control is required. This presentation will provide an overview of the contamination control attributes and developing containment hierarchy through different scales of processing. Large filling line Barrier systems, Small scale Aseptic processing and Pharmacy Isolators will be reviewed with consideration on the latest developments.

Bio-contamination control in Barrier separation technology has to consider integrated solutions in barrier design with integrated process equipment, material transfers in contamination and cross contamination control, integrated and automated gaseous disinfection (H_2O_2 vapour) and integrated monitoring systems.

There are more considerations to campaign processing and Aseptic hold operation that need new qualification strategies. In addition qualification rationale needs to be risk and scientifically based requiring a new level of process and risk knowledge to deliver GMP compliance. This presentation will discuss key aspects of a control strategy, including microbial control strategy now expected in GMP and particularly relevant to aseptic processing of biological products (GMP Annex 2 refers).

Literature

PHSS RABS Monograph 15, 2011 Restricted Access Barrier systems for Aseptic processing applications.

Pharmaceutical Isolators PHSS publications catalogue

Vaporized Hydrogen Peroxide Room Decontamination in Cleanrooms

Nicolas Toulze

Steris Corporation, France

Abstract

Traditionally formaldehyde has been used to decontaminate laboratories, clean rooms, and equipment. For a variety of reasons, including health & safety, formaldehyde is increasingly replaced by new technologies. Alternative equipment and services are now readily available for biodecontamination on a preventative or reactive basis.

Vaporized Hydrogen Peroxide (VHP) is a low temperature hydrogen peroxide vapour and proven broad-spectrum antimicrobial. This dry vapour process was developed by STERIS Corporation over 25 years ago and used is extensively in the pharmaceutical, research, and healthcare markets. Mobile and modular VHP equipment can effectively decontaminate a wide variety of applications, from an entire facility to a single piece of equipment and everything in between. Cases from pharmaceutical area will be discussed.

PHSS Biocontamination Technical Monograph 20

James Drinkwater

Pharmaceutical & Healthcare Sciences Society, UK &
Head of Aseptic Processing Technologies and GMP Compliance F Ziel Gmbh

Abstract

The PHSS – Pharmaceutical and Healthcare Sciences Society Bio-contamination monograph comprises five key sections in a circa 200 page document available as hard copy or in e-book format.

Section one is the introduction and scope detailing the context of Bio-contamination in GMP, the challenges in controlled processing environments and the risks to patients from products prepared in contaminated environments.

Section two presents Bio-contamination characterisation and risk profiling with a new initiative called; Risk Profiling and Proactive Response based on holistic review of monitoring data and response to risk escalation in contaminating critical EU Grade A or ISO 5 environments.

Section three covers: Bio-contamination control, setting out principles but also detailing best practice. Contamination control attributes are explained including Zonation in the Shell like Cleanroom barrier concept and different background environments based on the type of barrier technology used in the aseptic core e.g. Flow Hoods (with simple barrier screens) in conventional cleanrooms, Isolators, RABS or Blow Fill seal technology.

Section four covers: Environmental monitoring of Bio-contamination on Surfaces and airborne including techniques, tools, setting risk based sampling locations, incubation regimes and a perspective on new Rapid Micro Methods.

Section five covers: Bio-contamination deviation management with considerations on what is required for a comprehensive root cause analysis and CAPA – Corrective and preventative Action. Examples of deviation investigations and outcomes are included.

This monograph is the most comprehensive for some time providing guidance through the life cycle of bio-contamination characterisation, control, and monitoring with deviation management for GMP compliance.

Literature

Bio-contamination Risk Profiling and Proactive Response – European Pharmaceutical review, April 2014 edition.

HOSPITAL SESSION

Performance of Single-use Surgical Clothing Systems in On-going Surgery

Bengt Ljungqvist & Berit Reinmüller

Chalmers University of Technology, Sweden

Abstract

To prevent surgical site infection it is desirable to keep the number of airborne bacteria-carrying particles low in the operating room during surgery susceptible to infections. As the airborne bacteria are mainly derived from the skin flora of the personnel present in the operating room a reduction can be achieved by using surgical clothing systems with high filtration efficacy of the fabrics.

This study will discuss the performance of the single-use clothing system made of polypropylene in the context of operating rooms during surgical procedures.

A comparison between the single-use clothing system and the commonly used reusable clothing system made of mixed material (cotton/polyester) is discussed. The results show that the protection efficacy of the tested single-use clothing system is much higher than that of the compared reusable clothing system made of mixed material.

Introduction

In the operating rooms the number of airborne bacteria-carrying particles is considered an indicator of the risk of infections to the patients undergoing surgery susceptible to infections. Today when the supply air in the operating room is HEPA-filtered, the main source of airborne microorganisms is people (patient and personnel). The filtration efficacy of the fabric in surgical clothing systems plays an important role. The design of the clothing system also affects the number of particles emitted from people to the air of the operating room. In operating rooms for surgery susceptible to infections, the selection of clothing systems for the operating personnel should no longer only be considered in terms of comfort but also in terms of patient safety.

Clothing and clothing systems for cleanrooms and associated controlled environments such as ultraclean operating rooms are mainly tested with regard to material properties such as particle generation, particle filtration, and resistance to wear, tear-related damage, and comfort. Increasing cleanliness during operations on infection sensitive patients requires in-depth knowledge regarding the performance of surgical clothing systems.

The combined filtration efficacy of fabric, construction and design of the clothing can be evaluated in a dispersal chamber or "body box." A dispersal chamber has been used to study surgical clothing systems for operating rooms, where a comparison between singel-use non-woven and reusable mixed material systems has been performed. The source strength, which is described as the mean value of the number of total or viable airborne particles per second emitted from one person, has for the two clothing systems been presented by Ljunqvist and Reinmüller (2012).

The values of the source strength depend mainly on chosen clothing system and the persons' activity levels. One of the advantages of estimating the source strength with dispersal chamber tests is that the test subjects are performing standardized cycles of movements, i.e., the movements are the same in all tests. This is of importance in order to compare the filtration or protection efficacy of various clothing systems.

In the dispersal chamber the activity levels of the test subjects are often higher than those of the operating team during ongoing surgery. This results in source strength values for clothing systems evaluated in a dispersal chamber that are mostly higher than those estimated in operating rooms. The performance of the single-use surgical clothing systems in the context of operating rooms will be discussed in the following.

Material and methods

Operating room

The measurements were performed at South Hospital (Södersjukhuset), Stockholm. South Hospital is an emergency hospital with approximately 650 beds, where about 6000 orthopaedic surgical procedures are performed each year. The tests were performed during ongoing orthopaedic surgery in an operating room, where the air movements could be characterized as turbulent mixing, i.e., the dilution principle is applicable. The supply air was HEPA-filtered with an air volume flow of 0.7 m^3/s which gives about 17 air changes per hour.

Apparatus and sample collection

In the operating room airborne viable particles were collected using a slit-to-agar (STA) sampler (FH3®, d50-value 1.6 µm) and the total number of airborne particulates was determined using a particle counter (DPC; HiacRoyco 245). All instruments were operated according to the manufacturers' instructions.

Microbial growth medium for all tests was standard medium Tryptic Soy Agar (TSA) in 9 cm Petri dishes. The TSA plates were incubated for not less than 72 h at 32˚C followed by not less than 48 hours at room temperature. After incubation the number of colony-forming units (CFU) were counted and recorded as CFU/m^3. The STA sampler in comparison to other impaction air samplers has been discussed by Ljungqvist and Reinmüller (1998, 2008).

The probes of the STA sampler and the particle counter were situated just beside the operating table with a distance of approximately 0.8 m to 1.2 m to the wound site at two alternative locations depending on the position of the surgical team. The sampling probes were positioned just above the operating table 1.2 m above the floor. Figure 1 shows the principle arrangements of the location of the sampling probes.

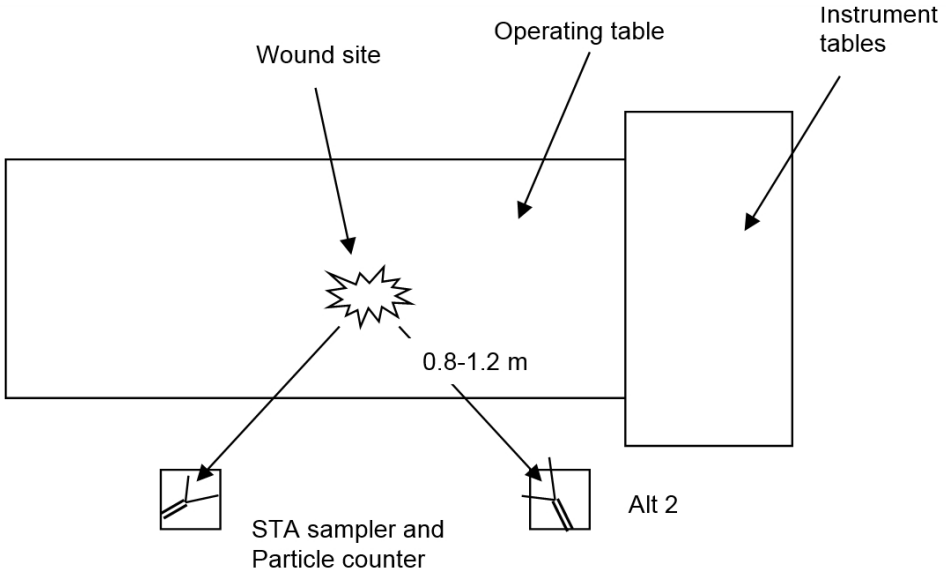


Figure 1. Principal arrangement of the placement of the sampling probes (STA sampler and the particle counter) beside the operating table.

Each simultaneous sampling periods for the two instruments was 10 min. The sampling volumes per period become for the STA sampler 0.5 m^3 and for the particle counter 10 ft^3 (0.28 m^3). Total ten operations (hip and knee) were monitored and during each operation five to nine samples were taken between incision and closure of the wound.

Clothing system

The people in the operating room (8 persons) had the same clothing system during ongoing surgery. The surgical team consisted of the surgeon, the assistance surgeon, and the operating nurse that had additional singel-use sterile gowns and sterile gloves.

No door openings were allowed during surgery (incision time). The studied single-use clothing system was single packed in plastic bags but not sterilized. The evaluated single-use surgical clothing system consisted of:

– MHC 2 Blue, Spun bonded polypropylene/Melt blown/ Spun bonded polypropylene (SMS), wieght 35g/m^2, antistatic treated (Mölnlycke Health Care AB, Göteborg, Sweden)

The trousers had cuffs at the legs and the short sleeved shirts had cuffs at the arms, bottom and neck-line. Additionally, singel-use head covers (type GIenn) and face masks were worn. Private socks and shoes were used.

Of the eight people in the operating room two “instrument technicians,” mostly sitting calmly, wore additionally hoods and knee-long boots of clean room quality.

Source strength

With the assumption of no leakage into the operating room and the HEPA filters having efficiency close to 100 percent, the simplest possible expression, which is applied on the dilution principle, describes the source strength (outward particle flow):

$$q_s = c \times Q/n \quad (1)$$

where

q_s = source strength; total particulates (number/s),bacteria-carrying particles (CFU/s)

c = concentration; total particulates, (number/m^3); bacteria-carrying particles, (CFU/m^3)

Q = total air flow (m^3/s)

n = number of persons present, (number).

The source strength is described as the number of total or viable airborne particulates per second emitted from one person. Data are given as mean values based on several persons dressed in specific clothing systems.

Due to the two technicians´ low activity and their additional clothing of cleanroom quality, in the calculations of source strength, the number of persons present in the operating room is reduced from eight to seven. This reduction is made in order to avoid overestimation of the protection efficiency of the studied clothing system.

Results

Colony-Forming Units (CFU)

Measurements of aerobic airborne CFUs were performed during ten operations, where eight were hip replacements and two were knee replacements. The total number of air samples was 69. The results of all measurements are reported by Ljungqvist and Reinmüller (2013).

Table 1 shows airborne CFU/m^3 mean values from ten operations and the calculated source strength, CFU/s in accordance to Equation (1), when the number of persons is seven and the air flow is 0.7 m^3/s.

From the data in Table 1 it can be calculated that the mean values and standard deviations for the CFU concentration and the source strength are 11.5 ± 6 CFU/m^3 and 1.15 ± 0.6 CFU/s, respectively.

It could be noted from the data in Table 1 that the median values for the CFU concentration and the source strength are about 12.6 CFU/m^3 and 1.26 CFU/s, respectively.

Mean value, standard deviation and 95% confidence intervals (t-distribution) from the data given in Table 1 are shown in Table 2 for the source strength of single-use surgical clothing system MHC-2.

Table 1. Mean values of airborne CFU/m^3 from ten operations (hip and knee) and the calculated source strength according to Equation (1) (n = 7, Q = 0.7 m^3/s) for single-use clothing systems, MHC-2.

Operation	Mean value (CFU/m^3)	Source strength (CFU/s)
No1, left hip	11.3	1.13
No 2, right hip	2.9	0.29
No 3, right hip	15.8	1.58
No 4, left hip	13.1	1.31
No 5, left hip	4.0	0.40
No 6, right knee	20.0	2.00
No 7, left knee	12.0	1.20
No 8, right hip	18.0	1.80
No 9, right hip	13.3	1.33
No 10, right hip	4.3	0.43

Table 2. Mean value, standard deviation and 95% confidence interval for the source strength of single-use surgical clothing system, MHC-2.

Source strength (CFU/s)			
Mean value	Standard deviation	Confidence interval	
		Lower	Upper
1.15	0.60	0.7	1.6

The source strength mean value of 1.15 CFU/s in Table 2 should be compared with the source strength mean value of 0.7 CFU/s given by Tammelin et al. (2013) calculated from five operations with the persons dressed in the same single-use surgical clothing system (MHC-2) as for the received data in Tables 1 and 2.

Total Number of Particles

Measurements of total number of airborne particles (≥0.5 µm, ≥5 µm, ≥10 µm) have simultaneously been performed with the CFU measurements during ten ongoing operations. The results of these measurements are reported by Ljungqvist and Reinmüller (2013). It could be noted that surgical activity such as diathermia, sawing and reaming increased the number of particles, especially the number of smaller particles (≥0.5 µm).

Figure 2 summarizes on a logarithmic scale, the mean value concentrations of particles (≥0.5 µm, ≥5 µm, ≥10 µm) and CFU per operation and their reciprocal relationships during ten operations.

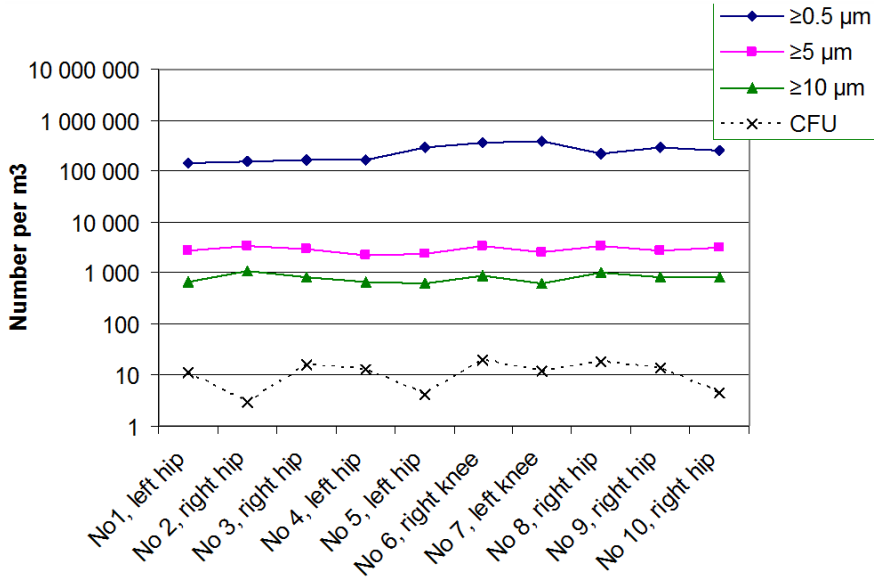


Figure 2. Mean values of measured concentrations during ten operations. Airborne particles (≥0.5 µm, ≥5 µm, ≥10 µm) and CFU.

Discussion

Comparison

The single-use surgical clothing system should preferably be compared with one of the most commonly used surgical clothing systems in Sweden. This clothing system is a reusable mixed material system and has the following specification:

Mixed material: Cotton 69%, polyester 30% and carbon fibre 1%, weight 150 g/m^2, (Mertex P-3477® Mercan AB, Skanör, Sweden).

Measurements during ongoing surgery with the operating team dressed in the reusable mixed material surgical clothing system were investigated by Ljungqvist et al. (2012) and Tammelin et al. (2012). These measurements were performed in three operating rooms

with turbulent mixing air with air flows in the range of 0.7 m^3/s to 1.0 m^3/s. The operating rooms were situated in the same operating department at South Hospital as the earlier described measurements were performed.

Ljungqvist et al. (2012) presented measurements with two different measuring methods for collecting airborne viable particle. The two measuring methods, filter sampler (Sartorius MD8®) and slit-to-agar sampler (FH3®, d50-value <2 µm), gave concentration values (CFU/m^3) in the same range, although values from the slit-to-agar sampler were slightly higher than those of the filter sampler.

The source strength of the clothing system of mixed material (cotton/polyester) was in the range of 4.6 to 6.2 CFU/s, where higher values were obtained from the slit-to-agar sampler.

Tammelin et al. (2012) accounts for measurements of CFU in air with a filter sampler (Sartorius MD8®) during 13 orthopaedic surgical procedures where the operating team was dressed with a clothing system of mixed material (cotton/polyester), which was either washed repeatedly or brand new. The mean value of the source strength was estimated to be 4.1 CFU/s.

If, as described earlier, in the calculations of the source strength the number of persons is reduced by one person, due to the fact that the instrument technicians have as low activity level, i.e., low contribution of airborne viable particles, the mean values of the source strength becomes 5.0 CFU/s. The results also show that the median value is the same as the mean value.

A comparison of mean values, standard deviations, 95% confidence intervals (t-distribution) for source strength from a reusable, mixed material surgical clothing system and from a single-use surgical clothing system during ongoing surgery are shown in Table 3.

Table 3. Comparison of mean values, standard deviations, 95% confidence intervals (t-distribution) for source strength from reusable mixed material surgical clothing system and from the studied single-use surgical clothing system. All measurements performed during ongoing surgery.

Clothing system (fabric)	Source strength (CFU/s)			
	Mean value	Standard deviation	Confidence Intervals	
			Lower	Upper
Mixed material (69% cotton, 30% polyester)	5.0	2.0	3.8	6.2
Single-use (polypropylene)	1.15	0.60	0.7	1.6

Measurements with the two surgical clothing systems in a dispersal chamber were performed by Ljungqvist and Reinmüller (2012). The received mean values of the source strength of mixed material clothing system, washed for about 50 times and of single-use clothing system were 10.9 CFU/s and 2.5 CFU/s, respectively. These higher values –

than those given in Table 3 – depend on the higher activity level of the test subjects in the dispersal chamber than the activity level of the operating team during ongoing surgery.

It should be noted that the source strength mean value for each of the two clothing systems from operating room measurements is about 46% of the mean value from dispersal chamber tests. Furthermore, the ratio of the source strengths between the two clothing systems is 4.36 from tests in the dispersal chamber and 4.35 from measurements during ongoing surgery.

Estimations

When the air movements are turbulent mixing, the dilution principle is applicable, i.e., Equation (1) is valid. The concentration of bacteria-carrying particles received from Equation (1) is the theoretical mean value during ongoing surgery from incision to wound closure.

The total air flow necessary can be calculated if the theoretical mean value of bacteria-carrying particles is determined and the number of people in the room and their source strength is known. In this case, the Equation (1) becomes:

$$Q = \frac{n \cdot q_s}{c} \tag{2}$$

In the following example some estimations are given with Equation (2).

Example Calculate the total air flow necessary in an operating room with turbulent mixing air and the theoretical mean value of the concentration should be 10 CFU/m^3, when the operating team of six persons has different kinds of surgical clothing systems according to Table 4.

Solutions *Mixed material clothing system*

With the source strength of 5 CFU/s, the total air flow according to Equation (2) becomes 3 m^3/s. If the source strength of 4.1 CFU/s (Tammelin et al. (2012)) is used , the total air flow will be about 2.5 m^3/s.

Single-use clothing system

With the source strength of 1.15 CFU/s, the total air flow according to Equation (2) becomes 0.7 m^3/s.

It should be noted that at low air flows (<1 m^3/s) changes of the activity level created by the operating team can give relatively high variations of the CFU concentration measured by single samples. This implies that the value of upper confidence level preferably should be used at low air flows. For the case in the Example with single-use clothing system the total air flow thus becomes 0.96 m^3/s.

If, for example, the theoretical mean value of the concentration should be 5 CFU/m^3 the air volume flow values will be twice the above given values.

Most of the recently installed unidirectional air flow systems in Europe are with partial sidewalls or without and have air velocities below 0.3 m/s. During ongoing surgery this often results in a disordered air flow pattern above the operating table resembling that of

total mixing air. Furthermore, entrainment of air can occur from the outer zone to the inner zone. This shows that the dilution principle starts to become valid, see Nordenadler (2010).

Conclusions

The chosen clothing system plays a determining role and it is possible to classify the clothing systems by the definition of their source strength of bacteria-carrying particles. This study shows that the source strength of the tested single-use surgical clothing system is much lower, i.e., the protection efficacy is much higher, than that of the compared reusable mixed material surgical clothing system, which today is one of the most commonly used clothing systems in Sweden. The achieved resultats are in agreement with results presented by Tammelin et al. (2013).

As a first approximation, when calculating the necessary air volume flow in operating rooms, one can assume that the dilution principle is valid in the “sterile zone” during ongoing surgery. In such cases the number of people in the operating rooms, and the selected clothing system should be taken into consideration.

References

Ljungqvist, B. & Reinmüller, B. 1998. Active Sampling of Airborne Viable Particles in Controlled Environment; a comparative study of comon instruments. Europ. J. Parent. Sci. 3(3): 59–62.

Ljungqvist, B. & Reinmüller, B. 2008. Monitoring efficiency of microbiological air samplers. European Journal of Parenteral & Pharmaceutical Sciences, 13(4): 93–97.

Ljungqvist, B. & Reinmüller, B. 2012. People as a Contamination Source, Surgical clothing systems for operating rooms – a comparison between singel-use non-woven and reusable mixed material systems. Technical report, Report No D2012:4, Building Services Engineering, Chalmers University of Technology, Gothenburg.

Ljungqvist, B. & Reinmüller, B. 2013. People as a Contamination Source, Performance of single-use surgical clothing systems in operating rooms. Technical report, Report No D2013:03, Building Services Engineering, Chalmers University of Technology, Gothenburg.

Ljungqvist, B., Reinmüller, B. & Tammelin, A. 2012. Comparison between two measuring methods with active air sampling of airborne viable particles. Europ. J. Parent. Pharmaceut. Sci. 17(2): 56–59.

Nordenadler, J. 2010. Some observations on safety ventilation in operating rooms, PhD-thesis, Bulletin No 74, Building Services Engineering, KTH, Stockholm, (in Swedish).

Tammelin, A., Ljungqvist, B. & Reinmüller, B. 2012. Comparison of three distinct surgical clothing systems for protection from air-borne bacteria; A prospective observational study. Patient Safety in Surgery 6: 23.

Tammelin, A., Ljungqvist, B., Reinmüller, B. 2013. Single-use surgical clothing system for reduction of airborne bacteria in the operating room. J. Hospital Infect. 84: 245–247.

Evaluation of Clothing Systems – Results from Dispersal Chamber Tests

Bengt Ljungqvist & Berit Reinmüller

Chalmers University of Technology, Sweden

Abstract

A dispersal chamber or “body-box” has been used for studying the protective efficacy of different types of clothing systems for cleanrooms and associated controlled environments such as operating rooms. Measurements were carried out in order to relate airborne dispersal of particles and bacteria-carrying particles to the quality of fabrics and the design of clothing systems. The results show the relevance of dispersal chamber testing in the evaluation of clothing systems.

Introduction

People disperse fragments from the skin and the airborne dispersion will vary from person to person and from time to time. The prime function of clothing systems for controlled environments is as a filter, protecting product and processes from airborne contamination. However, the effectiveness of clothing systems will deteriorate due to factors such as aging, wear, washing, and sterilization.

Clothing and clothing systems for cleanrooms and associated controlled environments such as ultraclean operating rooms are mainly tested with regard to material properties such as particle generation, particle filtration, resistance to wear and tear-related damage, and comfort.

The combined filtration efficacy of fabric, construction and design of the clothing can be evaluated in a dispersal chamber or “body box”. A dispersal chamber or “body box” has been used to study cleanroom garment protection efficiency by, e.g., Whyte et al. (1976), Hoborn (1981), Whyte and Bailey (1985), Ljungqvist and Reinmüller (2004) and Whyte and Hejab (2007). Measurements are carried out in order to relate airborne dispersal of total particulates and/or viable particles (colony forming units (CFUs)) to the quality of fabrics and the design of the evaluated clothing system.

Disperal Chamber

The principal arrangement of the dispersal chamber is shown in Figure 1. The concentration of airborne particles is measured in the exhaust duct of the dispersal chamber (body-box), where the air is turbulently mixed.

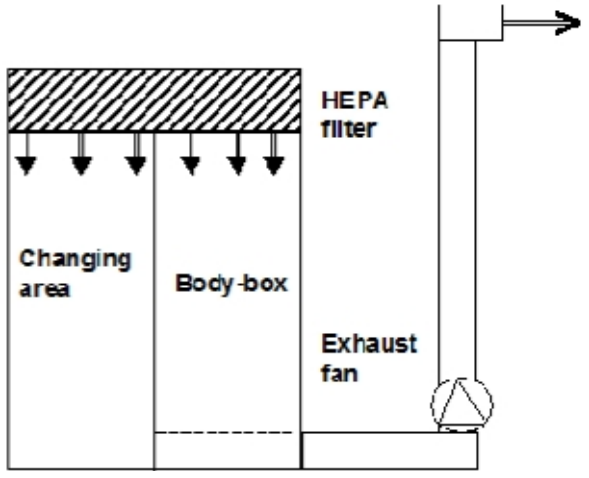


Figure 1. Principal arrangement of dispersal chamber (body-box).

With the assumption of no leakage into the dispersal chamber and the HEPA filters having efficiency close to 100 percent, the simplest possible expression, which is applied on the dilution principle, is describing the source strength (outward particle flow):

$$q_s = c \times Q \quad (1)$$

where

q_s = source strength; total particulates (number/s), bacteria-carrying particles (CFU/s)

c = concentration; total particulates, (number/m^3); bacteria-carrying particles, (CFU/m^3)

Q = total air flow (m^3/s).

The source strength is described as the number of total or viable airborne particulates per second emitted from one person. Data are presented as mean values based on several test subjects dressed in specific clothing systems.

During the measurements the test subjects (men) performed standardized cycles of movements that included arm movements, walk in place and knee bends at a set speed. These movements are, in principle, comparable with those described in IES-RP-CC003.2 (1993). Prior to each cycle of movement, the test subject stood still to avoid the influence

of particle generation from the previous test cycle. The evaluated clothing systems each had five test subjects performing the standardized cycles of movements four times.

In the test chamber viable particles were collected using a slit sampler (FH3®, d50-value 1.6 µm) and the total number of airborne particulates was determined using a particle counter (DPC; HiacRoyco 245). All instruments were operated according to the manufacturers' instructions.

Microbial growth medium for all tests was standard medium Tryptic Soy Agar (TSA) in 9 cm Petri dishes. The TSA plates were incubated for not less than 72 hours at 32˚C followed by not less than 48 hours at room temperature. After incubation the number of colony-forming units (CFU) were counted and recorded as CFU per m^3.

By using the air volume flow in the dispersal chamber in combination with the measured concentrations, the source strengths of each clothing system were estimated. The source strengths reported are mean values per clothing system in number per second for one person of airborne aerobic colony-forming units (CFUs) and in total number per second for one person of airborne particles (≥0.5 µm, ≥5 µm, and sometimes ≥10 µm, ≥25 µm).

Clothing Systems Tested

The clothing systems evaluated were:

- Clothing Systems used in Food Industry
  - Clothing system of mixed material consisting of 35% cotton and 65% polyester. Weight 245 g/m^2. The clothing system was washed once and 40 times.
- Surgical Clothing Systems
  - Common clothing systems with mixed material consisting of 69% cotton and 30% polyester and 1% carbon fiber. Weight 150 g/m^2. The clothing system was evaluated after being laundered up to approximate 50 times.
  - Common clothing system of mixed material consisting of 50% cotton and 50% polyester. Weight 160 g/m^2. The clothing system was evaluated after being laundered once, 25 and 50 times.
- Single-use Surgical Clothing Systems
  - Blue, Spun bonded polypropylene/Melt blown/ Spun bonded polypropylene (SMS). Weight 35 g/m^2, antistatic treated.
- Cleanroom Clothing Systems
  - High quality cleanroom clothing system consisting of 100% polyester. Weight 120 g/m^2. The clothing system was evaluated after being laundered and sterilized by steam once, 25 and 50 times.
  - High quality cleanroom clothing system consisting of 99% polyester and 1% carbon fiber used with underwear consisting of 100% polyester. Weight 100 g/m^2 for clothing system and 99 g/m^2 for underwear. The clothing system was evaluated after being laundered and sterilized by steam 25 and 50 times.

Results of Performed Tests

Comparison of the source strengths for people dressed in various clothing systems are shown in Table 1.

The results in Table 1 show that the source strength varies with the clothing systems. Values of the clothing systems for food manufacturing are much higher than those for operating rooms and in pharmaceutical cleanrooms. The results also show that the level of source strength increases with the number of washing cycles and surgical clothing systems of mixed material (cotton/polyester) reach after 50 washes almost the same CFU-values as those received for clothing systems used in food manufacturing.

It can also be noted that for surgical clothing systems (cotton 50%/polyester 50%) and cleanroom clothing systems (polyester, 100%) the particulate levels reach higher values at 25 washes and sterilizing cycles than after 50. This might be explained by the fact that after certain number of washes and sterilizing cycles the fabric releases particles. When these particles have been washed away, the performance of the fabric stabilizes.

Conclusion

An important conclusion is that fabric/clothing specification should include performance after e.g., 50 washing cycles. However, the washing cycle should be specified with regard to maximum temperature and time.

The values of the source strength depend mainly on chosen clothing system and the persons' activity levels. One of the advantages of estimating the source strength with dispersal chamber tests is that the test subjects are performing standardized cycles of movements, i.e., the movements are the same in all tests. This is of importance in order to compare the filtration or protection efficacy of various clothing systems.

In the dispersal chamber the activity levels of the test subjects are often higher than those of the operating team during ongoing surgery. This results in source strength values for clothing systems evaluated in a dispersal chamber that are mostly higher than those estimated in operating rooms. It should be noted that the source strength mean value of a specific clothing system from operating room measurements seems to be slightly less than half the mean value received from dispersal chamber tests.

Table 1. Comparison of data (mean values per person) of the source strength (number of particles/s and CFU/s) from people dressed in various clothing systems laundered once, 25 times and approximately 50 times, respectively. (Ljungqvist & Reinmüller (2004, 2008, 2012) and Hallberg Borgqvist (2010)).

Clothing system	Contaminant	Number per second		
		1 wash	25 washes	Approx. 50 washes
Food industry, cotton (35%) polyester (65%)	Particles ≥0.5µm	99 885	-	49 531
	Particles ≥5 µm	2 790	-	1 780
	Particles ≥25µm	738	-	506
	CFU	11.8	-	13.8
Surgical clothing system cotton (69%) polyester (30%) carbon fiber (1%)	Particles ≥0.5µm	-	-	29 467
	Particles ≥5 µm	-	-	1 653
	Particles ≥10µm	-	-	608
	CFU	-	-	10.9
Surgical clothing system cotton (50%) polyester (50%)	Particles ≥0.5µm	4 060	13 875	12 207
	Particles ≥5 µm	270	535	698
	CFU	1.7	4.2	9.0
Single-use surgical clothing system polypropylene antistatic treated	Particles ≥0.5µm	1 072	-	-
	Particles ≥5 µm	113	-	-
	Particles ≥10 µm	53	-	-
	CFU	2.5	-	-
High quality cleanroom clothing system polyester (100%).	Particles ≥0.5µm	585	3 950	2 860
	Particles ≥5 µm	9	70	36
	CFU	0.4	0.5	1.1
High quality cleanroom clothing system polyester (99%) carbon fiber (1%) and undergarment polyester (100%)	Particles ≥0.5µm	-	143	153
	Particles ≥5 µm	-	7	10
	CFU	-	<0.2*	<0.2*

* Values below detection limit.

References

Hallberg Borgqvist, J. 2010. Some observations on the impact of clothing systems on the concentration of airborne bacteria-carrying particles during surgery. M Sc-thesis, Report No 112, Building Services Engineering, KTH, (in Swedish).

Hoborn, J. 1981. Humans as Dispersers of Microorganisms – Dispersion Patterns and Prevention. Ph.D. Thesis, Department of Clinical Bacteriology, Inst. of Medical Microbiology, University of Gothenburg, Sweden.

IES-RP-CC003.2. 1993. Garment System Considerations for Cleanrooms and Other Controlled Environments. IES Institute of Environmental Sciences, Illinois.

Ljungqvist, B. & Reinmüller, B. 2004. Cleanroom Clothing Systems; People as a Contamination Source. PDA/DHI Publishing, LLC, River Grove, IL. ISBN 1-930114-60-5.

Ljungqvist, B. & Reinmüller, B. 2008. Evaluation of product protection efficiency for two clothing systems used in food manufacturing. RenhetsTeknik 3: 14–17.

Ljungqvist, B. & Reinmüller, B. 2012. People as a Contamination Source, Surgical clothing systems for operating rooms – a comparison between disposable nonwoven and reusable mixed material systems. Technical report, Report No D2012:04, Building Services Engineering, Chalmers University of Technology, Göteborg.

Whyte, W. & Bailey, P. 1985. Reduction of Microbial Dispersion by Clothing. J. Parent. Sci. Technol. 39: 51–60.

Whyte, W. & Hejab, M. 2007, Particle and microbial airborne dispersion from people. Europ. J. Parent. Pharmaceut. Sci. 12(2): 39–46.

Whyte, W., Vesley, D. & Hodgson, R. 1976. Bacterial dispersion to operating room clothing. J. Hyg., Camb. 76: 367–378.

Rapid and Sensitive Detection of Antibodies against Norovirus using VLP-functionalized Biolayer Interferometry Biosensor

Sanna Auer[1,3], Tiia Koho[1], Vesna Blazevic[2], Hanni Uusi-Kerttula[2], Timo Vesikari[2] & Vesa P. Hytönen[1,3,*]

[1]BioMediTech, University of Tampere, Finland
[2]School of Medicine, University of Tampere, Finland
[3]Fimlab Laboratories Ltd., Finland
* correspondence to vesa.hytonen@uta.fi

Abstract

We describe the use of a biolayer interferometry biosensor (ForteBio Octet Red) for fast and sensitive detection of analyte from serum samples. Norovirus-like particles were used to functionalize biosensor surface to detect norovirus antibodies from human serum. Two different His-tagged norovirus-like particles were used. With metal chelator (DAB) enhancement specific antibodies could be detected nearly as sensitively as with enzyme-linked immunoassay (ELISA) using serum dilution up to 1:100 000. With Octet system the analysis is though much faster compared to the conventional ELISA: the analysis could be performed in less than 30 min using prefunctionalized sensors.

Introduction

Noroviruses (NoV) infect people of all ages and are a major cause of acute epidemic gastroenteritis worldwide. Symptoms appear 12–48 h after viral infection and are characterized by acute onset of nausea, vomiting, abdominal cramps and diarrhea. Infection can be severe for small children and elderly people as well as for immunocompromised persons leading to hospitalization in order to prevent severe dehydration. The virus is spread by contaminated food and water and from person to person via the fecal-oral pathway

and can be further transmitted to food and food contact surfaces by virus-contaminated hands [1,2].

Noroviruses possess a high level of genetic and antigenic diversity. Based on the amino acid sequence of the capsid VP1 protein, norovirus genotypes can be classified into five genogroups (GI–GV) [3,4]. Structural studies have shown that the viral capsid is almost entirely composed of the 58 kDa VP1 capsid protein. VP1 consists of two domains, the shell (S) domain and the protruding (P) domain. The two domains are linked by a short hinge. The S domain is involved in the formation of the icosahedral shell, whereas the P domain is involved mainly in dimeric contacts to stabilize the capsid. The dimers of the P domain project out from the icosahedral shell, and are predicted to contain the antigenic determinants of the immunological response of the host [5].

The recombinant expression of VP1 major capsid protein results in self-assembly of empty, non-infectious virus-like particles (VLPs) that are morphologically and antigenically similar to the infective virion [6]. Because the biological characterization of human noroviruses has been hampered by the lack of an appropriate cell culture system and animal model for the propagation of the virus, VLPs have been used extensively to study norovirus structure and stability, host-cell interactions, and as a tool in diagnostic serological assays [2,5,7,8]. When expressed recombinantly in *E. coli*, P domain dimers form larger complexes called P particles, which have unique repetitive structure [9].

Biolayer interferometry (BLI) is a label- and fluidics-free, real-time detecting and monitoring system based on light intensity interference [10–13]. Interference changes between the intensities of the reflected light beams are used to measure the changes in the attached molecular layer on the sensor surface. Histidine-tagged NoV-VLPs or NoV P-particles were immobilized on Ni-NTA sensors (Figure 1) and used to detect norovirus-specific antibodies from human serum samples. The signal was further enhanced with DAB (3,3´-diaminobenzidine) metal chelator [14] via anti-human-HRP conjugated antibodies detecting the surface bound norovirus antibodies. DAB enhancement signal was not generated on the norovirus-negative serum samples assayed at dilutions 1:100 and 1:500 (Figure 2). These preliminary results strongly suggest that BLI can be used with clinical samples giving almost as sensitive detection as ELISA, but in much shorter time frame.

Methods

Interferometry biosensing is based on the measurement of light intensity produced by an interference of light beams reflected from a reference surface and a biofunctionalized sensor surface. Fortebio Octet RED384 instrument equipped with 16 parallel biosensors was used in this study.

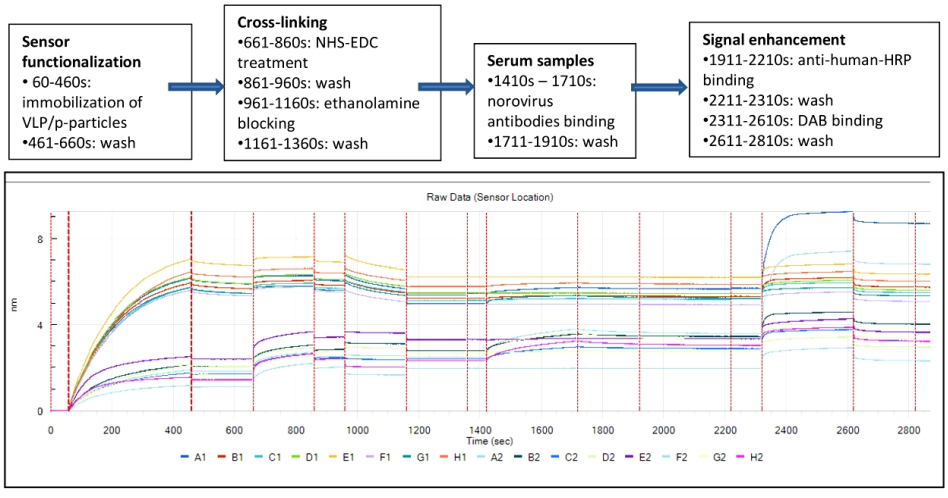


Figure 1. Biolayer interferometry-assay workflow showing the virus-like particle immobilization, covalent amine-coupling, binding of serum antibodies and anti-human HRP and enhancement of the detection with DAB.

All the used reagents were of analytical grade. In our experimental setup the Ni-NTA sensor surface was functionalized with histidine-tagged NoV-VLPs or NoV P-particles in concentrations of 60 and 80 µg/ml respectively. The production and purification of the recombinant virus-like particles is described by Koho et al. 2012 [15]. NHS(N-hydroxysuccinimide)–EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride]) coupling and ethanolamine blocking were performed according to Johnsson et al. 1991 [16] in distilled water.

Convalescent serum samples from norovirus-infected subjects were collected in 2006–2008 at the Tampere University Hospital and stored at −20 ◦C until used [17]. ELISA-assays were performed essentially as described in Nurminen et al. 20118. For Octet analysis the norovirus-related particles, serum samples and anti-human HRP labelled antibody (Vector laboratories Inc. USA, dilution 1:800 was used) were all diluted in 10 mM NaPO3, 150 mM NaCl, 0.02% Tween 20, 0.05% Sodium azide, 1 mg/ml BSA (pH 7.4) and the temperature at Octet system was set at +25°C. Measurement plates were black, tilted-bottom 384-well plates supplied by Fortebio.

Results and discussion

The BLI was found to be rapid and sensitive method for detection of norovirus antibodies from serum samples. Dilution up to 1:100 000 yielded a measurable signal, which is comparable to that observed for ELISA (Figure 2). However, BLI assay can be performed significantly faster. Large dynamic range was observed for BLI (Figure 2). Serum dilutions up to 1:100 gave almost perfectly linear response in BLI biosensing, while ELISA signal was saturated already at dilution 1:1000. In practice, this means that quantitative measurement of antibody concentration with BLI can be performed by using few or just one

dilution(s) of each sample, while quantitative ELISA would require assaying of several samples to avoid signal saturation.

BLI is a robust technology and the instrumentation allows use of heterogeneous and complex sample materials (whole blood, serum, urine, saliva) and simple setup of assays. Therefore we believe BLI may offer a reasonable alternative for ELISA in the analysis of clinical samples.

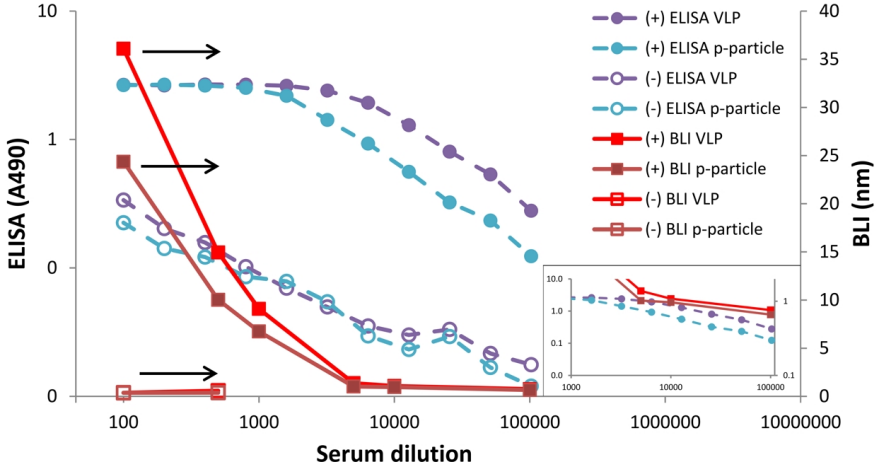


Figure 2. Comparison of the BLI and ELISA for detection of norovirus antibodies from human serum samples. Samples were collected from norovirus-positive and norovirus-negative patients.

References

1. Koopmans, M. & Duizer, E. 2004. Foodborne viruses: an emerging problem. Int. J. Food Microbiol., 90: 23–41.

2. Patel, M.M., Hall, A.J., Vinje, J. & Parashar, U.D. 2009. Noroviruses: a comprehensive review. J. Clin. Virol., 44: 1–8.

3. Fankhauser, R.L., Monroe, S.S., Noel, J.S., Humphrey, C.D., Bresee, J.S., Parashar, U.D., Ando, T. & Glass, R.I. 2002. Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. J. Infect. Dis., 186, 1–7.

4. Zheng, D., Ando, T., Fankhauser, R.L., Beard, R., Glass, R.I. & Monroe, S.S. 2006. Norovirus classification and proposed strain nomenclature. Virology 346: 312–323.

5. Prasad, B.V., Hardy, M.E., Dokland, T., Bella, J., Rossmann, M.G. & Estes, M.K. 1999. X-ray crystallographic structure of the Norwalk virus capsid. Science 286: 287–290.

6. Jiang, X., Wang, M., Graham, D.Y. & Estes, M.K. 1992. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J. Virol. 66: 6527–6532.

7. Ausar, S.F., Foubert, T.R., Hudson, M.H., Vedvick, T.S. & Middaugh, C.R. 2006. Conformational stability and disassembly of Norwalk virus-like particles. Effect of pH and temperature. J. Biol. Chem. 281: 19478–19488.

8. Nurminen, K., Blazevic, V., Huhti, L., Räsänen, S., Koho, T., Hytönen, V.P. & Vesikari T. 2011. Prevalence of norovirus GII-4 antibodies in Finnish children. J Med. Virol. 83: 525–31.

9. Tamminen, K., Huhti, L., Koho, T., Lappalainen, S., Hytönen, V.P., Vesikari, T. & Blazevic, V. 2012. A comparison of immunogenicity of norovirus GII-4 virus-like particles and P-particles. Immunology 135: 89–99.

10. Lotze, M.T. 2009. US Patent 2009/0147264A1.

11. Abdiche, Y., Malashock, D., Pinkerton, A. & Pons, J. 2008. Determining kinetics and affinities of protein interactions using a parallel real-time label-free biosensor, the Octet. Anal. Biochem. 377: 209.

12. Rich, R.L. & Myszka, D.G. 2007. Higher-throughput, label-free, real-time molecular interaction analysis. Anal. Biochem. 361: 1.

13. Farkas, A.M., Kilgore, T.M. & Lotze, M.T. 2007. Detecting DNA: getting and begetting cancer. Curr. Opin. Invest. Drugs 8: 981.

14. Nadkarni, V.D. & Linhardt R.J. 1997. Enhancement of diaminobenzidine colorimetric signal in immunoblotting. BioTechniques 23: 385–388.

15. Koho, T., Huhti, L., Blazevic, V., Nurminen, K., Butcher, S.J., Laurinmäki, P., Kalkkinen, N., Rönnholm, G., Vesikari, T., Hytönen, V.P. & Kulomaa, M.S. 2012. Production and characterization of virus-like particles and the P domain protein of GII.4 norovirus. J. Virol. Metho.: 179, 1–7.

16. Johnsson, B., Löfås, S. & Lindquist, G. 1991. Immobilization of proteins to carboxymethyldextran- modified gold surface for biospecific interaction analysis in surface plasmon resonance sensors. Anal. Biochem. 198: 268–277.

17. Blazevic, V., Lappalainen, S., Nurminen, K., Huhti, L. & Vesikari, T. 2011. Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. Vaccine 29: 8126–8133.

Compounding of Pediatric Oral Formulations

Minna Helin-Tanninen

KYS Pharmacy, Finland

Introduction

Compounding, i.e. extemporaneous preparation, has remained an integral part of pharmacy practice. Extemporaneous compounding in hospitals and community pharmacies is important since it allows the provision of age-appropriate dosage forms when suitable authorised medicines are not available. The compounding of customised medicines is needed in particular situations in which the proprietary medicines available do not meet the specific needs of the patients: need for particular strengths, alternative dosage forms, ingredients or organoleptic characteristics.

Although the tradition of compounding “lege artis” can be traced over a period of hundreds of years as a part of the professional skill of the pharmacist, the quality of the extemporaneous products being compounded has been inadequately studied. Many of the medicines used for neonates and children have not yet been licensed for this purpose and their use is considered off-label or unlicensed. Off-label and unlicensed medicine use is more likely to occur in newborn infants, who may be predisposed to suffer adverse drug reactions due to their physiological immaturity. The off-label and unlicensed use of medicines in paediatrics is common in many countries: in hospital and neonatal care studies, the proportion has ranged from 5% up to 65% of all prescriptions. This leads to a situation where attempts are made to modify an existing “adult” dosage form or an active ingredient and excipients are converted to an age-appropriate paediatric formulation. The dose required for a child may be delivered in a portion of a tablet designed for adult.

A positive trend in the approval of safe and efficacious manufactured medicines for children seems to be in progress in Europe, but achieving this goal takes time. The Regulation issued by the European Parliament and of the Council on Medicinal Product for Paediatric Use came into force in January 2007, nearly ten years later than the corresponding mandatory regulation in the USA. It obliges the pharmaceutical industry to undertake clinical trials in the paediatric population.

Need for standards

The skill and judgement of physicians and pharmacists are critical in ensuring that the patient receives the appropriate drug, the best dosage form and an optimal dosing regimen. In the absence of specific clinical trial-based data in children, clinicians are forced to rely on experience from adult patients, although children have different pharmacokinetics to adults and their response to many medicines can be unpredictable. Medication with extemporaneous preparations in hospital is an acute multidisciplinary process where decisions need the input and co-operation between physicians, pharmacists and nurses, i.e. all of the professionals involved have a duty of care to the patient within their area of responsibilities.

Currently, there are neither appropriate nor comprehensive published standards about the process of extemporaneous preparation; in fact not all pharmacies compound according to published formulations. In order to ensure product quality, it has been recommended that there should be harmonization of extemporaneous formulations and quality control procedures and collected data should be published as standards and uniformly implemented in all countries.

Harmful excipients

Thousands of different excipients are used in different medicines. In recent years, excipients have proved to be anything but inert. It has been studied that almost all treated hospitalised neonates received medicines with at least one potentially harmful excipient.

The amount of preservatives should be kept at minimum in paediatric formulations. Adverse toxic effects have been reported in paediatric patients due to the use of inappropriate excipients both in extemporaneous and commercial products. In many products, the high osmolality associated with irritating effects on the gastrointestinal tract is not due to the active ingredient but to “inactive” excipients such as propylene glycol or sorbitol.

Different dosage forms

Medicines intended for adults can be modified for children into oral suspensions, oral powders or capsules the contents of which are emptied for use. However, the quantity of the active ingredient per dosage can vary in these preparations, depending on the dosage form and handling of the product. The lower the proportion of active component present in the mixture, the more difficult it is to achieve an acceptably low deviation in the active content.

The same drug may be compounded in liquid, capsule or powder form according to different standards and monographs across Europe or even within the same European country. These differences reflect the different traditions of extemporaneous preparation. In addition, many different concentrations may be compounded for each dosage form.

Hand crafted procedures are widely used in hospital and community pharmacies. Crushing of tablets is the critical point in the powder mass preparation. The importance of the crushing technique becomes emphasized in small size oral powders, where more variation in content can be observed. The loss of active ingredient may be significant. Commercial tablets have a specific content uniformity variation of their own, which may lead to some variation in the modified dosage forms.

For infants and children under 5 years old, pharmaceutical liquids are traditionally preferred for oral administration. However, it has been noted that uncoated mini-tablets seemed to be a very promising alternative to liquid formulation even for infants aged 6–12 months. Solid dosage forms such as oral powders, fast dissolving granules, granules, mini-tablets, orally disintegrating mini-tablets or dispersible dosage forms may be considered as alternatives to oral liquids.

Small capsules to be emptied and powders are suitable extemporaneous formulations for infants, since they can be easily added to milk, other liquid or to some pleasant-tasting semi-solid food to prevent aspiration of a powder and to mask the taste. Oral solids are simple to use at home, and the stability of the drug is usually good. However, administration through a nasogastric tube requires a liquid form of drug.

A suspension should dispense drug particles uniformly after brief shaking of the bottle, so that the desired dose can be measured accurately. In practise, the compounding pharmacist does not have the possibilities of the industrial formulator to control flocculation. Special caution is needed in dispensing extemporaneously prepared oral suspensions in multidose containers since the variation between doses may be considerable. Incorrect dosages may be dispensed from poorly mixed suspension bottles. However, the quality of many commercial vehicles seems to be good when compared to compounded vehicles. Suspensions should always be shaken well before use to ensure uniform distribution of the solid in the vehicle. However the command “Shake well before using may be understood in different ways in the pharmacy, on the ward and particularly at home.

Literature

Brion, F., Nunn, A.J. & Rieutord, A. 2003. Extemporaneous (magistral) preparation of oral medicines for children in European hospitals. Acta Paediatrica 92: 486–490.

Giam, J.A. & McLachlan, A.J. 2008. Extemporaneous product use in paediatric patients: a systematic review. Int. J. Pharma. Pract. 16: 3–10.

Glass, B.D. & Haywood, A. 2006. Stability considerations in liquid dosage forms extemporaneously prepared from commercially available products. J. Pharma. Pharmaceut. Sci. 9: 398–426.

Haywood, A. & Glass, B. 2011. Pharmaceutical excipients – where do we begin? Australian Prescriber, 34(4): 112–114.

Helin-Tanninen M. 2013. Compounding of paediatric oral formulations: extemporaneous nifedipine capsules, powders and suspensions in the hospital pharmacy. University of Eastern Finland. Dissertations in Health Sciences. Juvenes Print, Tampere.

Nahata, M.C. & Allen, L.V. 2008. Extemporaneous drug formulations. Clin. Therapeut. 30(11): 2112–2119.

Salunke, S., Hempenstall, J., Kendall, R., Roger, B., Mroz, C., Nunn, T. & Tuleu, C. 2011. European Paediatric Formulation Initiative’s (EuPFI) 2nd conference commentary – Formulating better medicines for children. Int. J. Pharmaceut., 419: 235–239. doi:10.1016/j.ijpharm.2011.06.040

Standing, J.F. & Tuleu, C. 2005. Paediatric formulations – Getting to the heart of the problem. Int. J. Pharmaceut., 300: 56–66.

Testing of Hand Hygiene of the Personnel in an Internal Medicine Ward using Automatic Non-Touch Dosing Devices in Comparison with Hand-Operated Dispensers

Gun Wirtanen*, Päivi Godden[1], Satu Salo[2] & Hannu Salmela[2]

* R[3]Nordic Board, Finland

[1] KiiltoClean Oy, Espoo, Finland

[2] VTT Technical Research Centre of Finland, Tampere & Espoo, Finland

Abstract

KiiltoClean Oy developed an automatic non-touch dosing device Kiilto Non-Touch for use in improving hand hygiene at public places like hospitals, healthcare centres, day care centres, canteens etc. in the HighTech-Hospital project 2010–2013. The device promotes good personal hygiene in health care premises, public spaces, toilets and bathrooms. The testing of hand hygiene of the personnel at an internal medicine ward was performed by researchers from VTT. The testing was first performed using hand-operated devices and thereafter using non-touch dosing devices. Hand washing movements are also shown in the presentation.

Background

Hand hygiene is the most effective mean in prevention of spreading infections. The most important single measure to cut the transmission of diseases is to correctly use alcohol-based hand rubs. An efficient way of action is to rub 3–5 ml of hand rub all over the hands, which takes 20–30 s if the spreading and rubbing is correctly perform. The hand rub is rubbed until all the alcohol has evaporated from the skin. Unfortunately, only 50% of the hand disinfections within the healthcare sector are performed properly. Campaigns

in hand hygiene improve the situation only provisionally. New measures and also new techniques are therefore needed to improve hand hygiene.

Introduction

In the VTT-led HighTech-Hospital project, KiiltoClean Oy developed an automatic dispenser called Kiilto Non-Touch dosing device (Fig. 1). This dosing device functions with 1L dispenser packages. It is drip proof and battery-powered. The hinged lid has a front opening, which makes it easy to install the dosing device also in narrow spaces. The dose adjustment is between 1.5–3 ml. The piston engine of the device is set to release a dose equivalent to one or two strokes. The flexible material of the drip tray is safe to use, removable and can be washed or wiped clean. The device has a service button that prevents dosing during drip tray cleaning and includes a standard type key that fits every Kiilto Non-Touch dosing device.

Research study

The testing (Fig. 2) was performed in an internal medicine ward. The efficacy of the hand hygiene practices was tested by comparing the results obtained using hand-operated dispensers with results from automatic non-touch devices. The same hand rub was used in both types of dispensers during the whole testing period. The personnel tested was informed about the testing days in advance.

Figure 1. The dosing of the disinfectant with the Kiilto Non-Touch dispenser is based on impulses from the movement sensor and thus the users' hands do not touch the surfaces of the device. This dispenser is designed for use in public places, i.e. it is locked so that the disinfectant bag cannot be removed by visiting customers.

Figure 2. The testing was performed using a modification of the standard EN1500 i.e. without using artificial inoculation. Microbes from fingertips were transferred to dilution solution and cultured on 3M™ Petrifilm™ Aerobic Count Plates. The results from fingers before and after disinfection showed that the testing with the manual dispensers gave a reduction of up to 0.7 log-units and with the Kiilto Non-Touch device up to 1.1 log-units.

The efficacy of the disinfection was most clearly visible in the beginning of the work shift. The results were also better after using the automatic devices than after using the manually operated ones. The measurements performed during the work shifts did not show statistically better results for the automatic device compared to the manually operated devices. The most important practice is to spread the hand rub correctly on hands, but the non-touch system of course reduces the spreading of possible infections, since the device is not touched.

In the user questionnaire at the internal medicine ward the users stated that they found the automatic Kiilto Non-Touch device more suitable for the hospital environment than the manually operated dispensers. The daily use with the automatic dosing devices was also found easier than that of the other versions tested in the premises.

Application areas

The Kiilto Non-Touch dosing devices are suitable for use in hospitals, medical centers, healthcare facilities, elderly homes, other public places e.g. daycare centers and schools as well as food manufacturing premises.

Conclusions

This automatic Non-Touch dispenser gives an adequate amount of hand rub (3 ml) for disinfecting hands. The time used for rubbing the disinfectant took 12–20 s in the study performed. The recommendation in guidelines is 30 s. The use of hand rub was more frequent with the automatic device than with the manually operated device. The use increased 56% as compared to the same time a year before. Furthermore, the personnel taking part in the study stated that they gave more thoughts on their actions. However, the microbiological samples taken from fingertips in this study showed that the fingertips were not always properly disinfected. The importance of spreading the hand rub evenly can be taught through regular exercises in hand hygiene and thus the efficacy of hand disinfection can be improved.

Literature

Anon. http://www.kiiltoclean.com/images/pdf/pro_kiilto_non_touch.pdf. April 1, 2014.

Enbom, S., Heinonen, K., Kalliohaka, T., Mattila, I., Nurmi, S., Salmela, H., Salo, S. & Wirtanen, G. 2012. High-tech sairaala: Korkean hygienian hallintakonseptit sairaalassa. Tutkimushanke high-tech konseptien benchmarkkaamisesta sairaaloissa. http://www.vtt.fi /inf/julkaisut/muut/2012/VTT-R-02058-12.pdf. VTT, Tampere. 58 p. + app. 8 p. (In Finnish)

Salmela, H., Enbom, S., Heinonen, K., Kalliohaka, T., Mattila, I., Salo, S. & Wirtanen, G. 2013. Proactive prevention of hospital infections. http://www.vtt.fi/inf/pdf/research-highlights/2013/R10.pdf. In: Research highlights in safety and security. Ed. Rouhiainen, V. VTT Research Highlights 10. VTT. Espoo. Pp. 20–21.

Wirtanen, G., Nurmi, S., Kalliohaka, T., Mattila, I., Heinonen, K., Enbom, S., Salo, S. & Salmela, H. 2012. Surface and air cleanliness in operating theatre environments. Europ. J. Parent. Pharmaceut. Sci. 17(3): 87–93.

Effect of Door Opening in Hospital Isolation Room

Petri Kalliomäki*, Pekka Saarinen & Hannu Koskela

Finnish Institute of Occupational Health, Finland

Abstract

This project studying airflow patterns across the isolation room doorway was carried out in co-operation between Finnish Institute of Occupational Health (FIOH) and National University Hospital of Singapore (NUH). Experiments at FIOH were made in a full-scale isolation room model and passage through the doorway was simulated with a moving manikin. The airflow patterns across both hinged and sliding doors were studied with measurements and numerical simulations. Different environmental conditions e.g. ventilation rates, pressure difference, door and passage speed and temperature difference between rooms were tested to find out how they affect the doorway flows.

Smoke visualizations illustrated well the door generated flows and the difference in airflow patterns between hinged and sliding doors. Tracer gas measurements showed quantitatively that the amount of airflow through the hinged door (on average) was between 1.3–2.5 m^3 and across sliding door 0.3–1.1 m^3 depending on the environmental parameter values. Passage increased the amount of airflow notably. Less airflow was detected with sliding door setup. Results of the numerical simulations will also be shown. These results were in agreement with the results from the experiments.

Introduction

Recent pandemic outbreaks of SARS and A/H1N1 viral infections have increased the use of hospital isolation rooms dramatically. Although isolation rooms are designed to prevent the spreading of the diseases, containment failures do still happen and door-opening and passage through the door are among important factors (Tang et al. 2005, 2006). Opening

of the door allows airborne infectious agents to pass out of the isolation room due to strong airflow created by the door and person passing through the doorway.

This study was a part of an international project measuring, visualizing and modelling the airflow patterns across the isolation room doorways. In the project the partner institutions were FIOH from Finland and NUH from Singapore. Studies on the airflow patterns using full-scale air were carried out at FIOH and small-scale water models at NUH (Tang et al. 2013). Results of the numerical simulations will also be shown. In this article the authors summarize the findings obtained so far at FIOH.

Method

Airflow through the doorway between isolation and anteroom was studied in laboratory environment with a full-scale model. The model consisted of two adjacent rooms with a dividing wall and a doorway in the middle between the rooms. Two different door types were used on the doorway (one at a time), i.e. single hinged and sliding -doors. The two rooms were identical (4.00 m long, 4.70 m wide and 3.00 m high each). The door between the rooms was automated and controlled by a computer. Supply air was distributed through radial diffusers located in the ceiling in the middle of the rooms (mixing ventilation). Exhaust grilles were located on the wall, close to the ceiling near the dividing wall. Human passage through the doorway was simulated with a manikin attached to a small cart moving along a motorized rail (Kalliomäki et al. 2013).

Tracer gas measurements were performed in order to quantify the airflow through the isolation room doorway. Measurements were made with two tracer gases, sulfur hexafluoride (SF_6) and nitrous oxide (N_2O). Utilizing two gases enabled defining the airflow to both directions through the doorway at once. First, measurements were made without ventilation. These measurements were important for uncovering the pure effect of various parameters tested (Kalliomäki et al. 2012, 2014).

After the basic, fundamental experiments without ventilation were made, ventilation was turned on and tracer gas measurements were performed in a more realistic isolation room environment. Only the results for single hinged door are presented in this paper. The experiments with sliding door will be reported later. First, the experiments were done with 12 ACH and -20 Pa pressure difference (between isolation- and anteroom) without manikin passage. Then the tracer gas measurements were performed with manikin passage through the doorway maintaining the same ventilation rate and pressure difference as earlier (Kalliomäki et al. 2013).

The flow patterns across the doorway were illustrated with smoke visualizations and computational fluid dynamics (CFD) simulation. Smoke was injected into one room at a time and the flow patterns were recorded with a camera from the other room as the door was opened. A CFD simulation was made by using incompressible, time accurate large eddy simulation (LES) solver with LES WALE subgrid model using 2 ms time step. CFD-simulations were carried out only without ventilation and manikin passage for single hinged door. Artificial smoke videos were generated to visualize the simulated flow patterns by creating several isosurfaces with decreasing transparency, corresponding to the increasing tracer gas concentration levels (Saarinen et al. 2014).

Results

Figure 1 shows a series of still images obtained from the smoke videos for the single hinged and sliding -doors (without ventilation). Qualitative visual examination of the figure indicates that the hinged door creates a pronounced flow through the doorway and the smoke spreads further inside the anteroom than with the sliding door. The drastic influence of the hinged door itself overshadows the impact of the passage close to doorway. However, after the manikin has stopped the passage induced flow spreads the smoke over the entire room. For the sliding door the influence of the passage is much more distinct (close to the doorway as well). Simulated smoke visualizations generated from the CFD-modeling (not shown here) show comparable flow structures with real smoke visualizations (Saarinen et al. 2014).

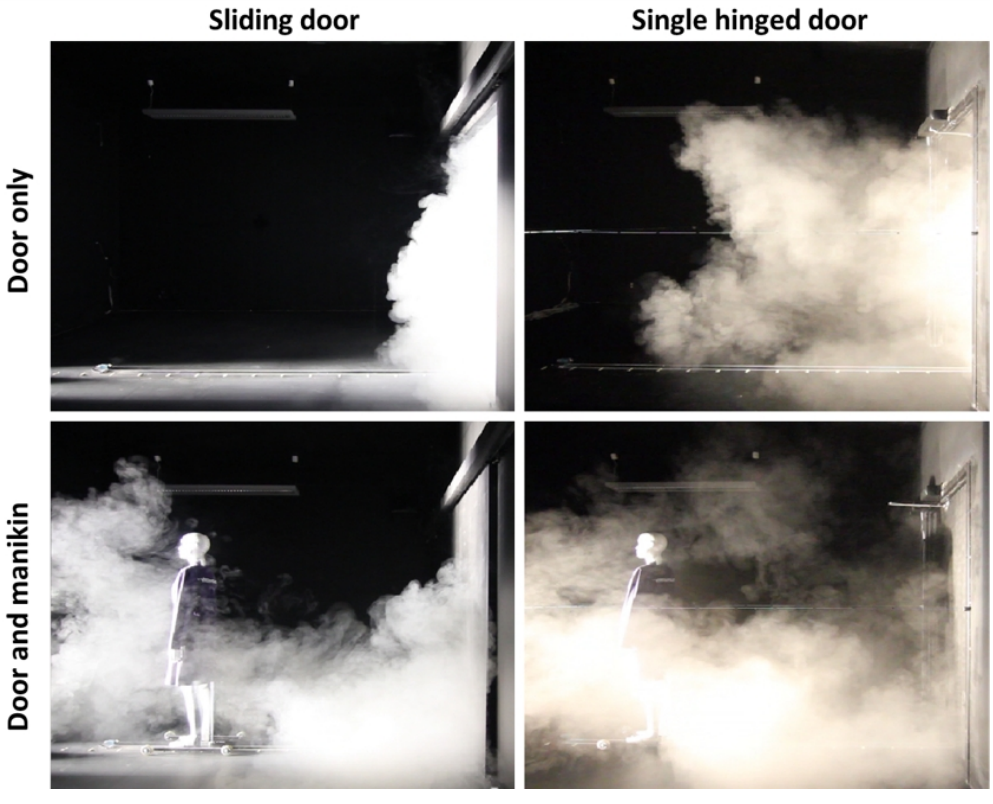


Figure 1. Smoke visualizations of the flow patterns (anteroom side view) for single hinged and sliding doors without ventilation (after Kalliomäki et al. 2014). Door was set to open in 3 s, stay open for 8 s and close in 5 s.

Figure 2 shows the effect of the door opening and the manikin passage on the amount of airflow through the doorway (from isolation room to anteroom) without ambient ventilation. The door opening, hold open and closing times were kept constant (3 s, 8 s and 5 s respectively). The airflow without passage (door operation only) was 0.56 m^3 and 1.39 m^3 on average for sliding and hinged -doors correspondingly. The airflow with manikin passage (speed 1 m/s) was 0.92 m^3 and 1.65 m^3 on average for sliding and hinged -doors respectively (Kalliomäki et al. 2014).

Figure 3 shows the airflow across the doorway for the single hinged door (with and without manikin passage) with ventilation and pressure difference between the rooms (12 ACH and -20 Pa). As in Figure 2, only the results from the isolation room to anteroom are shown. The airflow without (door only) and with manikin passage (1 m/s) was 1.31 m^3 and 1.56 m^3 on average respectively (Kalliomäki et al. 2014).

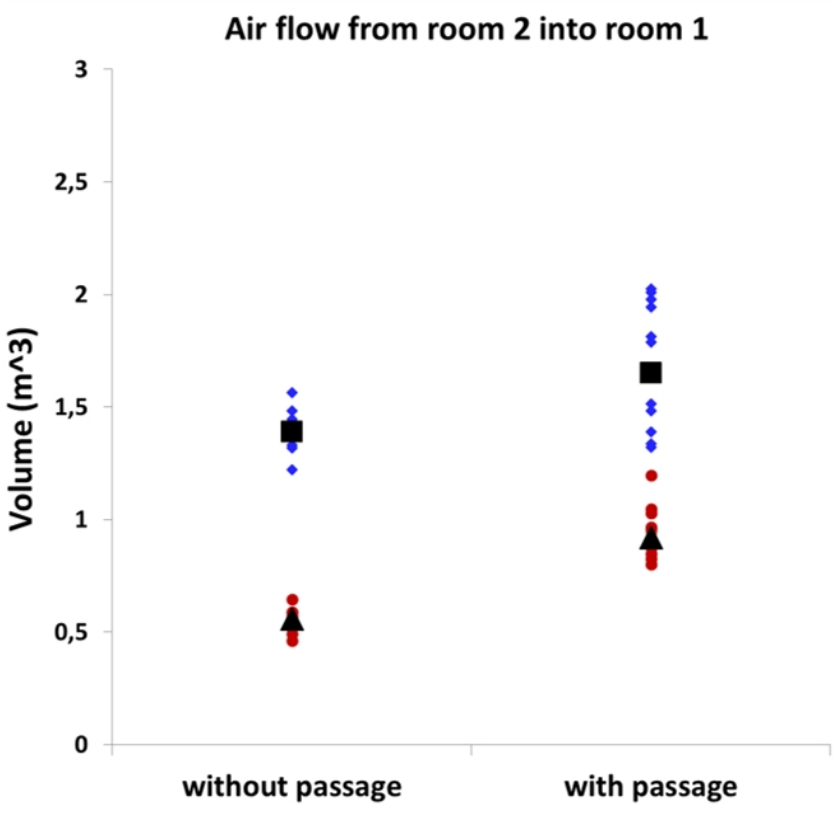


Figure 2. Airflow through single hinged and sliding -doors without ventilation. Blue diamonds (red dots) represent separate hinged (sliding) -door openings and black squares (triangles) their averages. The door parameters were as in Figure 1. Room 2 is isolation room and room 1 is anteroom.

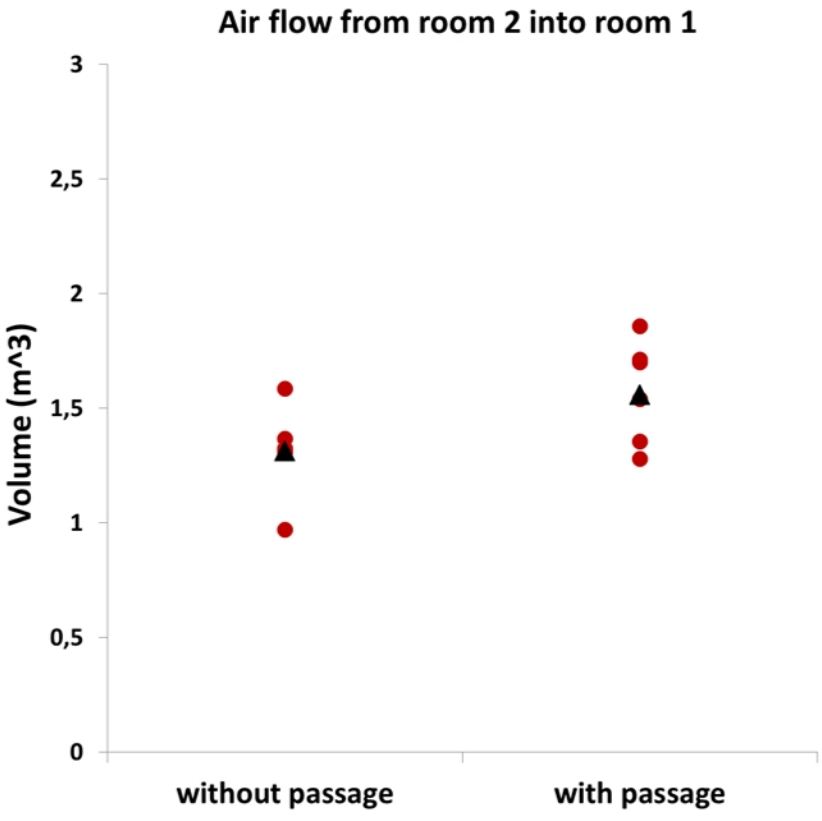


Figure 3. Airflow across the single hinged-door with ventilation and pressure difference between the rooms. Black triangles represents the averages for each case. The door parameters were as in Figure 1.

Conclusions

Smoke visualizations illustrated well the door generated flows and the difference in airflow patterns between hinged and sliding -doors. Similar flow patterns were found in the experiments, CFD simulations, which were conducted only for single hinged door without manikin passage, and small-scale water model visualizations, which were carried out at NUH. According to the tracer gas measurements the amount of airflow was on average, when measured with manikin passage and without ventilation, through the doorway 1.65 m^3 during hinged door operation and 0.92 m^3 during sliding door operation. The single hinged door operation with ventilation (12 ACH and -20 Pa pressure difference) and with manikin passage induced 1.56 m^3 airflow across the doorway on average. Thus passage increased the induced airflow notably in each case. Significantly less airflow through the doorway was measured during sliding door than with hinged door operation.

References

Kalliomäki, P., Koskela, H., Saarinen, P., Mustakallio, P., Sandberg, E., Tang, J.W., Nicolle, A., Klettner, C. & Tham, K. 2012. Air leakage from hospital isolation room during passage through a hinged door. Proceedings of Ventilation 2012, September 17–19 2012, Paris.

Kalliomäki, P., Koskela, H., Saarinen, P., Tang, J.W., Klettner, C. & Nicolle, A. 2013. Airflow Patterns through a single hinged door in hospital isolation rooms. Proceedings of CLIMA 2013, June 16–19 2013, Prague.

Kalliomäki, P., Saarinen, P., Tang, J.W. & Koskela, H. 2014. Airflow patterns through single hinged and sliding doors in hospital isolation rooms, Proceedings of Indoor Air 2014, 7–12 July 2014, Hong Kong. (Accepted).

Saarinen, P., Kalliomäki, P., Tang, J.W. & Koskela, H. 2014. Air leakage through isolation room doorway – Measurements and CFD simulations, Proceedings of Indoor Air 2014, 7–12 July 2014, Hong Kong. (Accepted).

Tang, J.W., Li, Y., Eames, I., Chan, P.K.S. & Ridgway, G.L. 2006. Factors involved in the aerosol transmission of infection and control of ventilation in healthcare premises. J. Hospital Infect. 64: 100–114.

Tang, J.W., Eames, I., Li, Y. Taha, Y.A., Wilson, P., Bellingan, G., Ward, K.N. & Breuer, J. 2005 Door-opening motion can potentially lead to a transient breakdown in negative-pressure isolation conditions: the importance of vorticity and buoyancy airflows. J. Hospital infect. 61: 283–286.

Tang, J.W., Nicolle, A., Pantelic, J., Klettner, C., Su, R., Kalliomäki, P., Saarinen, P., Koskela, H., Reijula, K., Mustakallio, P., Cheong, D.K.W., Sekhar, C. & Tham, K. 2013. Different types of door-opening motions as contributing factors to containment failures in hospital isolation rooms. PLoS One, 8, e66663.

Modern Concepts of Environmental Monitoring: QbD for Aseptic Processes

Gilberto Dalmaso

Particle Measuring Systems, Italy

Abstract

There is a real and growing need in pharmaceutical microbiology for the introduction of new analytical methods which can address the requirements of today's fast-paced industry. Changes in the industry are beginning to happen. Technology driven solutions to drug development and manufacture are beginning to take shape. The main Regulatory Agencies have recently published a series of guidelines with the purpose to facilitate the innovation in the pharmaceutical industries. If Good Manufacturing Practice (GMP) has been the light of Pharmaceutical Industry in the last 20 years, Quality by Design (QbD), i.e. how to build the quality into the drug product, will be the new paradigm for the next years. This approach will have profound effects on the future direction of the industry. These changes will have an impact on every area of drug manufacture, including microbiological analysis. The new QbD processes will require real time or near real time analytical data and very different types of analytical evaluation.

The scope of this presentation is to show the usefulness and potentialities of this new technologies/methods implemented in the pharmaceutical field for real time release in pharma industry and in particular applied on the manufacturing process investigation, using the real-time evaluation, to engage the suitable corrective actions and resolute remedy at the right time.

It is important to underline that the new technologies has been able to provide economic benefits in terms of "safe-costs" and the "stock-out" risk reduction of the products as well.

Cleanroom Classification Studies and Airborne Bioburden Particles in Operating Theatres

Hannu Salmela, Seppo Enblom, Satu Salo, *Tuomas Attila, *Jukka Vasara & #Gun Wirtanen

VTT Technical Research Centre of Finland, Finland

*Granlund Kuopio Oy, Finland

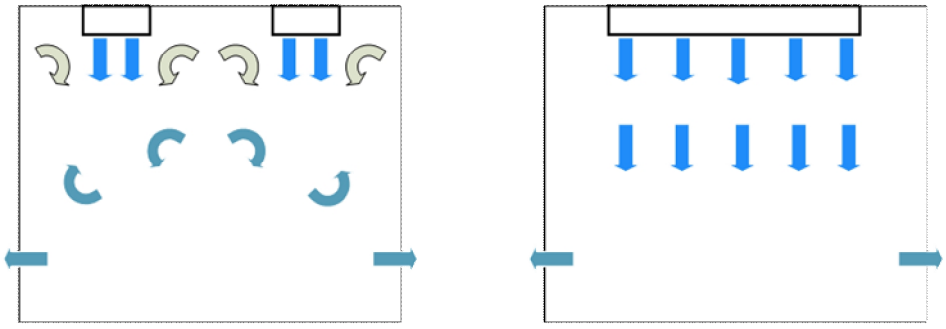
#R3-Nordic Board, Finland

Abstract

The objectives of the study were to evaluate cleanliness classes and airborne particle concentrations in typical operating theatre environments in Finland. At rest the cleanliness classes of the theatres fulfilled the guiding reference values when there was overpressure in the theatres. At operation the cleanliness classes were between ISO 7 and ISO 9. The airborne bioburden samples analysed showed that >85% of the sites evaluated had air of either good or inadequate quality. The results and suggested hygiene limits are benchmarked with methods and qualification limits of a standard draft prepared by the European hospital ventilation working group CEN/TC 156/WG 18.

Introduction

Air condition systems have an important role in control of air quality in operating theatres. There are two main principles to supply air into operating theatres (Figure 1). The mixing ventilation is typically used for less critical operations and laminar supply systems for operations which require especially high levels of aseptics. In both cases supply air systems should be provided with high efficient filters (HEPA). It's also obvious that in operating theatres there should usually be overpressure in relation to surrounding areas.



Mixing ventilation Laminar ventilation

Figure 1. Supply air distribution principles of operation theatres.

At the moment there are no mandatory air quality tests for operating theatres in Finland. In fact, there are no national regulations considering ventilation of special premises in hospitals like operating theatres, but the ventilation is planned on a case by case basis. A guideline that is used commonly is a thesis prepared by Jouko Ryynänen in 2007 [1]. The guideline introduces air cleanliness classes for operating theatres based on the standard EN ISO 14644-1 [2].

The European hospital ventilation working group FprCEN/TR 16244 has continued its work in 2013 and 2014 to prepare a draft standard [3]. The draft introduces acceptance tests for operating theatre ventilation and air conditioning. The acceptance limits are based mainly on existing standards (EN ISO 14644-1 for indoor air particle concentration at rest and ISO 15698 for microbiological qualification). The standard draft determines different areas in operating theatres according to cleanliness requirements. ”Protected zone” is reserved for patients, operating staff and instrument tables. “Periphery area” in the operating theatre is reserved for staff supporting the operation. In addition, the draft classifies the operations as 1) high risk operations that include high infection risk operations such as orthopaedic or neurological surgery, or 2) normal risk operations.

According to the standard draft, the microbiological qualification tests should be carried out at rest and in operation using active or passive air sampling methods. With active sampling the acceptance limits for bacteria in operation are <10 CFU/m^3 (Colony Forming Units) for high risk operations and <100 CFU/m^3 for normal risk operations. At rest the acceptance limits are <1 CFU/m^3 for high risk operations and <10 CFU/m^3 for normal risk operations. At rest, for high risk operations, the airborne particle cleanliness classes are determined as ISO 5 in the protected zone and ISO 6 in the peripheral area. At rest, for normal risk operations, the cleanliness class is determined as ISO 7 in the operating theatre.

Methods

Cleanroom classification studies

The airborne particle cleanliness classes of operating theatres were verified at rest conditions according to the standard EN ISO 14644-1 [1]. Also EU GMP Guide [4] was applied to the measurements. The particle concentrations were measured by using a particle counter (MetOne 3313) and the pressure differences between operating theatres and surrounding areas by using a micro manometer (DPM). The particle concentrations were also measured during operations. In those cases particles were measured only at one point near the exhaust (MetOne 3313) or near the operation area (MetOne 237).

In addition, video exposure monitoring (VEM) was utilized to determine particle concentration variation during operations. The VEM method combines video picture and different measurement data for e.g. examining different working practices and material solutions or in training purposes [5]. During VEM measurements optical particle counter MetOne 3313 or MetOne 237 was used. The counters were used in the continuous mode where the total particle count (all particles >0.3 µm) was obtained every 5 s.

Measurement results are compared to the Finnish design values of operating theatres as well as standard draft prepared by the European hospital ventilation working group. At rest conditions air cleanliness design values in Finland are ISO class 5 for operating theatres witch require especially high levels of aseptics and ISO class 7 for operating theatres with normal aseptics [1].

Airborne bioburden studies

Both the Klotz impactor FH5 (Markus Klotz GmbH, Bad Liebenzell, Germany) and the MAS-100 impactor (Merck KGaA, Darmstadt, Germany) were used in measuring the bioburden of the air. A volume of 200 L air per sampling site was impacted onto the agar plates with an airflow of 30 L/min when using FH5 or 100 L/min when using MAS-100 in all four operating theatres and surrounding areas. Moulds and yeasts were analysed using sampling on potato dextrose agar (PDA, Difco 0013; Difco, BD – Diagnostic Systems, Sparks, MD, USA). Chlortetracycline (0.01%) and chloramphenicol (0.01%) antibiotics were used to suppress the growth of bacteria on this agar. The total amount of mesophilic bacteria was determined on plate count agar (PCA, Difco 0479). In this growth medium, cycloheximide (0.05%) was added to suppress the growth of fungi. The plates were all incubated at 25 °C for 5 d.

Results

Cleanroom classification studies

The results of airborne particle cleanliness analysis are shown in the Table 1. In some cases the ventilation systems did not work properly and therefore there was periodically negative pressure in operation theatres. The cleanliness classes were in those cases ISO

class 6 or EU-GMP grade C in at rest conditions. After changes in ventilation operation the cleanliness classes reduced to the planned values of ISO class 5 or EU-GMP grade A (at rest).

Table 1. The results of airborne particle cleanliness analysis.

Location/situation	ISO Class	EU-GMP Grade	Pressure difference operating theatre – corridor, Pa	Observation
Laminar area, at rest	5 - 6	A - C	-11 - + 43	Periodically negative pressure in operating theatres – ISO Class 6 or EU-GMP Grade C
Area outside the laminar area, at rest	5 - 6	B - C	-11 - + 43	Periodically negative pressure in operating theatres – ISO Class 6 or EU-GMP Grade C
Operating theatres with mixing ventilation, at rest	6	C	0 … + 5	
Operating theatres during operations outside the laminar area	7 - 8	B - C	+ 3 … + 43	Emissions of operating personnel and operations
Operating theatre during operations near the operation area (laminar air flow system)	> 9	NA		Emissions of operating personnel and operations
Surrounding areas	7 - 8	B - C		

A snapshot of an airborne particle VEM analysis is shown in Figure 2. There is shown an example of short term relative concentration variations in different particle size ranges. The VEM analyses also showed that the use of diathermy equipment increases the concentration of particles range in 0.3 µm to 1 µm remarkably (Figure 2). The magnitude of short term rises were up to several thousand times compared to the particle concentration of operating theatre at rest.

The VEM analyses showed that the airborne particle concentration of particles diameter over 1 µm was increased by a level of 10–100 times in operating theatres during different operation and nursing periods (Figure 2). It was not possible to determine any particular practices releasing short term high concentration peaks in that particle size range. Thus, the rises of concentrations were quite steady.

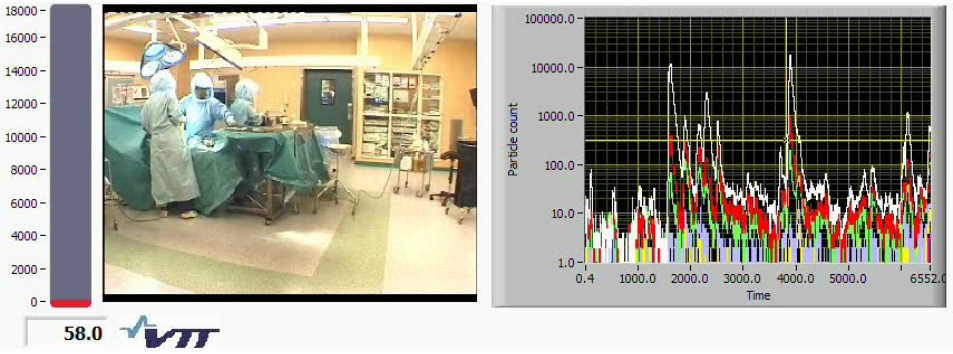


Figure 2. Variation of particle concentration near exhaust during an orthopedic operation.

In one operating theatre the particle concentration was also measured near the operation area and near the exhaust at the same time. The results of these measurements are shown in Figures 3 and 4.

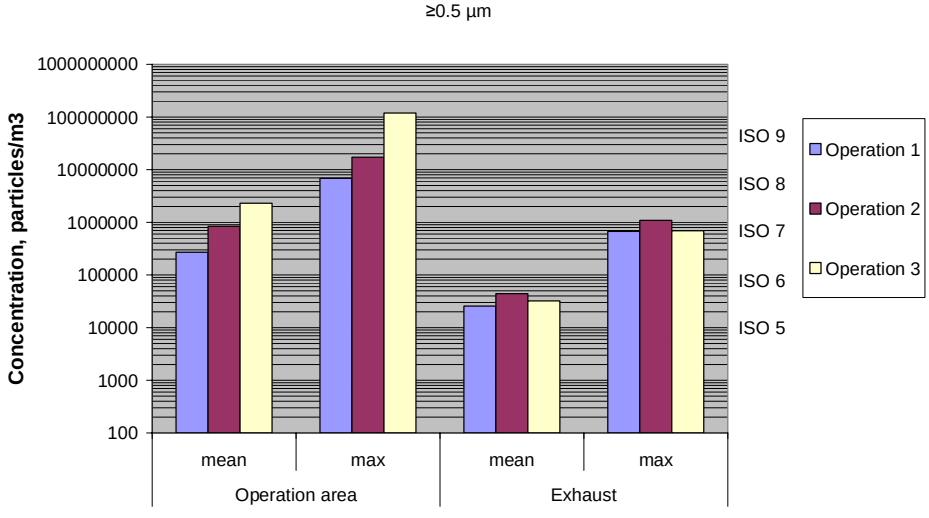


Figure 3. Particle concentrations in an operating theatre (laminar ventilation) during operations. Particle size ≥ 0.5 µm.

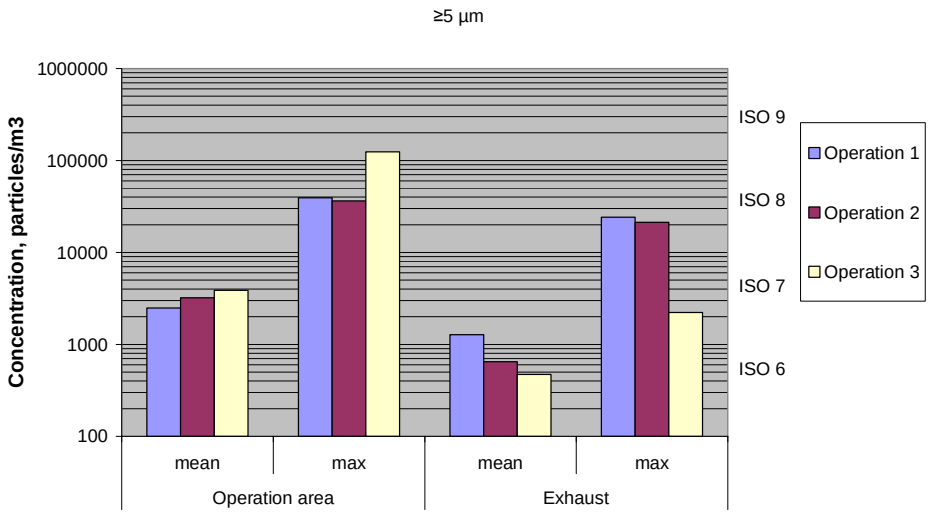
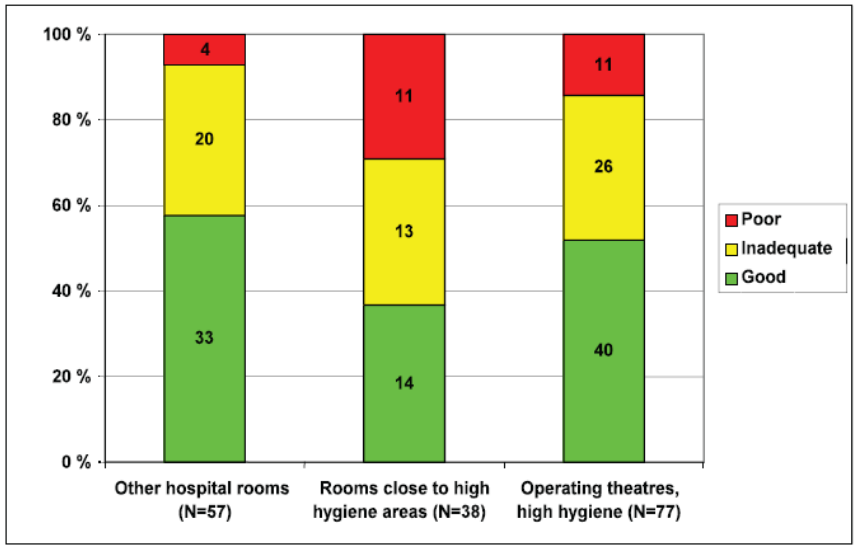


Figure 4. Particle concentrations in an operating theatre (laminar ventilation) during operations. Particle size ≥ 5 µm.

Airborne bioburden studies

The evaluated air samples were taken in three different areas: operating theatres, the areas surrounding the theatres and other hospital rooms (Figure 5). In total 180 air samples were collected in four hospitals (77 samples in operating theatres, 38 samples in rooms close to high hygiene areas and 57 in other hospital rooms). The microbial levels were defined as good, inadequate or poor depending on the total microbial bioburden and the area. Results from operating theatres with laminar and mixing ventilation (56 samples) are separated in Figure 6. The levels for the high hygiene rooms were set based on the levels used in aseptic processing in the pharmaceutical industry in the EU area [4].

Airborne bioburden particle measurements were also carried out during three orthopaedic operations in an operating theatre equipped with laminar air distribution. During these operations, no living microbes able to form colonies on agar plates were measured in the air of the laminar area. Colony forming units outside the laminar air varied from 1.7 CFU/m^3 to 8.6 CFU/m^3 depending on the measurement.



Sampling place / Microbial level in air (cfu/200L)	Microbes: Good	Inadequate	Poor
Other rooms in hospital	< 5	5-20	> 20
Rooms close to high hygiene areas	< 2	2-5	> 5
Operating theatres, high hygiene areas	< 1	1-2	> 2

Figure 5. The airborne microbial levels. The evaluated air samples have been divided into three levels: high hygiene areas i.e. the operating theatres; the areas surrounding the theatres; and other hospital rooms. The microbial levels have been defined as good, inadequate or poor depending on the total microbial bioburden in the 200 l sample taken.

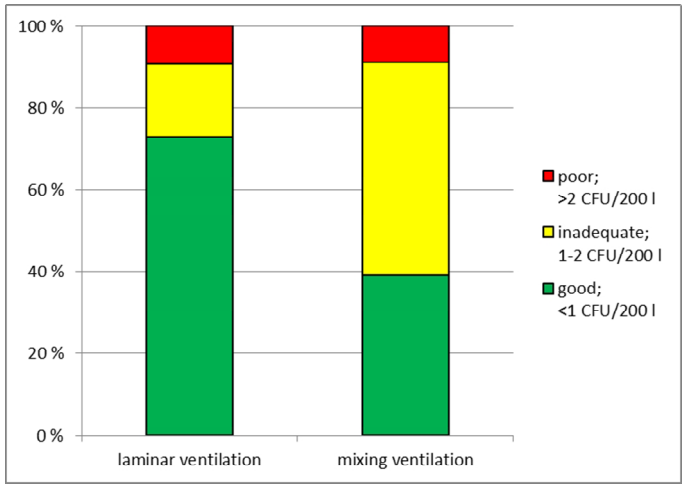


Figure 6. The airborne microbial level comparison between operating theatres with laminar ventilation and mixing ventilation. The microbial levels have been defined as good, inadequate or poor depending on the total microbial bioburden in the 200 l sample taken.

Discussion

At rest conditions the airborne particle cleanliness of operating theatres was normally ISO class 5 (laminar supply) or 6 (mixing ventilation), which values corresponds to the typical design values in Finland [1]. In some cases the air cleanliness in operating theatres with laminar supply was ISO class 6 because of control problems in the ventilation. When these problems were solved the air cleanliness reduced to the planned values of ISO class 5 in at rest conditions. Because the laminar supply operating theatres were designed for high risk operations and the mixing ventilation operating theatres for normal operations, they also corresponded to the standard draft of the European hospital ventilation working group.

Highest particle concentrations were near the operation area in spite of laminar supply air system. High particle concentrations indicate that it's difficult to reduce particle concentrations in operating theatres only by ventilation. Because the ventilation rates of operating theatres are already high (20 … 40 air changes per hour) and the particle concentration of supply air due to the effective supply air filtration (HEPA) is very low an increase of the ventilation or an increase of the supply air filtration rate have only a marginal influence on particle concentration.

The air flow rates of operating theatres are often reduced during night time in order to save energy. The results indicated that reducing the air flow rate can cause a negative pressure conditions in operating theatres and high particle leakage from surrounding areas to operating theatres. The airborne bioburden samples analysed showed that > 85% of sites evaluated had air of either good or inadequate quality. 86% of the samples collected in operating theatres had air of either good or inadequate quality. Both the impaction and the optical particle counter methods showed similar trends in the air quality. The European standard draft proposes acceptance limits in operation as 10 CFU/m^3 for high risk operation and 100 CFU/m^3 for normal risk operation. According to the airborne bioburden measurements during operation, the three operations studied in an operating theatre with laminar air distribution would have passed the qualification limit.

Conclusions

The study indicated that if the ventilation systems are working properly air cleanliness of ISO classes 5 (laminar supply) of 7 (mixing ventilation) can be maintained in operating theatres in at rest conditions. The air particle measurements showed that during operational conditions main particle sources are the personnel and the operations themselves. Particle emissions of these sources are so high that the ventilation systems have quite limited possibility to remarkably reduce the particle concentration of operating theatres during the operations. If lower particle concentrations in operating theatres are required, more attention should be paid for developing work practices and protective clothes for personnel in operating theatres. The airborne bioburden samples analysed showed that >85% of the sites evaluated had air of either good or inadequate quality.

Acknowledgements

The authors wish to thank the partners of the Finnish High Tech Hospital -project (2010–2012) that enabled the work. The work was supported by Tekes, the Finnish Funding Agency for Technology and Innovation.

References

1. Ryynänen J. 2007. Sairaalailmanvaihdon suunnitteluohjeita 2007. Mikkelin Ammattikorkeakoulu. 17 p. + appendixes. In Finnish.

2. Annon, Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness (ISO 14644-1:1999). CEN, 1999, 37 p.

3. Attila, T. 2014. Modulaarisen leikkaussalin ilmanvaihtoratkaisujen kehittäminen. Opinnäytetyö, Mikkelin Ammattikorkeakoulu. To be published, In Finnish.

4. Annon, EudraLex, The Rules Covering Medicinal Products in the European Union. Volume 4, EU Guidelines to Good manufacturing Practice, Medicinal Products for Human and Veterinary Use – Annex 1, Manufacture of Sterile medicinal Products. European Commission 2008. 16 p.

5. Rosén, G., Andersson, I.-M., Walsh, P.T., Clark, R.D.R., Säämänen, A., Heinonen, K., Riipinen, H. & Pääkkönen, R. 2005. A Review of Video Exposure Monitoring as an Occupational Hygiene Tool, Ann. Occup. Hyg. 49: 201–217.

Cleanroom Design of Hospital Pharmacies

Jukka Vasara

Granlund Kuopio Oy, Finland

The GMP guide for cleanroom design of hospital pharmacies

The EU Commission has adopted directives 91/356/EEC, 2003/94/EC and 91/412/EEC. These directives lay down principles and guidelines for good manufacturing practice of medicinal products for human and veterinary use. The “Guide to good Manufacturing Practice” (GMP), offers guidance to archive the requirements set out in these directives. In Finland, the “Finnish Medicines Agency” (Fimea) gave an order in May 2012, requiring compliance with GMP, for medicinal production and operations related to it. GMP in turn refers on many occasions to the widely used cleanroom standard ISO 14644.

The most important parts in GMP are in Chapter 3 and Annex 1. Chapter 3 covers clean room design, placement within premises, equipment and maintainability. Annex 1 covers manufacture of sterile medicinal products, classification of air cleanliness with regard to particle concentration and microbial contamination. Ref. Tables 1 and 2.

Table 1. Air cleanliness classification based on particle count.

	At Rest		In Operation	
Grade	Maximum permitted number of particles per m^3 equal to or greater than the tabulated size.			
	0,5 µm	5 µm	0,5 µm	5 µm
A	3 520	20	3 520	20
B	3 520	29	352 000	2 900
C	352 000	2 900	3 520 000	29 000
D	3 520 000	29 000	-	-

Table 2. Cleanliness classification based on microbial contamination.

	Recommended limits for microbial contamination			
Grade	air sample cfu/m^3	settle plates (diameter 90 mm) cfu/4 hours	contact plates (diameter 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	-

Basics of design

The first stage for hospital and pharmacy cleanroom designs begins with the drafting a "basis of design document". This document describes the operations that will take place in the space. Also covered are volume of production, personnel and material flows, large machinery requirements and preliminary options for structural and technical solutions. This document is drafted jointly between the pharmacy personnel, designers and customers. The document gives a good and firm foundation for continuing the designing process.

Cleanrooms in a hospital pharmacy

Premises and structures: A "cleanroom" is a working space with a tightly controlled environment within a secondary shell. In a typical hospital pharmacy cleanroom the height in the working space is about 2600 mm. A space usually in the range of 1800–2100 mm is reserved above the working space for technical equipment and maintenance. Ductwork for ventilation and other technical installations are placed in this space. The equipment room for air handling units is commonly situated above the cleanroom floor.

It is crucial for a successful layout design that the design group is experienced in cleanroom technology and operations in hospital pharmacies. Placement of airlocks and gowning rooms within a space are define by the routes of personnel, materials and finished products. Space and service areas for special equipment within production areas must also be taken into account at this stage.

Structures in hospital pharmacies are primarily designed by using sandwich elements. The boundary wall elements are made by using polyurethane elements which are coated with powder painted galvanized steel. Thickness of the element is usually 65 mm.

Neither the cleanroom standards nor the GMP defines a specific material or method of construction that should be used for the manufacture of the elements. The designer should consider carefully therefore which options to present to the customer and users. The structure is however required to meet the following criteria:

- Airtight envelope
- Internal surfaces to be smooth and easy to clean
- Resistance to wear and staining
- Resistance to chemicals
- Staticity or antistaticity.

Technical systems: Ventilation in hospital pharmacy cleanrooms is usually archived using a dedicated air handling unit (AHU) and ventilation system, which serves only the cleanroom. Contaminated or hazardous air such as the extract from laminar airflow cabinets is led outside using separate ductwork and a roof fan. If necessary, according to a risk analysis, the AHU can be duplicated. Most of the air supplied has been re-circulated and treated though the AHU, then supplemented with fresh air as required. Indoor air is usually conditioned to a relative humidity of 45% ±10% and temperature of 21°C ±1°C. The operating indoor conditions are decided upon with the user. Air exchange rates in air cleanliness classes D-B are 15–50 1/h. Supply air is normally brought into the room through ceiling diffusers which are equipped with HEPA filters. Extract air is normally removed by floor level extract grilles set within the walls

One of the basic principles of cleanroom design is to archive the required air cleanliness classification within the specific work spaces. The GMP guideline for pressure between different classes is 10–15Pa. Almost all recently built hospital pharmacy cleanrooms have a control system for regulating pressure differences. The system consists of VAV-dampers and controls for single rooms and a central overall control. VAV-dampers regulate the airflow so that the room pressure remains in its set point.

Cleanroom doors are often interlocked to ensure that only one door at a time can be opened from a single room. When a door is opened the pressure difference control is frozen for a specified period of time.

Cleanroom compliance with its given requirements must be demonstrated by regular measurements and documentation. Recently it has become customary to produce documentation of compliance with a validated monitoring system. The monitoring system collects data of pressure differences, particle counts, temperature and humidity. The system consists of room sensors and particle counters usually situated in the service space above the clean room.

Validation is testing and assurance with a goal of proving that the premises meet the requirements set in standards and guidelines and that the facility can be used to manufacture medicinal products of specified quality.

A cleanroom which is used to manufacture or in any way interacts with medicinal products has to comply with GMP and EU regulations. GMP requires process validation in chapter 5, Annex 15, “Qualification and validation”.

Validation is done by measurements, tests, and experiments. Documentation is a vital part of validation. It could be said that what hasn't been documented has not been validated. Validation is divided into four parts, design, installation, operational and performance qualification.

Validation in hospital pharmacies covers the clean room, its technical systems and the process to be carried out in it. All validation is based on a validation plan, which includes testing, measuring, simulating and above all documentation.

Case design project for Jyväskylä central hospital, cytostate dilution facility

In the presentation there will be material on the case study carried out for the Hospital in Jyväskylä.

New Classification of Operating Theatres

Dennis Andersen

Andersen Control ApS & R^{3}Nordic Board, Denmark

Abstract

The newly released standard “DS 2451-5, Infection hygiene in the healthcare sector, per operating procedures” combines, ventilation premises, infection risk, behaviour, garment and protective equipment into different classes. The aim is to provide the health care sector with knowledge and thereby taking the right precautions prior to invasive procedures. It has already been well received in Denmark. A follow through of the standard will be presented.

GENERAL SESSION

Modular Manufacturing System Compared to the Conventional Clean Room Construction

Esa Högel

M+W Process Industries GmbH, Switzerland

Abstract

The presentation describes principal explanation of modular manufacturing system. Examples of different projects are presented. The advantages of the modular system are discussed.

Advantages of modular system

- Highest degree of prefabrication
- Module approach moves ¾ of construction hours off site with highest productivity
- No weather impact during manufacturing
- Each layout, each facade, each hight and size of building can be build
- SAT partly in the factory
- Precommissioning of different systems are possible in the factory
- Limited risk of functioning
- Easy to reconstruct and reerect in different places
- Shortest erectionphase on site (only some weeks for commissioning + validation)
- Simple foundation works on site
- Enough time for finding the site, construct the foundation and suit the final site with water, sewage and electricity

- Only one combined transport
- Design, construction and erection in one hand
- Design meetings can be held everywhere
- Minimum activities on site in the country (risk minimizer)
- Only some concrete works for foundation are needed – can be executed in parallel to the construction of the modules –time saving
- Time saving due to the fact, that the earth and foundation works can be performed in parallel to the iconstruction of the modules in the factory
- Minimum issues for transport, visa and accomodation for expat staff
- Guarantee for quality and project in time!!!

Cleanroom Standardization: Where Does It Go?

Alexander Fedotov

Invar Project, Moscow, Russia

Abstract

The presentation discusses the ISO/TC 209 intention to change fundamental building bricks of cleanroom definition and other important issues. The community of cleanroom specialists should be well informed on it because nations will vote for new draft of ISO Cleanroom standards. This is challenging. The ISO/TC 209 meeting was held on 7–8 October 2013 in the USA. The solutions of that meeting are discussed.

History of cleanroom standards

Cleanroom standards exist for more than 50 years. Their development has its own logic. But sometimes it does not follow needs of practice and is not always based on proved ideas. USA Federal Standard 209 was the first and internationally recognized cleanroom standard. It passed several (A-E) revisions from 1963 till 1992 (209 E). It was cancelled in 2002 and replaced by ISO 14644-1:1999. Federal Standard 209 (till E version) had simple cleanroom classification (classes 100; 1000 etc.). Development of cleanroom standards in the world have had two stages (Figure 1).

1st Stage – In 1960–1970s there was an intention to create national independent cleanroom classifications and about 10 different variants appeared. It came in contradiction with international cleanroom market and co-operation, created difficulties and mixings. As a result nations outside USA used Federal Standard 209 together with national classifications.

2nd Stage – By the end of 1980th it was clearly understood that international cleanroom classification was necessary. In 1992 the Board meeting of International Confederation of Contamination Control Societies (ICCCS) decided to make application to ISO to create special Technical Committee on Cleanrooms. Initiative was supported and ISO/TC 209 “Cleanrooms and associated controlled environments” was approved in 1993. Num-

ber 209 in Federal Standard 209 and ISO/TC 209 was just a coincidence. So in 1993 the international era of Cleanroom standardization started.

The first standard ISO 14644-1 “Cleanrooms and associated controlled environments – Part 1: Classification of cleanliness classes” was approved in 1999. It established international classification and methods to determine cleanroom class. In 2002–2005 other key standards were approved (ISO 14644-2 on frequency of testing; ISO 14644-3 on test methods; ISO 14644-4 on design, construction and start-up; ISO 14644-5 on operation and ISO 14664-7 on isolators). By 2005 the first generation of ISO cleanrooms standards appeared. Not all of them were perfect, but they were simple and were accepted by professionals. Firstly, we will observe some confusions in existing cleanroom classification standard and then go to more complicated issues. Let us take a look at various directions of development in cleanroom standardization. I have divided them into three directions:

1. Changing cleanroom classification;
2. Widening cleanroom scope from cleanliness on particles to other attributes;
3. Developing other standards (energy saving, etc.).

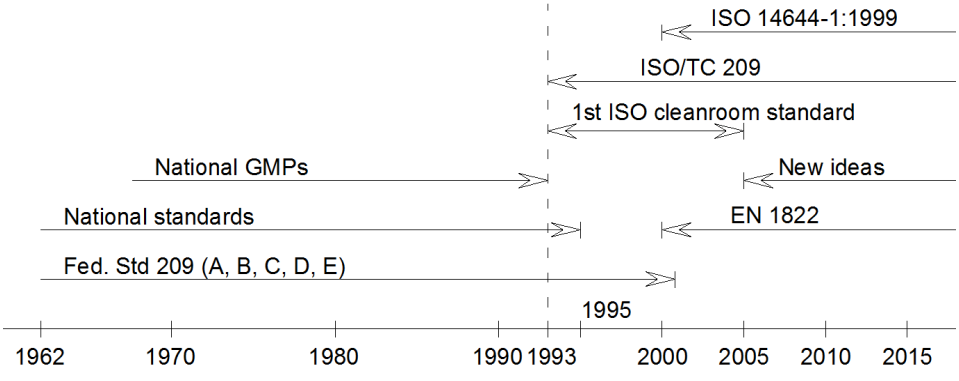


Figure 1. Development of cleanroom standardization.

Some confusions in existing ISO 14644-1 standard

Generally standard ISO 14644-1:1999 was accepted by professionals and serves as a basis for cleanrooms classification for 15 years. But it has some weak places that already have created confusion with GMP EC Annex 1. Some of them are just oversights and some are consequences of non-checking the correctness of formula for particles concentration Cn that is a basis for classification.

Intermediate classification

Existing standard has obvious confusion with intermediate classification. It allows to specify classes with 0.1 increments of classification number (for example, ISO Class 5.1, etc.). GMP EC Annex 1 mentions ISO Class 4.8. Does it have any sense?

Precision of parameter has sense only if it can be tested with available method and if random fluctuations of parameter do not go out of tolerances specified. The main rule says: it is not possible to specify limit if it cannot be checked. What is happening with cleanrooms?

ISO 14644-3 specifies inaccuracy of testing with particle counter as ± 20%. Real randomness of particle concentration can be estimated as ratio s/X_a (s – standard deviation; Xa – mean value of particle concentration). Different investigations showed that s/X_a= 0.4 for many applications but in the worst case s/X_a= 1.5 can happen. Table 1 shows particle concentrations for ISO Class 4.8 to Class 6 for particles ≥ 0,5 µm according to ISO 14644-1:1999, formula $C_n = 10^N \times (0{,}1/D)^{2{,}08}$, where N is classification number (class), D – particle size.

Table 1. Concentrations of particles ≥ 0.5 µm for decimal ISO Class 4.8 – Class 6.0.

N	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0
C_n	2220	2790	3520	4430	5570	7020	8830	11100	14000	17600	22200	27900	35200

For s/Xa = 0.4 total inaccuracy will be 1.2 x 1.4 = 1.68 (1.2 mean 20% inaccuracy of particle counter; 1.4 mean 40% real statistical deviations of airborne particles concentration). So for these conditions lower level of inaccuracy for class ISO 5 will be 3520/1.68 = 2100 particles/m^3 and for upper level will be 3520 x 1.68 = 5920 particles/m^3. It means that it is not possible to separate cleanrooms with classes ISO Class 4.8 and ISO Class 5.2 from cleanroom ISO Class 5.

For s/Xa = 1.5 total inaccuracy will be 1.2 x 2.5 = 3.0. So for these conditions lower level of inaccuracy for ISO Class 5 will be 3520/3 = 1170 particles/m^3 and for upper level 3520 x 3 = 10600 particles/m^3. It means that it is not possible to separate cleanrooms with ISO Classes 4.6 and ISO Class 5.4 from cleanroom ISO Class 5. ISO Class 4.8, ISO Class 5.1, etc. can never be checked or confirmed. Difference between them can never be proved (Figure 2). If so, why decimal class was included in standard and in Annex 1?

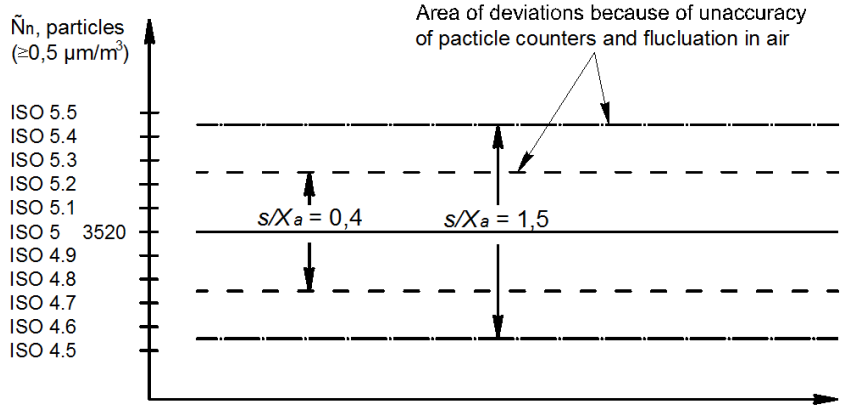


Figure 2. Intermediate classification and real randomness around ISO Class 5.

ISO Class 9

What is it and what is it for? ISO Class 9 specifies 35 200 0000 particles/m^3 as a limit for particles ≥ 0.5 µm. Offices, shops and many other dirty premises were never considered as cleanrooms, but they have cleanliness ISO Class 9 with big margin. One should make efforts to make a room worse than ISO Class 9. This is in contrary with normal expectation that a cleanroom is somewhat cleaner than usual premises.

But the story with ISO Class 9 is not so simple. There are evidences that some people and companies use ISO Class 9 for advertisement/image purposes. Looking around what is happening with standardization one can assume that ISO Class 9 was invented for such purposes deliberately. It is misleading and is in contrary with purposes of standardization! Some hot heads offer even ISO Class 10. If it will be approved, chimney with smoke can be classified as a clean zone.

Wrong formula

Class limits (particle concentration) are determined in ISO 14644-1 by formula:

$$C_n = 10^N \times \left(\frac{0.1}{D}\right)^{2.08} \quad (1)$$

Where

- C_n is the maximum permitted concentration (in particles per cubic meter of air) of airborne particles that are equal to or larger than the considered particle size. C_n is rounded to the nearest whole number, using no more three significant figures;
- N is the ISO classification number, which shall not exceed a value of 9;
- D is the considered particle size, in micrometers;
- 0.1 is a constant, with a dimension of micrometers.

GMP EC Table is based on ISO 14644-1 Table and formula for C_n. This formula travels from standard to standard and is considered to be correct. But who proved that it is correct? To check this we made analysis of numerous real cleanroom testing results that were collected by company “Invar-project” for almost 15 years.

The examples for some facilities are shown on Figures 3 and 4. All data are for “at rest” stage. Designations of cleanrooms (1, 2,…) of the same facility are shown on X axis. Analysis has showed that this formula is wrong. Formula gives results that differ from practice on orders of magnitude! If so, what is it for, especially in standard? A misleading standard causes confusions in other documents. It happened so with Annex 1 to GMP EC (2008) that tried to make bridge between GMP and ISO (20 and 29 particles/m^3 with sizes ≥ 5.0 µm instead of 1 particles/m^3). This confusion is the result of blind trust in wrong formula.

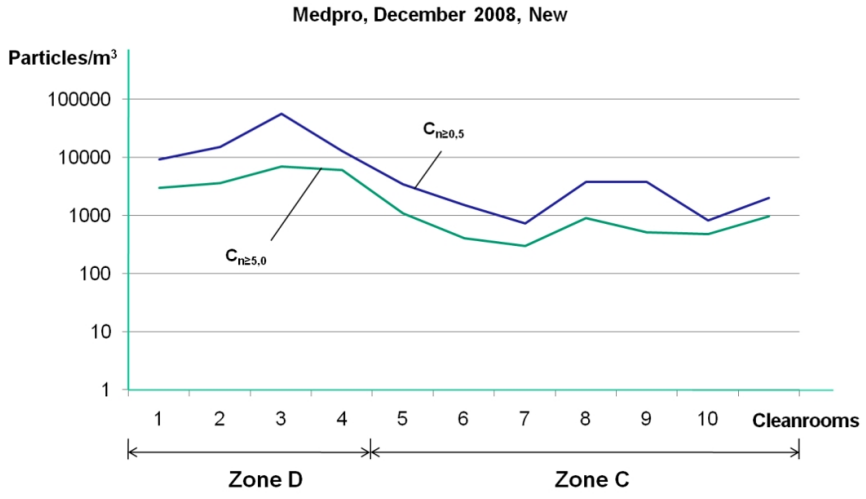


Figure 3. Example of particle concentration for cleanroom facility 1.

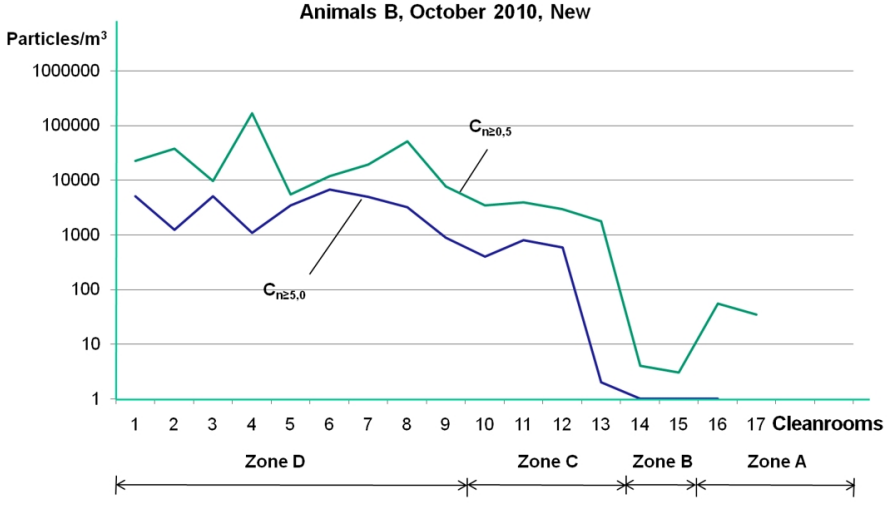


Figure 4. Example of particle concentration for cleanroom facility 10.

It is seen that difference between particles concentrations ≥ 0.5 μm and ≥ 5.0 is much less than expected by ISO 14644-1 (relation $C_{n0.5}/C_{n5.0} = 121.4$). This non-compliance of practice and ISO standard is significant and systematic.

- Figure 5 shows relations between $C_{n0.5}$ and $C_{n5.0}$. It gives interesting results:
- for dirty rooms (class ISO 9) real relations of particles concentrations are more or less grouped around ISO relation (121.4). The deviation is big, but mean value is close to ISO;
- for cleanrooms (zones A, B, C and D) real data are far away from ISO standard and this non-compliance increases with increasing of cleanliness level.

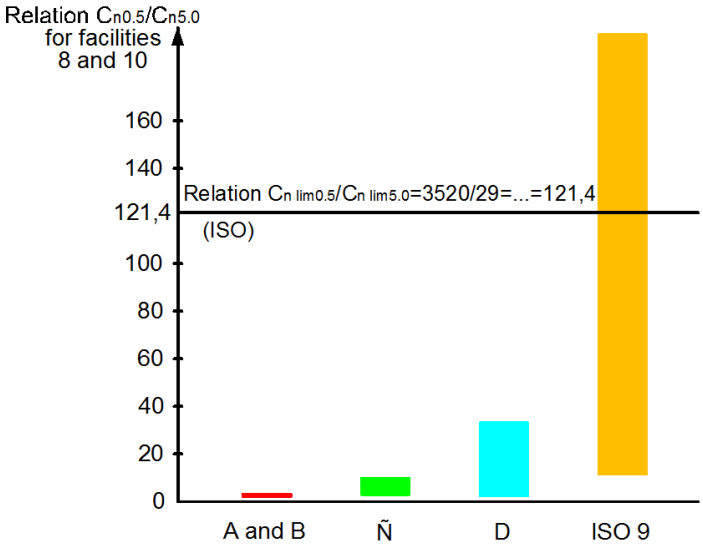


Figure 5. Relation $C_{n0.5}/_{n5.0}$ for facilities 8 and 10.

Table 2. Non-compliance of theory and practice is different for different classes.

A and B	C and D	ISO Class 9
50-100	10-50	1-5

So ISO 14644-1 formula works more or less well for dirty rooms (ISO Class 9) but for cleanrooms it does not reflect practice (Table 2). It seems that for-mula was discovered long ago for dirty rooms and then extrapolated to-wards realistic cleanrooms without any analysis. In contrary, how to explain real statistics that we observe for years? Many people travel around the globe for decades trying to brush up GMP EC and trusting the correctness of ISO formula. But the formula is not correct.

GMP and ISO 14644-1

Tables 3 and 4 show GMP EC limits for air cleanliness with adding ISO classes. GMP 2003 had huge contradiction between limits for concentration of particles ≥ 0.5 µm and particles ≥ 5.0 from ISO 14644-1 point of view:

- limits for particles ≥ 0.5 µm correspond to ISO Class 5 (ISO 4.8 precisely);
- limits for particles ≥ 5.0 µm correspond to ISO Class 3 approximately.

ISO Class 3 limit for particles ≥ 5.0 µm is not specified by ISO 14644-1 standard, but can be approximately extrapolated for illustration purposes. Such contradiction was consid-

ered as confusion in GMP and harmonization was done simply by increasing limit for particles ≥ 5.0 from 1 to 20 and 29.

Table 3. GMP EC 2003 requirements for air cleanliness.

Grade	Maximum permitted number of particles per m^3 equal to or greater than the tabulated size			
	At rest		In operation	
	0.5 µm	5.0 µm	0.5 µm	5.0 µm
A	3 500 (ISO 5)	1 (ISO 3)	3 500 (ISO 5)	1 (ISO 3)
B	3 500 (ISO 5)	1 (ISO 3)	350 000	2 000
C	350 000	2 000	3 500 000	20 000
D	3 500 000	20 000	Not defined	Not defined

Table 4. GMP EC 2008 requirements for air cleanliness.

Grade	Maximum permitted number of particles per m^3 equal to or greater than the tabulated size			
	At rest		In operation	
	0.5 µm	5.0 µm	0.5 µm	5.0 µm
A	3 520 (ISO 5)	20 (ISO 4.8)	3 520	20 (ISO 4.8)
B	3 520 (ISO 5)	29 (ISO 5)	352 000	2 900
C	352 000	2 900	3 520 000	29 000
D	3 520 000	29 000	Not defined	Not defined

The background for harmonization was assumption that ISO 14644-1 relationships between particles concentrations for class limits is correct and reflect real practice, i.e. if ISO Class 5 zone has 3500 particles ≥ 0.5 µm, then expected concentration of particles ≥ 5.0 µm should be somewhat around 10–40, but not 100 or 1000, otherwise setting of such relations is misleading and has no sense. Relation between concentration limits for particles ≥ 0.5 µm and ≥ 5.0 µm according to ISO 14644-1 standard is equal to $C_{n0.5}/C_{n5.0}$ = 121.4. So it is expected that real mean data should be somewhat around this figure, not 5 or 1000. Such expectation was taken on trust for harmonization of GMP EC with ISO and setting limit of 20 (29) particles ≥ 5.0 instead of 1.

Everybody take correlation $C_{n0.5}/C_{n5.0}$ = 121.4 on trust. But ISO 14644-1 Table and formula do not correspond to practice on orders of magnitude. If so, the next question appears: Was it a good idea to write 20 particles instead of 1? Users and designers will, of course, say: “Yes, it is good, it is easier for us”. But this opinion is of secondary importance. Quality of product is of only priority. Practice for many facilities proved that limit “1 particle at rest” is realistic for zones A/B that are well designed, constructed and cleaned. Annex 1 in 2008 became worse for quality of products and safety of patients and helps only

poor designers, constructors and users. Is it really worth to count ≥ 5.0 µm particles together with ≥ 0.5 µm ones? Observing statistics and FDA experience we can state:

- NO! There is no scientific/technical background for counting ≥ 5.0 µm particles any more. This is only dogma without any background.

Can cleanroom classes be tested in a simple way?

Changing cleanroom classification

Existing ISO 14644-1 standard sets two different procedures for determining cleanroom class depending on number of sample locations:

- to calculate 95% Upper Confidence Level (UCL) if number of sample locations is equal to 2 or more but is equal or less than 9;
- to calculate mean values of particle concentration only and look whether they are with class limit if number of sample locations is equal of more than 10.

The second procedure is very simple and practical. The first one requires some basic knowledge of statistics to understand it and is not attractive for wide family of practitioners. Is it really necessary to calculate 95% UCL or is it possible to get rid of it? There are three ways to abandon UCL calculations:

1) To increase number of sample locations deliberately to 10. Number of sample location is equal to $\sqrt{S}$, where S is a cleanroom square in m^2 with rounding to bigger value. For cleanroom with square S = 15 m^2, number of sample location is 4 and standard requests to calculate 95% UCL: 4 is less than 10. But it is possible to increase number of sample locations. If to specify 10 locations we calculate mean values only and forget about 95% UCL. This is a good idea, but it requires more samples and is not always practical.
2) To make class limit tighter to the level that will allow to calculate mean values only. This method was offered by Dr. Alexander Fedotov. Its sense is rather simple but requests to change point of departure on cleanroom class thinking.
3) To follow logic of method 2, but increase number of sample locations instead of making cleanliness class limit tighter. The sense of method 3 is similar to method 2. Both offer some redundancy, but for method 2 redundancy sits in tightening of class limits, and for method 3 it sits in increasing number of sample locations without tightening class limits.

Method of safety margin

The idea of method is very simple: existing standard requires to calculate 95% UCL each time. Why not to solve the reversal task? That is to calculate assurance limits C_a as mean values of particles concentration that will correspond to 95% UCL equal to class limit C_L

(Figure 6). Difference $C_L - C_a$ gives safety margin. Its use simplifies classification process in the field. Detailed description can be sent by the author on request: fedotov@invar-project.ru.

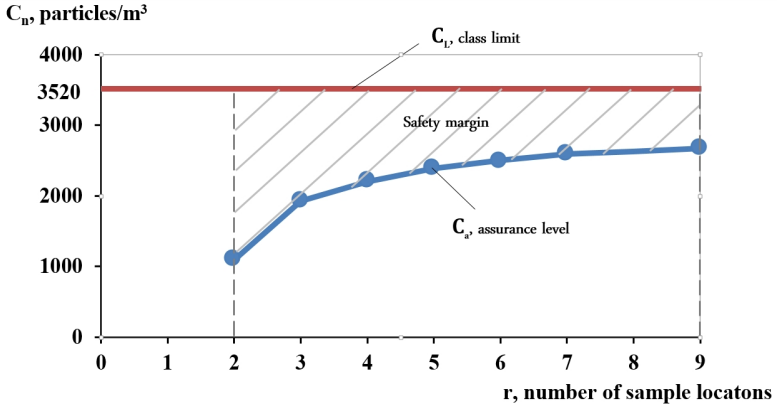


Figure 6. Sense of safety margin method.

Randomization model

In 2007–2012 an attempt to create a simpler method of testing to determine cleanroom class by randomization model was undertaken. This method was published in several magazines and was widely announced as a scientifically based. It even came to DIS voting as the draft of ISO 14664-1 standard. The author of this paper made analysis of randomization model in 2012 and this method failed as the result. Key points of this analysis are shown in Table 5. The main disadvantages of this model are:

- random selection of sample locations instead of fixed ones has sense only for multiple testing, that is some 20–30 repetitions of tests are necessary in comparison with one test now; this is absolutely not practical;
- scientific background for selecting distribution law is absent;
- no trend analysis is possible because of changing sample locations each time;
- no practical verification how this method works was presented.

Randomization model failed as a result.

Table 5. Comparison of methods to determine sample locations.

		ISO 14644-1:1999	Randomization model
1	Number of tests	1	20-30
2	Distribution law	Normal	Even or hypergeometric
3	Rationally for statistics	No prove that normal law is correct, but it is widely accepted by practice and there is no evidence that this assumption makes harm	Even distribution is not correct for airborne particles. To do so is the same as to approximate mountains with plane and then to make any conclusions
3	Simple/complicated	Simple and well accepted	Overcomplicated
4	Trend analysis	Possible	Not possible

To widen sense of term “cleanroom” or not?

Some members of ISO/TC 209 suggested to widen scope of cleanroom term to surfaces and other contaminants (chemical and viable), specifying for them cleanliness classes ACP, CCP, etc. (at least six classification systems) with designations ISO Class 5 ACP and so on. As a result multiple cleanliness classes for the same room could appear instead of existing one class on particles concentration. For chemical concentration it means that there can be as many classes as chemical substances are be in air. Practical example for this is shown in Figure 7. It means absolute nonsense and was not accepted.

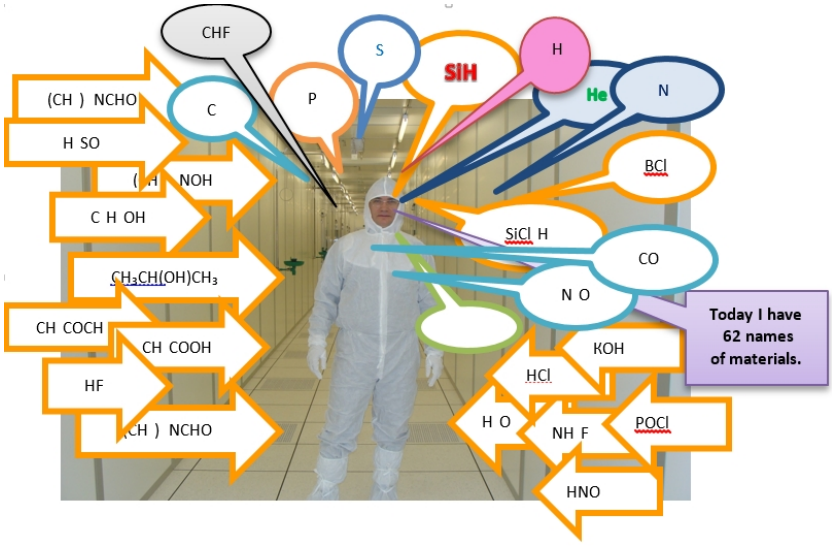


Figure 7. Chemical contaminants in cleanroom: do we need 62 new classes for chemicals only?

New standards

New generation of standards is at the preparing stage. This process is not simple. Sometimes new suggestions reflect only wishes of authors and are not needed or not correct. Special attention should be paid to classifications with chemicals and surfaces. They are not supported with practical study, but were already approved as ISO standards. Some new drafts, such as on energy saving, are necessary. The advice that can be done from observation above that any suggestion shall be well verified and discussed before moving to standard. Only wide involvement of professionals can avoid confusions and create good standards.

Acknowledgements

The author thanks specialists of “Invar-project” V. Yakukhina and K. Isakova for help.

Hygiene Survey in Food Plants

Satu Salo

VTT Technical Research Centre of Finland, Finland

Abstract

Food safety is related to the absence or presence of levels of foodborne hazards in food at the point of consumption. The EC Regulation 852/2004 covers the principal objective of the general hygiene rules to ensure a high level of consumer protection with regard to food safety. However, food safety is the joint responsibility of many people and it is principally ensured through the combined efforts of all the parties in the food chain. Legislative demands set the basic requirements for the manufacturing of safe food products whereas food safety management systems and food safety guidelines and standards based on given legislation help the food industry to keep up with current food safety requirements. Hygiene survey is a practical tool for controlling hygiene in food plants. By using an efficient sampling of the surfaces of the process line, it is possible to reduce the amount of low-quality food leaving the plant. Hygiene survey can include checking of the amount of surface-attached soil including protein, polysaccharides, other organic and inorganic residues, biofilm, dead and/or living microbes in general, or specific pathogens and other harmful microbes. Hygiene survey also helps with tracing contamination sources and in optimising cleaning systems. There are several sources of microbial contamination: raw materials, process equipment, environmental surfaces, air, personnel and the final product. In some cases demands for better hygiene have been made because of prolonged shelf-life of products, centralized production and long-distance transportation, less time spent on cleaning and demands for environmentally safe cleaning agents (Salo, 2006).

Quantification of the actual number of microbes from surfaces is difficult due to strong microbial adherence of biofilms. In addition, detection of biofilms using traditional swabbing method often gives incorrect results due to strong microbial adherence. Most techniques underestimate the number of microbes on a surface. Hygiene surveys from surfaces are challenging; the measuring should be quickly performed, directly from the surface and without damaging the surface, and the microbes need to be detached completely. However, it is difficult to measure biofilm and biotransfer potential because the conventional microbiological methods used to assess equipment hygiene have not been devel-

oped for detecting biofilm. Reliable results are only possible if the biofilm is properly detached and the cultivation is performed under reproducible conditions or if the measurement can be performed without detaching the microbes. Microscopy is very often used as a reference method for swabbing and cultivation. It has been reported that the cells counted by direct microscopy consistently give results one log unit higher than the cultivation methods. Moreover, observations of surfaces using epifluorescence microscopy have clearly revealed that even when vigorous swabbing is applied only a small part of the actual biofilm including the cells in it is detached (Wirtanen, 1995). On the other hand, use of excessive agitation and strong chemicals for detachment of surface-adherent cells may harm the cells, thus making them unable to grow in the cultivation procedure.

Choosing sampling sites in food plants and especially in equipment with complicated structures is challenging, since most likely the microbial residues are in curvatures, connections, propellers, or on uneven surfaces which are not easy to reach with sampling tools. Quantification of the swabbed areas in places like these is challenging. Contact agar applications with ridged frames are only suitable for sampling of smooth and straight surfaces (Salo *et al.*, 2008). Preventive risk-based food safety management systems such as HACCP require that hygiene monitoring should provide results rapidly in order to be able to perform corrective actions. ATP bioluminescence and protein detection kits for instance can provide a real time estimation of overall cleaning efficacy or protein residues, respectively. The detection and enumeration of indicator organisms is widely used to assess the efficacy of sanitation procedures. *Escherichia coli* counts can be used as an indirect measure of faecal contamination. The use of *Enterobacteriaceae* as hygiene indicators instead of coliforms or *E. coli* yields much more precise results.

Interpretation of the results from hygiene monitoring is often carried out case by case since there are quite many factors affecting an acceptable level of cleanliness. The acceptable level depends on the purpose of the surface. The surfaces in contact with ready-to-eat food products must be much cleaner than other surfaces in the process plant in contact with products which will be pasteurised or surfaces in no direct contact with foods. Special attention should also be paid to the surfaces next to food contact surfaces since there is a high risk of spreading contamination to food products (Salo *et al.*, 2006). The cleanliness level of the processed product depends also on the spoilage sensitivity and the wanted self-life of the product. The available recommended guidelines and standards for aerobic colony counts for clean surfaces vary widely, being 0 - 80 CFU/cm^2 (Griffith, 2005). The threshold limit for clean surface must be based upon a perception of a specific risk and the decided acceptable level. Alternatively, microbial yield obtained from surface after correct implementation of a well-designed cleaning programme can be used as a desired value (Griffith, 2005). Comprehensive studies performed by Griffith (2005) have indicated that in many cases levels of <2.5 CFU/cm^2 for general surface count are attainable and these are relatively close to majority of suggested standards. In the microbial survey described in Salo *et al.*, 2006 the threshold limit for clean surface was set based in perception of risk to <2 CFU/cm^2.

Suitable methods for detecting cleanliness of process surfaces vary according to the situation studied. All available methods have limitations and the suitability of the method strongly depends on the type of microbes and the microbial load present. Traditional sampling with swabs prior to culturing does not detach all microbes attached to the surface. Swabbing can be improved by moistening the surface with mild surfactant solution.

Also flexible contact agar is suitable for the detection of microbes from process equipment, and if the microbial load is less than 30 CFU/cm^2 the microbial colonies can be counted. It is recommended to use more than one detection method in new environment to validate the results obtained. A clean surface has to be free of microbes, physical particles and chemicals. Chemical residues remaining after cleaning and disinfection can be detected using rapid methods based on bioluminescence of sensitive photobacteria. Visual observation of the cleaning results is an important practical method which can be improved by using UV-light and possibly fluorescent stain. This type of assessment is not very accurate but is a practical choice especially for large equipment such as fermentation tanks.

References

Griffith, C. 2005. Improving surface sampling and detection of contamination. *In:* Lelieveld, H.L.M., Mostert, M.A. and Holah, J. (eds.) Handbook of hygiene control in the food industry. Cambridge: Woodhead Publishing Limited. Pp. 588–618.

Salo, S. 2006. Evaluating hygiene and cleaning efficiency of food process surfaces based on experimental data and modelling. Lyngby, Denmark; VTT, Espoo, BioCentrum-DTU, Technical University of Denmark, VTT Technical Research Centre of Finland. 55 p. + app. 189 p.

Salo, S., Ehavald, H., Raaska, L., Vokk, R. and Wirtanen, G. 2006. Microbial surveys in Estonian dairies. LWT – Food Science and Technology 39(5), 460–471.

Salo, S., Friis, A., Wirtanen, G. 2008. Cleaning validation of fermentation tanks. Food and Bioproducts Processing 86(3): 204–210.

Wirtanen, Gun. 1995. Biofilm formation and its elimination from food processing equipment. Espoo. VTT Publications 251. VTT. 106 p. + app. 48 p.

Particle Deposition Monitoring, the Missing Link in Contamination Control

Koos Agricola

International Confederation of Contamination Control Societies, the Netherlands

Abstract

Cleanrooms are monitored by measuring the concentration of airborne particles. However contamination of products is mainly affected by falling particles. The construction of a cleanroom has a strong impact on the air cleanliness. The way the cleanroom is used determines the deposition of falling particles. Therefore deposition of particles should be measured as well. The deposition of particles larger than 20 µm is determined by the operational quality of a cleanroom. The operational quality is determined by the number of persons, their garments, their discipline, the cleaning program of the cleanroom and the execution of this program.

New imaging techniques make it possible to measure particle deposition in terms particle size distribution. Particle deposition is measurement by detecting the change of the surface cleanliness of a witness surface by particle concentration according to 14644-9. Particle deposition events over relative short periods are recorded at places in the cleanroom. The sum of these events leads to a particle deposition rate that is representative for the actual operational quality of the cleanroom at critical locations.

Introduction

Cleanrooms are monitored by measuring the concentration of airborne particles. However contamination of products is mainly affected by falling particles. The construction of a cleanroom has a strong impact on the air cleanliness. The way the cleanroom is used determines the deposition of falling particles. Therefore deposition of particles should be monitored as well.

Product contamination

Vulnerable product surfaces can be contaminated by particles. The smallest critical particle size is determined by the function and constitution of the product. Particles can be transferred by contact with unclean surfaces and can be deposited onto vulnerable surfaces during exposure. To control the contamination risk of a product or parts of a product, that are exposed, the air cleanliness of the room is controlled and classified according to ISO 14644-1. The air cleanliness can be monitored by measuring the concentration of airborne particle using particle counters.

In cleanrooms, where people are working, particles in the size range of 10 µm and 500 µm are found on surfaces. These particles, especially the larger ones, are not detected by particle counters because of their low concentration and deposition in sampling tubes, at the entry, and within the particle counter. The cleanliness of relevant surfaces can be classified by ISO 14644-9. This cleanliness will change by particle deposition. In product contamination particle deposition is an important factor.

Mechanisms of particle generation and deposition

Particles are generated in cleanrooms by humans, machinery or by reactive, chemical processes such as corrosion or abrasion of a surface. Particles are distributed by air across the cleanroom. Particulate contamination may be distributed by people and other moving surfaces. These particles can also carry microbes. Particles are deposited onto surfaces in cleanrooms by various mechanisms such as gravitational, electrostatic attraction, diffusion and thermophoresis. However, the main mechanism is usually gravitational, especially where particles are greater than 5 µm. Where there is an electrostatic surface charge, electrostatic attraction may occur.

Particle smaller than about 5 µm remain suspended in cleanroom air and are removed in the exhaust flow. However, many particles are larger than 25 µm, and owing to their size and mass will deposit by gravitational sedimentation onto horizontal surfaces like floors, furniture, equipment and products, although these particles can be re-dispersed by movements such as walking, sitting and wiping. These particles can only be removed by cleaning. Particles close to 5 µm will most likely be removed by air, and close to 25 µm will most likely deposit onto a surface.

The influence of human activity and machinery on generation and distribution of particles throughout the cleanroom is important. In a cleanroom ‘at rest’ there is no machinery working and no people in the room. The concentration of particles larger than 5 µm will quickly drop to practically zero and there will be no significant particle deposition. Therefore, only in the ‘in operation’ occupancy state should particle deposition rate measurement and classification be considered.

When machines are working, particles are emitted. Personnel also disperse particles from their skin and cleanroom clothing, this dispersion being dependant on the person’s activity and the occlusive nature of their cleanroom clothing. For most cleanrooms, human activity is an important factor in the generation and distribution of particles and therefore in determining the particle deposition rate. Food breaks give a drop in particle concentrations but cleaning may cause peak concentrations. To reduce particle deposition on

vulnerable surfaces, working procedures need to be improved and this can be achieved by the detection of particle deposition peaks. The particle deposition rate (PDR) is related to the airborne concentration in a cleanroom by the following fundamental equation:

PDR = Airborne concentration x deposition velocity

PDR varies with the airborne concentration, and it may be difficult to obtain the exact airborne concentration at the location where the PDR is measured. In addition, the airborne concentration will vary over the several hours needed to measure the PDR. The deposition velocity depends on the particle size distribution and this will vary with activities and airflow in the cleanroom.

The concentration of particles in cleanroom air is monitored. However, this measurement gives an indirect measurement of the likelihood that particles will deposit on horizontal surfaces. By measuring the deposition rate of particles, a direct method can be obtained and used to measure the airborne contamination of vulnerable surfaces, and the **equation applies for total airborne deposition onto a surface = PDR × t × A**; where, PDR is particle deposition rate, t is the time the surface is exposed to particle deposition, and A is the surface area exposed to airborne contamination.

Classification of Particle Deposition

The PDR at a location in a cleanroom is determined by the change of Surface Cleanliness by Particle concentration over time. The maximum deposition rate can be used to classify a cleanroom in a similar way to ISO 14644-9, where surface cleanliness is used to classify cleanrooms. The following analogous equation can be used:

$$PDR_{MAX;\,D} = k.10^{M} / D,$$

where $PDR_{MAX;\,D}$ is the maximum permitted Particle Deposition Rate (PDR)/m^2 of surface/h, of particles that are equal to or larger than, the considered particle size; $PDR_{MAX;\,D}$ is rounded to the nearest whole number, using no more than three significant figures; M is the PDC classification number, which is limited to PDR Class 1 through PDC Class 6; D is the considered particle size, in micrometres and k is a constant 1, in micrometers. The Particle Deposition Class (PDC) is determined by the ^{10}log of the change of the concentration of particles ≥ 1 µm per m^2/h. Selected PDC classes are shown in Table 1 and Figure 1.

Table 1. Selected particle deposition class (PDC) classes and associated maximum values of particle deposition rate (PDR) at considered particle sizes.

PDC	Particle size							
	≥ 1 µm	≥ 5 µm	≥ 10 µm	≥ 20 µm	≥ 50 µm	≥ 100 µm	≥ 200 µm	≥ 500 µm
Class 1	10	2	1					
Class 2	100	20	10	5	2	1		
Class 3	1.000	200	100	50	20	10	5	2
Class 4	10.000	2.000	1.000	500	200	100	50	20
Class 5			10.000	5.000	2.000	1.000	500	200
Class 6			100.000	50.000	20.000	10.000	5.000	2.000

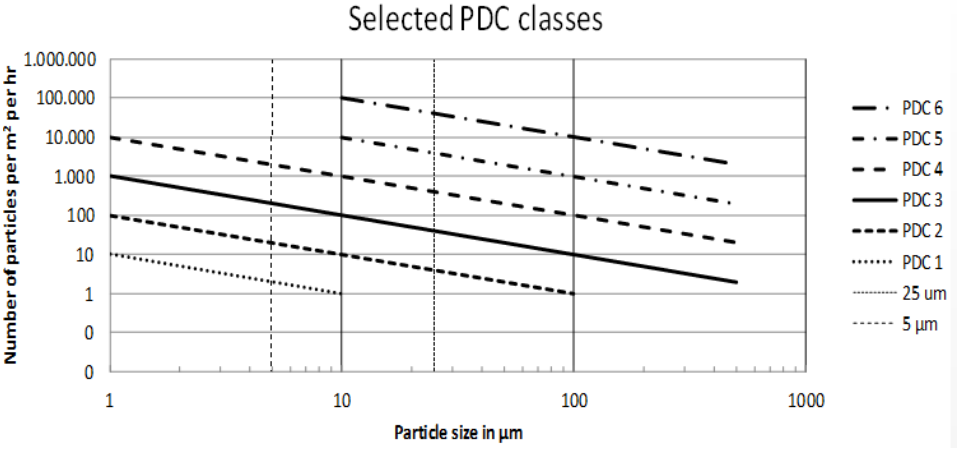


Figure 1. Selected particle deposition class (PCD) classes in graphical format.

The deposition rates of different sizes of particle are interrelated. To interpolate or extrapolate the PDR to sizes that have not been measured, the following equation is used:

$$PDC = {}^{10}\log (PDR_D * D)$$

The exact rate of deposition of particles ≥ D µm per m^2/h is calculated as follows:

$PDR_D = (C-Ci)/(t-t_i)$, where, PDRD is the deposition rate per m^2/h of particles ≥ D µm, C is the final particle concentration/m^2, Ci is the initial particle surface concentration/m^2,

t is the final time (hours), t_i is the initial time (hours).

Measurement of Particle Deposition Rate

The basic of a particle deposition measurement is the collection of particles on a witness plate over a known time period. The method is described in ISO 14644-3 2005 and DIS 2014.

The particle deposition measurement method describes procedures and apparatus for sizing and counting particles that are or can be deposited from the air onto product or work surfaces in the installation. Deposited particles are collected on witness plates with appropriate surface characteristics similar to those of the at-risk surface under consideration, and are sized and counted using optical microscopes, electron microscopes, or surface scanning apparatus. A particle fallout photometer or optical particle deposition meter may be used to obtain particle deposition rate data. Data for deposited particles should be reported in number of particles or particle concentration per unit surface area per unit of time.

The witness plate should be placed in the same plane as the at-risk surface and as close as possible to the risk surface. The witness plate should be at the same electrical potential as the test surface. Counting and sizing of particles collected on witness plates is carried out to obtain reproducible data that can be used to categorize the cleanliness of the area being tested.

When using an optical light microscope, calibrated linear or circular graticules may be used for the particle sizing measurements. Data from counts over a partial area of the witness plate may be extrapolated to the entire plate surface area (statistical counting). The particles on all witness plates are counted and enumerated on the total area of all witness plates and categorized in appropriate particle size ranges. The surface concentration of deposited particles for each witness plate is determined by subtracting the concentrations before and after exposure. The net concentration is divided by the test witness plate exposure time. The result is expressed as $PDR_{MAX;\,D} = 10^{M} / D$.

$PDR = PDR_{MAX;\,D} * D$. This calculation yields a particle deposition rate in terms of particles deposited per square meter per unit of time.

Apparatus for PDR measurement

Various apparatus may be used for counting and sizing particles that have settled onto the witness plate surface. These fall into four general categories, depending upon the size of the particles of concern:

- light microscopes (particles ≥ 2 µm);
- electron microscopes (particles ≥ 0.02 µm);
- surface analysis scanners (particles ≥ 0.1 µm);
- particle fallout photometer (up to 1% covered of surface area);
- particle deposition meter (particles ≥ 10 µm).

When choosing the counting and sizing apparatus to be used, consideration should be given to the detection of particles in the relevant size range. Other factors to be considered include the time required for sample collection and analysis.

The smallest particle size of interest that can be measured and the effective sampled surface of the witness plate determine the sensitivity. The lower the Particle Deposition Class the larger the required exposed surface A and/or the larger the exposure time texposure. The product A* texposure should not be too small to determine the PDC:

$$A * t_{exposure} \geq 20 / PDR_{MAX;\,D}$$

A given measurable witness surface and expected PDC determine the minimal time texposure. If a PDR or PDC needs to be within a certain time the measurable surface should be large enough.

New measurement instruments

For regular monitoring of particle deposition there was a need for new measurement instruments. In laboratories it was already possible to perform these measurements, but these involve expensive test apparatus and trained analysts. The problem is the optimisation of the smallest particle size and field of view. In the Netherlands two new measurement instruments are developed. One is based on machine vision technology and the other is based on digital holography.

The instrument using machine vision uses Compact Disc shape glass plates. Through light is used to image the particles on the top surface of the rotating witness plate onto a line camera. Particle larger than 10 µm can be measured on a surface area of 49 cm^2. One measurement can be within one minute.

After the initial measurement several glass plates can be placed at locations of interest in the cleanroom. After exposure the glass plates can be collected and measured. The change of the particle size distribution divided by the hours of exposure gives the PDR. These type of calculations are incorporated in the instrument. Also images of the particles are available.

The instrument that uses digital holography measures every 5 min the deposition of particles larger or equal to 20 µm on a set of 6 glass plates. The total measured surface is 25 cm^2 (in a rectangular area of 80 cm^2). This instrument monitors the particle deposition real time at the location of the sensor. A sensor consists of an digital holographic measurement unit and a disposable with 6 fixed glass witness plates at an angle of 45°. The sensor can communicate wireless or wired though a data network with a base station. One base station can communicate with a maximum of 6 sensors. When the base station is connected to the computer network the particle deposition monitor can be viewed from any location. This instrument calculates and shows all the important parameters of particle deposition measurement (Figure 2).

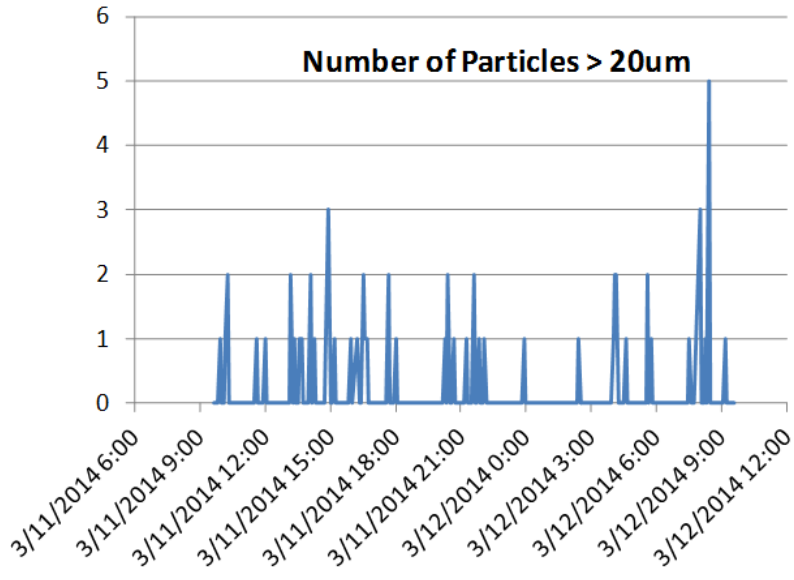


Figure 2. An example of a real time measurement.

Applications of particle deposition measurements

Particle deposition measurement can be used to measure various locations in a cleanroom to determine points of control. After that the critical locations can be monitored by regular or real time measurements. Real time measurements are also useful in creating operator awareness. Particle deposition of particles > 10 µm is determined by operational aspects such as number of people, their garments and discipline, working methods, cleaning methods and frequency and logistics. PDR values can be used to determine the risk of product contamination during exposure. From a product or process risk calculation the required Particle Deposition Class can be derived. Below some examples of applications are given.

Calculation of the PDR and PDC at a location

A witness plate with a measurable surface area of 64 cm^2 is exposed for 4 at a location in a cleanroom and the following particle sizes are measured ≥ 10 µm, ≥ 20 µm, ≥ 50 µm ≥ 100 µm, ≥ 200 µm and ≥ 500 µm.

The initial surface cleanliness of the witness plate was measured prior to exposure and given in Table 2. Also given in Table 2 are the particle counts measured on the witness plate after exposure in the cleanroom for 4 h. By deducting the initial count from the count after 4 h, the number of particles that actually deposit on the witness plate in 4 h is obtained.

The rate of deposition per m^2 per hour for each considered particle size (PRD_D) is then calculated. The particle deposition rate (PDR_D) for each particle size is then multiplied by the particle size (D) and the result given in Table 2. The PDC for each particle size is calculated by taking the ^{10}log of each PDR_D*D. The overall PDC of the location is

obtained by selecting the highest value of PDC from the various particle sizes measured. In this example, the highest PDC was obtained from both the ≥25 µm and ≥50 µm particles, and was Class 4.

Table 2. Calculation of particle deposition class (PDC).

Particle size	≥ 10 µm	≥ 20 µm	≥ 50 µm	≥ 100 µm	≥ 200 µm	≥ 500 µm
Initial count on witness plate / 64 cm^2	3	1	0	0	0	0
Count on witness plate after exposure for 4 hours	15	13	5	2	1	0
Number deposited / 64 cm^2 over 4 hours	12	12	5	2	1	0
Particle deposition rate (PDR_D) in no/m^2/hr	469	469	195	78	39	0
$D*PDR_D$	4 688	9 375	9 766	7 813	7 813	0
$PDC = {}^{10}\log(D*PDR_D)$	3,7	4,0	4,0	3,9	3,9	-

When testing often the PDR is expressed by the number of particles deposited per dm^2/h times D. This would lead to PDR 100 per dm^2/h.

Calculation of the Particle Deposition Classification of a clean zone

In an area of interest or clean zone of a cleanroom the particle deposition is measured in the same way as in above. At the 5 locations is the particle deposition PDR_5, PDR_{25}, PDR_{50} and PDR_{100} per m^2/h is determined. The $D*PDR_D$ and PDC can be calculated per location and the average value for all 5 locations. The measured particle size distributions can also be shown graphically (Figure 3).

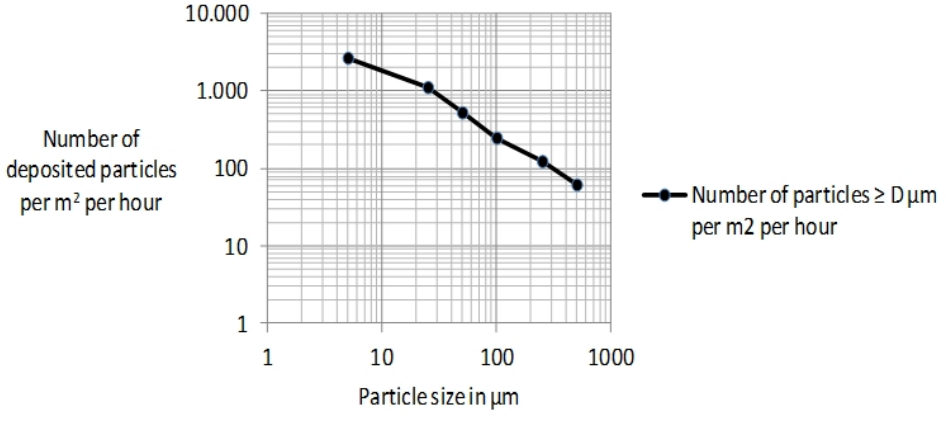


Figure 3. An average particle size distribution over a clean zone.

Representing daily or periodic particle deposition data to monitor the operational quality. In a cleanroom the PDR can be measured at critical locations. For monitoring the total performance the average of various location and/or various days can be taken. If a target is set measures should be taken when the target is not met. An example of the average weekly Particle Deposition Rate for a period of 13 weeks is given in Figure 4. After PDR increase general and local measures were taken to reduce the average PDR again.

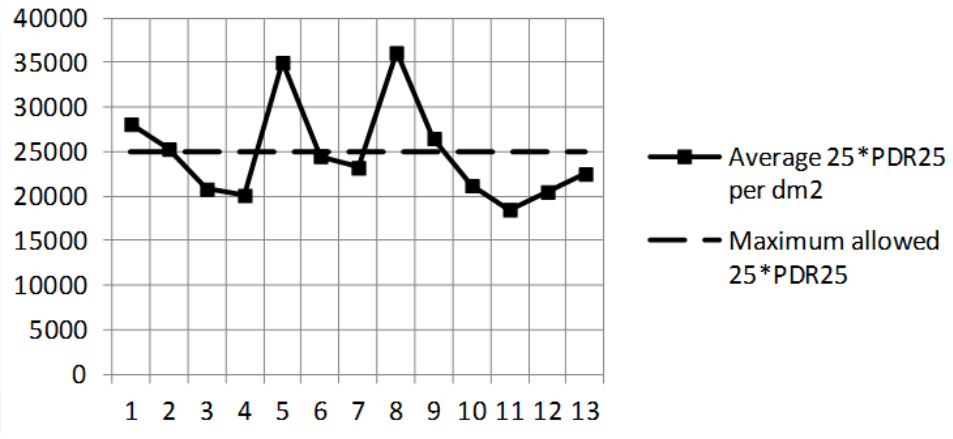


Figure 4. An example of the average weekly Particle Deposition Rate (D x PDR_D) for a period of 13 weeks. The average is taken from the daily average over five measurement location in a cleanroom.

Calculation of the risk of particle contamination at a location in a cleanroom deriving from measurements

The PDR determines how many particles will deposit from the air onto a surface in a given time, and the PDC classifies the deposition rate. This information can be used to determine the degree of risk of airborne particle deposition onto any surface, especially a vulnerable surface such as manufactured product. This estimate of the airborne contamination of a product can then be used to reduce the risk of contamination to an acceptable level. To calculate contamination, it is necessary to obtain the area of the vulnerable surface, the exposure time during working hours, and the PDR or PDC for particle sizes of interest. The equation that calculates the deposition rate onto a vulnerable surface, when the PDC is known, is as follows:

$$N_D = 10^{PDC} * A * t / D$$

where, N_D is the expected particle deposition of a given particle size (≥ D µm), PDC is the PDR class, A is the vulnerable surface area (m^2), and t is the time of exposure.
Alternatively, if the PDR is known, the equation is as follows: $N_D = PDR * A * t$.

Calculation of the degree of risk of airborne deposition onto a vulnerable surface

A product that is manufactured in a cleanroom has an exposed surface area of 10 cm^2 (10–3 m^2), and exposed during working hours for 5 h. The product is vulnerable to particles ≥ 10 µm, but one particle of ≥ 50 µm will lead to an immediate reject. The PDC was found to be 4 and, using equation B1, the expected particle deposition of particles ≥ 10µm is:

$N_{10\mu m} = 10^{PDC} * A*t/D = 104*10^{-3}*5/10 = 5$ particles per product and, for particle ≥ 50µm:

$N_{50\mu m} = 10^{4}*10^{-3}*5/50 = 1$ particle per product.

Alternatively, the expected particle deposition can be calculated from the PDR, which was 1000/m^2/h for particles ≥ 10µm, and hence, $N_{10\mu m} = 1000*10^{-3}*5 = 5$ particles per product.

In this example, the degree of risk of unwanted particle contamination is too high. It can be reduced by decreasing the time of exposure, or the particle deposition. For instance, if the time of the process is reduced to 30 min, then the particle contamination will be reduced by 10 fold. Alternatively, or additionally, if the product is manufactured in a clean area with lower particle deposition, the degree of risk can be reduced. Use of more occlusive clean-room clothing, fewer personnel, or a clean air device, are options that can be used.

Calculation of the PDC required for a given amount of surface contamination

The procedure given above can be reversed and the desirable air cleanliness calculated in terms of PDC, if the acceptable amount of surface deposition on a vulnerable surface of products is known. For example, consider a product that has a vulnerable horizontal surface area of 10 cm^2 (10^{-3}m), and is exposed in cleanroom air for 4 h. The airborne surface contamination of the product should not be more than 1 particle ≥10µm over 5 h.

Maximum $PDR_{10\,\mu m}$ can be calculated: $PDR_{10\,\mu m} = N_D/A.t = 1/0.001*5 = 200/m^2/h$

Maximum PDC can now be calculated: $PDC = \log_{10} (PDR_{10\,\mu m} * D) = {}^{10}\log (200 * 10) = 3.3$

Therefore, the PDC of the cleanroom should be better than 3.3.

Conclusions

In this article it is demonstrated this particle deposition measurement is the missing link in contamination control. Deposited particles can also carry microbes. The VCCN guideline on particle deposition proposes a particle deposition classification system. New measurement instrument will decrease the thresholds of performing particle deposition measurement and monitoring.

Acknowledgement

The author thanks W. Whyte for the fruitful discussions and his help in the wording of some paragraphs.

Literature

Agicola, K. & Geilleit, R. 2007. Classification of Contamination Control Practice. VDI Wissenforum Reinraum Technik, Basel.

Agricola, K. & Weling, P. 2008. Particle Deposition Monitor to reduce cleanroom costs. Cleanrooms Europe 2008, Stuttgart.

Agricola, K. 2009. Quality Assurance of Product Cleanliness. Cleanrooms Europe 2009, Stuttgart.

Agricola, K. 2012. Determination of operational quality of cleanroom by particle deposition monitoring, ICCCS 2014, Zürich.

Whyte, W., Whyte, W.H., Blake, S. & Green, G. 2013. Dispersion of Microbes from Floors when Walking in Ventilated Rooms. International Journal of Ventilation 12(3), December 2013.

VCCN Guideline 9, Particle Deposition 2014.

Selection and Use of Cleaning Agents and Disinfectants

Jennie von Fielitz

Miclev AB, Sweden

Extended abstract

Keeping the cleanroom clean requires that several factors be taken into consideration when designing a cleaning and disinfection regime, such as user requirements and types of disinfectants available. The principle aims of an effective cleaning and disinfection regime are microbiological and particulate control appropriate to the grade of area, and also using correct, safe and well documented materials as not to damage the surfaces or harm operators.

Basic cleaning is the process of removing residues and soiling from surfaces to the extent that they are visually clean. This will slightly reduce the microbial population, but will not achieve the same level of kill as a disinfectant. It is important that surfaces to be disinfected must be clean as disinfectants can be chemically inactivated by the presence of soiling and in turn create a physical barrier preventing the disinfectants reaching the microbial cells.

When selecting a cleaning agent it is significant to choose a known composition that is compatible with the surface to be cleaned along with the disinfectants in use. Low-foaming or non-foaming agents are preferred, since they are easy to rinse which avoids residue formation. If the cleaning agent is to be used in a Grade A and B area, the product must be sterile.

The process of removing or destroying microorganisms and reducing them to an acceptable level, efficacy is dependent on several factors. These are concentration, temperature, time, soiling, mode of application and type of organism. The ideal disinfectant kills all microorganisms, is non-corrosive, fast acting, safe and leaving no residue. It is also compatible with other cleaning agents/disinfectants and available in a variety of formats. Different disinfectants are effective against different types of organisms, so check the biocidal claims made by the manufacturer.

On what type of surfaces will the cleaning agent be used is an important factor to look at along with what format that is best suited. To avoid waste and cut costs, use of the appropriate pack size such as wipes for sessional use in sterile areas (e.g. LAF, isolators); large packs of tub wipes for areas where high volume usage is expected (e.g. transfer disinfection). Keep in mind that bulk sterile concentrates are no longer sterile once opened; unit dose concentrates ensure fresh sterile solution is used every time. Trigger sprays: available in 500ml, 1L and 4L in a protected system ensuring the contents remain sterile during use.

Small surfaces are typically treated by spraying and/or wiping and have significant variation in techniques. BEST PRACTICE is to spray and wipe to ensure maximum bioburden reduction and particulate removal, since microbes are killed by exposure to the disinfectant and those that are not killed are physically removed from the surface due to the mechanical action of wiping. Particulates are removed from the surface due to mechanical action.

Spraying onto a dry wipe shows significant variation in wetting of the wipe between operators. However, results show lower levels of airborne alcohol expected in comparison to surface spraying. It is ideal for flat, smooth surfaces (e.g. benches) and will prevent biocides from pooling in crevices. Spraying onto a surface is likely to achieve a more consistent surface coverage. Higher levels of airborne alcohol is expected in comparison to spraying onto the wipe. It is ideal for small items and uneven surfaces, but can result in pooling of biocides in crevices resulting in corrosion (not an issue with alcohol).

Use of impregnated wipes (without spraying) will significantly reduce operator exposure to the biocide/alcohol. Also the use of a pre-impregnated wipe in conjunction with spraying will result in maximum bioburden reduction.

Wipe in unidirectional over-lapping strokes (10–25%) and use slow, deliberate movements so as not to generate particulates and minimise disruption to airflows. A careful technique is required to maximise mechanical action and ensure good contact of the surface with the biocide. The wipe should be folded to allow maximum surface usage and ideally a fresh surface of the wipe should be used for each stroke. Keep in mind that the order of wiping just as important as it is with mopping (back > front, top > bottom, cleanest > dirtiest).

Points to consider are that the wipe should be folded to roughly hand size (this will vary depending on the size of your hand and the size of the wipe). How often the wipe must be re-folded to expose a “clean” side will depend on how dirty the surface is and the criticality of the surface. There is a potential for contamination from the used surface of the wipe to be transferred to the gloved hand! Some customers prefer to use a small wipe and not fold it all for use in for Grade A areas for this reason.

Ideally a fresh “side” of the wipe should be used for each stroke, but in practice this is not cost effective or necessary for all applications. In reality cost must be balanced against risk. Surface area to be covered by 1 stroke will depend on the criticality of the surface. Staff training and SOPs should define company practice to ensure consistency between operators.

There is no such thing as a “particulate/lint free wipe”, however some grades of wipe will shed more particulates that others – dependant on material of construction and method of manufacture. Ensure that the wipe selected is compatible with the disinfectant/alcohol in use. Wipes manufactured using chemical binders may not be compatible. Wipes

should be single use – re-used wipes will degrade and shed more particulates each time they are re-processed.

- When disinfecting large surfaces the some basic principle apply:
- Kill of microbes due to exposure to biocide
- Physical removal of microbes due to mechanical action
- Removal of particulates due to mechanical action
- For this reason the spray system should ideally be used in conjunction with a mopping system (mopping can be used as the residue removal step).

When using a single bucket system, the disinfectant/cleaning solution quickly becomes contaminated due to soiling reducing the efficacy of the solution, which is not suitable for use in a GMP environment. The double bucket system is a slight improvement on the single bucket system due to the addition of a waste bucket so the mop head is rinsed in "clean" solution. There is a declining use of this system in pharmaceutical industry. Using a triple bucket system the mop is dipped into "clean" solution, wrung out and applied to the surfaces. Then it is rinsed in middle bucket and wrung out into the waste. Use of disinfectant as a rinse agent is best practice as it avoids the potential for the solution in the front bucket to become diluted. Water may be used as a rinse agent to control cost. Note that some disinfectants are more sensitive to dilution than others, resulting is significant reduction in efficacy (e.g. phenol-based disinfectants).

Concentration Exponents is a measure of the impact of biocide concentration on biocidal activity. Products with a relatively high concentration exponent are more susceptible to dilution errors. Biocides with a high concentration exponents rapidly loose efficacy when diluted. This is important for preparation of the disinfectant, as well as use. The rinse/disinfectant solutions and mop heads should be replaced when they become visibly dirty. Solutions/mops may be taken from a higher grade area/surface to a lower grade area/surface, but where it is necessary to control cross-contamination equipment should be dedicated to a specific area. To ensure consistency company procedures should define the maximum area to be mopped with one bucket of solution/mop head (with the proviso that the solution is replaced earlier if found to be visibility dirty).

There is no such thing as a "particulate/lint free mop", as some mops will shed more particulates that others – dependant on material of construction and method of manufacture. Ensure that the mop material is compatible with the disinfectant in use. Mop fabrics manufactured using chemical binders may not be compatible. Select the appropriate grade of mop for the task at hand – sterile mops for applying sterile solutions. Mops should be for single use as re-used mops will degrade and shed more particulates each time they are used. Pre-impregnated mop wipes avoid over-application, reduces operator exposure, there is no need for disinfectant preparation and associated costs, and there is no need to dispose of liquid. This can be ideal for small cleanrooms that may not have space for a bucket system.

Regulatory requirement (EU GMP/ PIC/S Annex 1, USP <1072>) originally introduced due to concerns regarding the potential build-up of microbial resistance (as observed with antibiotics). No scientific evidence to date of resistance occurring due to lack of rotation in a cleanroom setting. Unlike the use of antibiotics, disinfectants are used at relatively high

concentration on a relatively small population, therefore the selective pressure for mutation is reduced. Disinfectant rotational schemes are still recommended as they ensure that the full spectrum of microbicidal activity is maintained, without the need to continually use a sporicidal agents, which are traditionally unpleasant to use. It is recommended that a bactericide/fungicide be rotated with a sporicide.

USP <1072> Antiseptics and Disinfectants: The rotation of an effective disinfectant with a sporicide is encouraged. It is prudent to augment the daily use of a bactericidal disinfectant with weekly (or monthly) use of a sporicidal agent. The daily application of sporicidal agents is not generally favoured because of their tendency to corrode equipment and because of the potential safety issues with chronic operator exposure. Other disinfection rotation schemes may be supported on the basis of a review of the historical environmental monitoring data.

PhaMIG survey revealed that there is no industry standard for rotation. EM data should be used to justify the frequency – if EM is in control then the current frequency is appropriate. Frequency of detergent cleaning will depend on risk factors associated with area, such as the amount of traffic (e.g. material transfer airlocks and gowning rooms higher risk), nature of processes that occur in the area (e.g. washrooms or areas where powders are handled are higher risk) and type of biocides used (low residue disinfectants = lower risk) and whether rinsing is performed (traditional disinfectants without rinsing = high risk).

Contact times is the time needed for the required level of kill to be achieved. A product may have different contact times depending on the target organisms (e.g. extended contact time for sporicidal action). Whether a “wet” contact time is required depends on how efficacy validation was performed. NOTE: the surface is not re-wetted during the contact time for EN 13697 testing. To achieve longer “wet” contact times re-wetting may be required. The surface does not need to be completely saturated. Alcohol is very fast acting, some customers have validated full bactericidal activity at > 1min contact. For some products (Glucoprotamin) microbial kill may continue after the surface has dried.

Effective cleaning and disinfection is a critical part of contamination control and GMP and to achieve the maximum benefit it is necessary to select the right products and use them appropriately. Deciding on the appropriate technique requires a pragmatic approach balancing risk, practicality and overall benefit and in order to be effective a controlled and consistent technique must be employed. Simple processes encourage compliance and it is essential that cleaning staff know not only HOW to perform these techniques, but WHY.

Basics Regarding Clean Room Ventilation and Biosafety Contamination Control

Conny Lindqvist* & Kari S. Aune**

* Exengo, Sweden & **COWI A/S, Norway
both are members of the R^3Nordic Board

Abstract

Ventilation systems are essential for achieving the special climate conditions needed in cleanrooms and biosafety laboratories. This presentation gives an overview of the key features and the basic design technique for these ventilation systems. Among the topics covered are air changes, air velocity and air movements as well as pressure differences between the cleanroom zones, and where to put the inlet and outlet devices to ensure the functionality needed. There will be some theoretical background and some project examples presented.

Why ventilation

The normal purposes of air handling systems are mainly to carry away exposed airborne pollutions and to reduce the increasing of indoor temperature in the situation of a high heating load.

Air handling system supplying clean room premises shall create areas free from particles and bacteria's in addition to the normal purposes.

To design suitable facilities you need an overall view regarding the entire process with detailed knowledge concerning; - The demands of operators/process, - Rules and regulations, - Clean room classification, - The nature of particles and bacteria, and Skilfulness regarding; - air-changes, - air velocity, - air movements as well as pressure difference between premises.

You need also to be familiar with the way to express clean room technique and the relationship between different parts of the technique

Air velocity and air changes

The regulations points out the importance of sufficient air velocity and air movement when designing clean room areas. Regarding air velocity/movement the character is also pointed out. Unidirectional (U) air movement is the most common, but when the demands are extremely high (semiconductor processing zone) Laminar Air Flow (LAF) could be required. A decade ago air changes were more in focus especially when you looked at the entire clean room premise. When designing it is of big importance that areas with higher cleanliness are selected from areas with less cleanliness.

Design – technique, air movement and climate

Air handling systems supplying air to create areas free from particles and bacteria's are mainly based on filter technique, high air flow and controlled air movements. To reduce the operation costs the main part of the air flow is recirculated. Some ventilation is needed in order to carry away exposed airborne pollutions such as emissions and gaseous pollutants. Those pollutants are not filtrated with common HEPA filter (or equal) located in the recirculated air. The ventilation is normally arranged via so called make-up air within the air handling system and a suitable situation is when the exhaust air needed for polluting processes (not to be re-circulated) can be balanced to the make-up air.

When designing clean room areas the presence of the operator has to be taken in consideration. The air movements have to arranged and controlled so that contamination generated from the operator not will occur (Figures 1 & 2).

Clean room technique has mainly the focus to create areas free from particles and bacteria's. But also the climate (temperature, humidity) is to be controlled as a preventive measure to defeat bacteria growth.

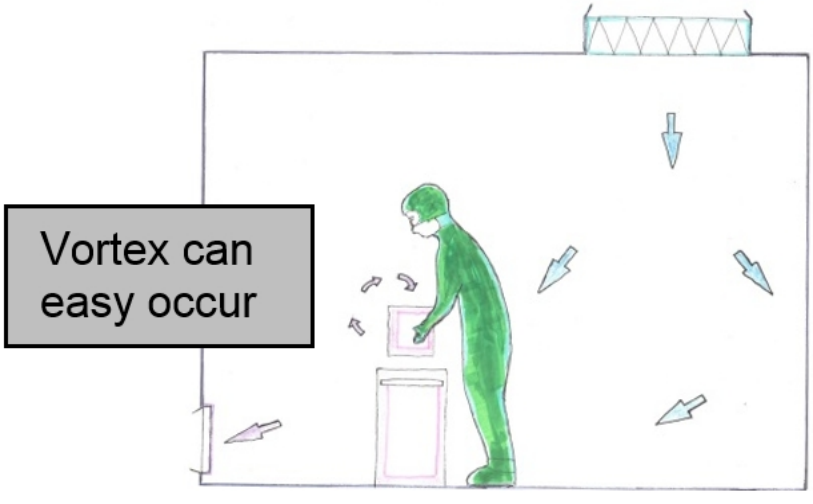


Figure 1. The presence of the operator has to be taken in consideration.

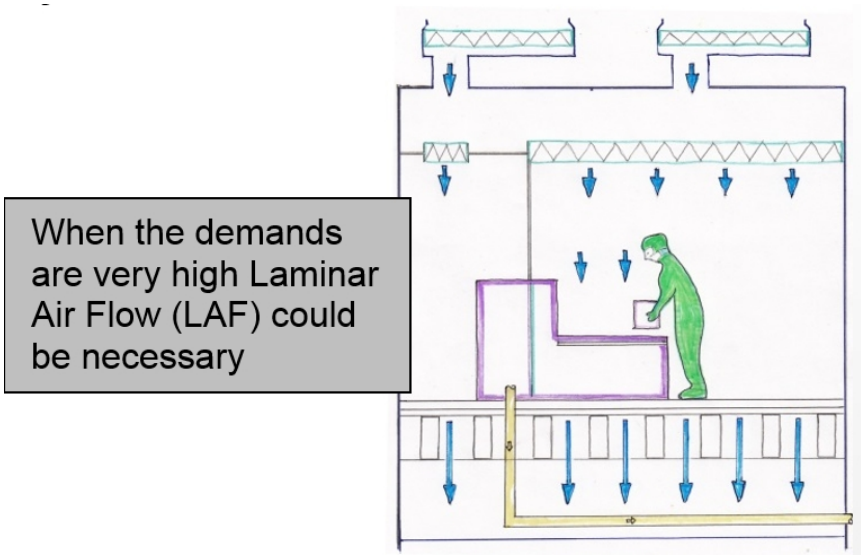


Figure 2. Controlled air movements will give less contamination generated from the operator.

Good Manufacturing Practices

During the sixties the Food and Drug Administration (FDA, USA) established the regulations we today knows as Good Manufacturing Practice (GMP; Figure 3). For the manufacture of sterile medicinal products 4 grades are used:

- Grade A: The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections
- Grade B: For aseptic preparation and filling, this is the background environment for the grade A zone
- Grade C and D: Clean areas for carrying out less critical stages in the manufacture of sterile products.

Figure 3. GMP is very heavy in pharmaceutical industry.

Cases

Examples are shown in Figures 4–6:

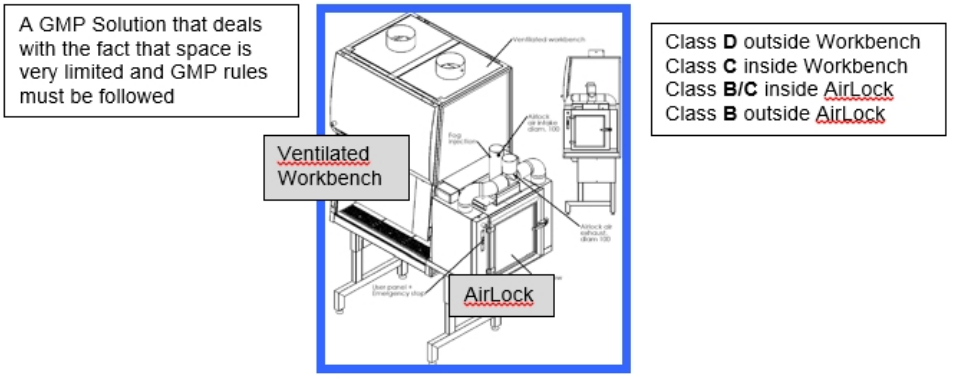


Figure 4. Combi WorkBench – AirLock case.

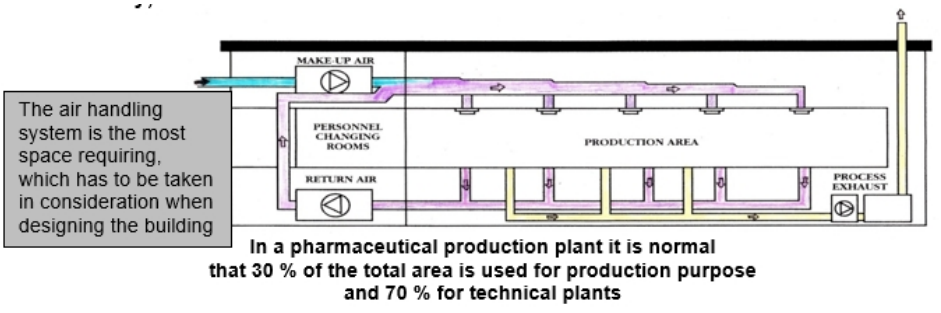


Figure 5. Production plant case.

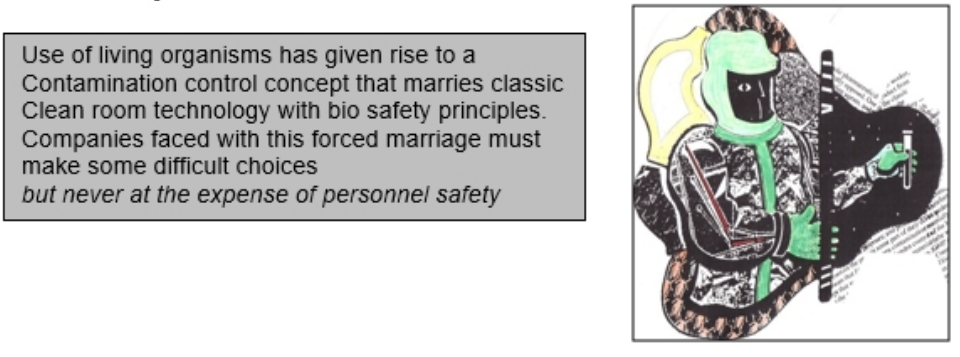


Figure 6. Bio safety/clean room hybrid case.

Basics Regarding Cleanroom Clothing – Why, What and How

Heidi Tuomi

Turku University of Applied Sciences, Finland

Introduction

People are the greatest source of contamination in a cleanroom. To maintain the specified level of cleanliness during operations, protective clothing of adequate efficiency must be used by the personnel. Different kinds of clothing regimes are selected for cleanrooms of different cleanliness classes. Cleanroom apparel are high-tech products that must meet many somewhat conflicting requirements. Gowning for the cleanroom is a skill that needs training and validation.

People as a source of contamination

The sources of contamination in a cleanroom are people, supply air, room surfaces, raw materials, packaging, tools and equipment, utilities, production machinery, production processes and adjacent, dirty areas.

Personnel have an important role in the production process, but they are the greatest contamination source in a cleanroom and thus can seriously compromise the product. Around 80% of the impurities present in a cleanroom originate from people and they are also the major source of viable particles present in a cleanroom. Everything else in the cleanroom can be sterilized or disinfected, except personnel.

People shed skin flakes, microbes, cosmetics particles, hair and textile fibers from their clothing. The outermost layer of skin epidermis is renewed every 24 hours which causes people to shed up to 100 g of dead skin cells per week. As hair regenerates, old hair falls off.

The microbes in a human body belong to either the normal or transient flora. Normal flora is the harmless, permanent bacterial population present in the human body and it is important for the well-being on a person. The composition of normal flora varies between

people by age, gender, region and season. Transient flora (mainly on the skin) can contain any microbes including pathogens and it transmits from the environment or from other people. It can be removed from the skin by washing and disinfection.

The number of particles people generate is increased by movement. A person walking generates 10 times more particles than one sitting down. Talking, coughing, smoking and the use of cosmetics also increase the amount of particles dispersed.

Ways to decrease the amount contamination originating from people are efficient and correctly worn protective clothing, slow movements and correct work methods and good personal hygiene. Training the employees for cleanroom behavior is most important.

Good personal hygiene means taking care of one's personal cleanliness and health. One should not work in a cleanroom when ill, not use cosmetics or wear jewelry in a cleanroom. Eyeglasses should be kept clean and nails short. Having s beard/moustache is not recommended. The use of moisturizer to prevent skin scaling is a good practice.

Cleanroom clothing

The functions of cleanroom clothing are to protect the cleanroom environment and products from contamination generated by people and to protect the personnel from hazardous materials or products. Cleanroom clothing should:

- protect the cleanroom environment from contamination,
- shed no particles,
- be easy to put on,
- be comfortable to wear,
- be dirt repellent and easily cleanable,
- withstand washing and sterilization,
- have adequate strength and resistance to break-up and
- have desirable electrostatic properties.

Cleanroom clothes are made of either woven fabrics, spun bonded fabrics or laminated fabrics (with membrane). Garments act as filters and retain the particle dispersions from personnel. The "filtration efficiency" is dependent on the fabric the garments are made of. Some amount of air escapes from under the garment unfiltered, via closures and holes in the garment. This can be reduced by careful design and good construction. A cleanroom clothing design consists of:

- Body garments; coat and trousers or coverall (bunny-suit),
- Headgear; hair cover and/or hood, facemasks and googles,
- Footgear; shoe covers or boots and
- Gloves; knitted/woven or barrier gloves made of latex, nitrile or vinyl.

The protection efficiency needed depends on the cleanroom class and the product/ process (sterile product? aseptic process?). It defines the material and model of the clothing. Garment systems may include several layers of garments. By using cleanroom undersuits, the effectiveness of the cleanroom apparel can be increased. By combining various types of garments and accessories a suitable combination for different cleanroom classes and applications can be found. Clothing manufacturers give recommendations on which products to use at different cleanroom classes.

Gowning for the cleanroom

Cleanroom garments are donned prior to entering the cleanroom. There is no single right practice to put on cleanroom clothing, but it varies with the clothing regime used. The best method of changing into cleanroom garments is the one that minimizes the amount of contamination getting onto outside of the garments.

The number of consecutive changing rooms depends on the cleanroom class and the gowning procedure. The changing rooms should act as air-locks. Personnel entering and exiting the cleanroom should be separated; either by time interval or in different changing rooms. The changing rooms must have a cleanliness classification high enough not to compromise the cleanliness of the cleanroom. A written instruction must be available in the changing rooms. The changing rooms should be divided into three zones:

1) pre-change zone; for removing clothes that are not to be worn underneath the clenroom garment,
2) changing zone; storing and donning/taking off cleanroom garments and
3) Entrance zone; checking of the garments and entrance to the cleanroom.

A suggested gowning protocol will be presented.

How to Bring Zones of Ozone Closer to Everyone's Everyday Life

Juhana Haukioja

Turku University of Applied Sciences & Hygio Oy, Finland

Abstract

The technology to produce ozone in different methods is known for over one hundred years but still the potential of using ozone in different areas is not completely utilized. Until recent years ozone devices have been controlled by authorized personnel but now there are a lot of different devices in market which allow consumer to use such devices as home appliances without any training. These devices use ozone in different concentrations and phases. As in any other technological devices some of these products are less successful than others.

Presentation discusses how to combine two effective abilities of ozone into one consumer level easy-to-use device. Antimicrobial effect of ozone gas reduces microbial activity in processed products and therefore increases general hygiene level while also deodorizing the product at the same time. Products that require ozonizing are placed inside the device in closed system so the area around the device is still useable. In the process the produced ozone is generated from ambient atmosphere so no additional oxygen is required. In the end of the process hygio (the actual device) is safe to open because the last few minutes of the process are used to catalyze the remaining ozone in the device. Because there are these additional above mentioned properties in the product it is decided to call the process hygiofying rather than ozonizing. It is wanted to make clear that the device does more than just ozonizes. There are plenty of different ways and places to use such device to prevent unwanted odors and to help prevent diseases from spreading.

The effectiveness of dry ozone gas against microbes e.g. *Escherichia coli* and *Pichia pastoris* is discussed. Time vs. concentration play an essential role in finding the right combinations for optimal utilization. Finally these results can be used to increase or decrease time and concentration whatever the purpose is.

Introduction

The need for ozonizing products by gaseous ozone is not well recognized or known because of lack of knowledge that such an option is even available. There are several areas where ozonizing would be a great method to decrease contamination chance or just generally to increase work welfare in terms of deodorizing. For example in food industry both of these abilities would be a welcome choice of treating working shoes and partly working clothes. Though the problem in this area is the fact that ozone gas alone won't remove dirt and therefore some mechanical actions are required to remove it. Also organic dirt or stain weakens the effectiveness of ozone because ozone affects to organic material therefore consuming available ozone in the ambient atmosphere. (Gynther 2010, 5). According to FDA guidance in food industry foot wear should be easily cleanable and therefore cleaned if they are dirty and disinfectant baths should be harnessed. Also in dry processing areas such baths should not be used at all and dry sanitizers should be used. (Anon 2008, II A.)

In laundry industry ozone has been used for a very long time as in fluid phase and at the moment there are more than 2000 laundries in USA that are using ozonized water and there are another 2000 laundries in Great Britain. According to these numbers ozone technology is well known when ozone is dissolved in water. In laundry industry there are several benefits for using ozone. Economical benefits are achieved because no chemicals are needed and low washing temperatures can be used. This means that the process is also more environmental friendly than traditional washing techniques. Also higher hygiene level is achieved in terms of destroying microbes. (Rice et al. 2009, 339). Hygio can be the answer for treating laundry in terms of inactivating microbial activity after removing stains and dirt with the new upcoming 20°C wash cycle.

While destroying microbes is important in above mentioned areas it's also important in nurseries, nursing homes, gyms, bowling halls and in places where people are near each other and/or use rental clothes and shoes. In these places hygiofying also prevents spreading of epidemics and athlete's foot. Also in such areas deodorizing is more than a welcome property.

Test methods and materials

The method of testing microbial inactivation in hygiofying process was done with VTT. Gram-negative bacteria *Escherichia coli* (ATCC 25922) and yeast *Pichia pastoris* (X-33) were used in the test and tests with different microbes are still running.

Two different test materials were used. First material was normal laboratory coat (35% cotton and 65% polyester) which was received from VTT. The second material was bought from Askelklinikka and was shoe insole material which was meant for sports. Pieces from both materials were cut (approximately 15 cm * 20 cm) and holes on the longer side were cut. Holes were made to make inserting the tests materials to coat hanger possible. Three pieces of each material were used as replicate. Materials were then sterilized in autoclave for 15 min 121°C. Sterilized materials were dried in biological safety cabinet on disinfected surface. Dry test pieces were then put on disinfected hanger (Figure 1). On dry test materials 5 ml of microbe suspension was pipetted on targeted

areas. The suspensions contained about 10^8 cfu/ml of the target microbe. These material pieces were then placed inside hygio device with 4 laboratory coats which acted as an extra load for the process (Figure 2).

Processing time was 60 min and ozone concentration was around 400 ppm (parts per million). In the process last 5 min were used to neutralize remaining ozone back to oxygen. After the process test samples were put into stomacher bags and 95 ml of peptone saline were added. Then the samples were driven with Stomacher 400 for 60 s with high speed. Then 100 µl from the stomacher bags were pipetted on PCA plates and were incubated o/n in 37°C. Test method was exactly the same for *P. pastoris* but with different culture medium, agar, incubating temperatures and incubating time.

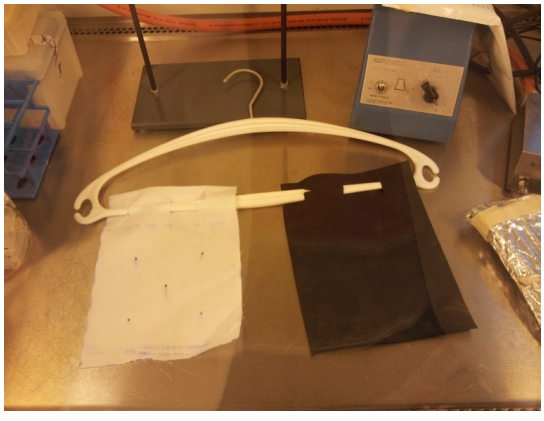


Figure 1. Test materials on hanger with marketed pipetting spots.

Figure 2. With microbe suspension contaminated test samples in the device.

Results, discussion and conclusions

In the first tests where processing time was 60 min and ozone concentration was around 400 ppm and almost sterilization level was achieved for *E. coli*. Suspension had 4.2 * 10^8 cfu/ml of bacteria and sample directly taken from stomacher bags had only total of 2 cells on Petri dishes per material on total of 6 plates. Inactivation of microbes without processing on materials was also tested and the reduction of microbes was only around 1 * 10^1 cfu/ml. Processing time was then reduced to 20 min and counts on laboratory coat material still remained same than in 60 min process time whereas insole material counts were as high as 2.4 * 10^4 cfu/ml.

According to good results with 60 min process time ozone concentrations were decided to lower to ensure durability of all the components in the device and to make difference between different programs in terms of deodorizing and drastically increasing hygiene level (Figure 3).

With these new parameters 1 * 10^3 to 1 * 10^6 reduction was achieved for the coat material and 1 * 10^2 for the insole material according to two tests with *E. coli*. Yeast *P. pastoris* was also used in this test parameter. Reduction for *P. pastoris* was only around 1 * 10^1 cfu/ml in insole material and 1 * 10^2 for the laboratory coat material.

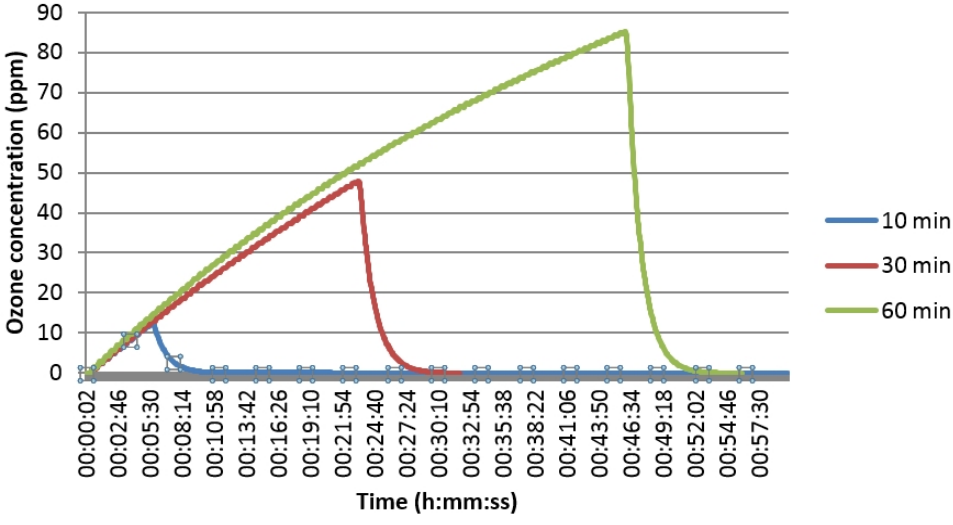


Figure 3. Ozone concentrations in different programs measured with Teledyne Model M465L.

In conclusion these concentrations and processing times are too low for inactivating *P. pastoris* effectively. Also it should be noted that in insole material microbial reductions were much lower than in laboratory coat material. Reason for this is that laboratory coat material is thinner and more porous than insole material. Therefore ozone can penetrate into laboratory coat material much easier than in insole material therefore inactivating more microbes in laboratory coat material. It is planned to increase the ozone concentra-

tion in 60 min program so it would better inactivate microbes and other two faster programs would work as deodorizing programs.

All in all via hygiofying general hygiene level can easily be increase drastically and over 90% inactivation of microbes can easily be achieved. Hygio can therefore reduce the amount of washing of clothes that are not stained and increase general hygiene level on the go. With modifications to ozone concentration and processing times very high inactivation rates can be achieved to even full fill food industry needs in the future.

References

Gynther, N. 2010. Otsonivesikäsittely välidesinfiointiaineena; elintarvikkeen ja pintojen hygieeninen laatu. Bachelor Thesis in Environmental Technology at Turku University of Applied Sciences (in Finnish).

Anon. 2008. Guidance for Industry: Control of Listeria monocytogenes in Refrigerated or Frozen Ready-To-Eat Foods; FDA Draft Guidance.

Rice, R., DeBrum, M., Cardis, D. & Tapp, C. 2009. The Ozone Laundry Handbook: A Comprehensive Guide for the Proper Application of Ozone in the Commercial Laundry Industry. Ozone Sci. Eng. 31: 339–347.

Indoor Air Quality Research in Laboratory Environment

Vuokko Lappalainen[1,2], Elina Sohlberg[1], Helena Järnström[3], Jarmo Laamanen[1], Hannu Viitanen[1] & Pertti Pasanen[2]

[1] VTT Technical Research Centre of Finland, Finland

[2] University of Eastern Finland, Finland

[3] VTT Expert Services Ltd., Finland

Abstract/

In the control of clean processes or environments, the real-time detection for biological airborne contaminants is invaluable. Rapid microbiological methods (RMMs) are commonly used in cleanroom environments, but now this method is applied also for indoor air quality measurements in laboratory simulation studies.

In the Microdiverbuild project at VTT and UEF, the release and drift of biological, gaseous or other airborne contaminants from hidden mould growth inside the wall structure were studied. RMM was used in real-time measurements of biological and non-biological particles in the Indoor Air Quality simulator (IAQ simulator). The real-time measurement enabled us to follow changes in emissions during dehydration of contaminated material.

Introduction

RMMs are invaluable in clean environments and processes. In Microdiverbuild project, the RMM was used in real-time measurements of biological and non-biological particles in the IAQ simulator. In IAQ simulator tests, the complex relationship between mould growth inside the wall structure and indoor air quality was simulated and the source strength of a mould growth inside the wall structure was assessed. The impact of relative humidity changes of the material with mould growth on IAQ were evaluated under normal pressure difference between indoor and outdoor air.

Material and methods

The IAQ simulator gives a new tool for indoor air quality assessment. In the Microdiver-build project, the simulator was used to assess IAQ when there is active mould growth inside the wall structure under various material relative humidity and pressure difference over the structure. The simulator was developed at VTT (Paavilainen et al. 2007), and it is based on the standard ISO 16000-9 (ISO 2006). The simulator consists of a supply air purification unit and a pressurisation unit and two test chambers (volume 0.5 m^3 per chamber). The tested structure was placed in a frame between the chambers (Figure 1).

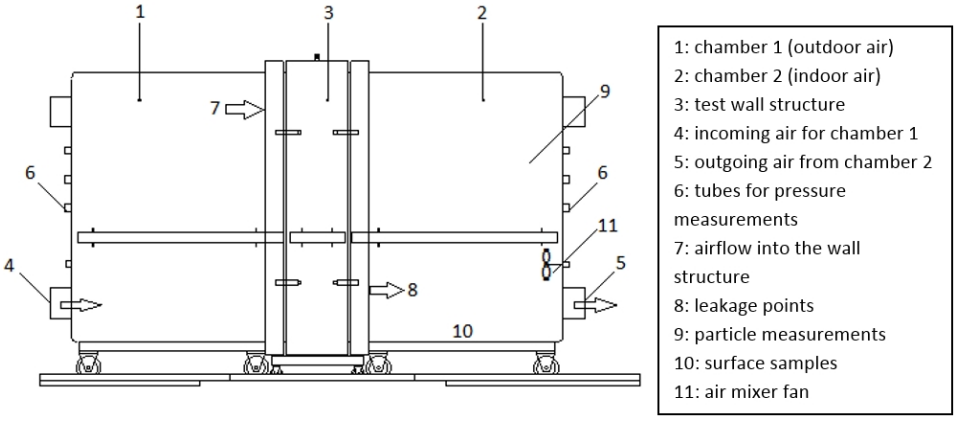


Figure 1. Principle of the IAQ simulator.

In the case presented in this paper, a wall structure of two gypsum boards and five upstanding pine sapwood laths with mould growth was tested. Leakage routes of the wall envelope were seven 6 mm in diameter drilled holes. The borders of the gypsum boards were sealed carefully with the aluminium tape to avoid other, uncontrolled leakages. The computational leakage rate at 50 Pa pressure difference (n50) was 2.87 h^{-1} for this wall structure. This is a typical compactness for this kind of structure. The pressure difference was at normal, low under pressure level (-2…-5 Pa) in this test. The air change rate was 1.0 1 h^{-1}, and incoming air flow was 8 l/min. The incoming air was purified and humidified for clean and constant air quality during the tests. All surfaces of the chamber and structures inside the simulator were cleaned with steam and 70% ethanol dilution before and after every test runs.

In the bottom of the wood laths, 0.5 l of water in the beginning of both tests was added. During the test, water dehydrated and the wood material dried out. Particle emissions during the drying were measured continuously. Both wall structures, with a mould damaged and an undamaged wood laths, were tested. In the moisture damaged wall, a mixture of 6 mould species (*Aspergillus versicolor, Penicillium brevicompactum* ATCC 58606, *Chaetomium globosum* D-81079, *Cladosporium sphaerospermum, Paecilomyces variotii* D-83214 and *Trichoderma viride*) was inoculated on two sides of the wood laths and incubated for 4–6 weeks in high relative humidity before the start of the test. An undamaged wall structure was used as a control case (no mould inoculated).

The particle concentrations in the indoor air chamber were measured continuously in three size fractions: 0.5–1 µm, 1–3 µm and 3–5 µm. Measurements were done with BioVigilant® IMD-A™ (Instantaneous Microbial Detection; Azbil BioVigilant, Inc., Tucson, AZ, US) particle counter what fractionates biological particles (i.e. microorganisms) from non-biological particles by fluorescence detection. The most of fungal spores and fragments infiltrated through the building envelope are in size fraction of < 4 µm (Airaksinen et al. 2004). The relative humidity of the material was measured with a surface sensor (Tinytag TV-4506, Gemini Data Loggers Ltd., Chichester, UK) in a height of 10 cm from the bottom of the wood lath. The pressure difference between indoor and outdoor chambers was measured continuously (SwemaFlow 300, SWA10, Swema AB, Farsta, Sweden).

Results

The average pressure difference over the wall structure varied in the control case between +0.4 and -3.5 Pa (average -2.2 Pa), and in the damage case between -2.4 and -4.6 Pa (average -3.5 Pa). The minor distinction in pressure differences between control and damage cases was caused by a little failure in the sealing of wall borders. The RH was > 90% during first 6 days when added water was absorbed into the wood. In next few days, the wood material dried slowly (RH 60–90%) reaching eventually the normal humidity level (RH 40–60%). Visible mould growth was not detected in the control case. In the damage case, strong visible mould growth was detected before and after the simulation test (mould index 4–5 on the scale of 0–5; Viitanen et al. 2010).

The particle concentrations were measured with BioVigilant® IMD-A™ in three size fractions (Figure 2). Biological and non-biological particles were measured separately based on fluorescence of the particle. Concentrations of non-biological particles were higher in two smallest measured size fractions in the damage case at high relative humidity of wood laths (RH >90%). However, during the test and decreasing of RH, concentrations in the damage case decreased also. At the two lower RH levels, the concentrations of non-biological particles were even higher in the control case. Biological particle concentrations were in same order of magnitude in control and damage cases.

Conclusions

The IAQ simulator is a tool to evaluate the impact of failures in the building structure on IAQ. The simulator is designed initially for VOC measurements and it is optimal for example to investigate emissions from a multilayer structure at various ventilation rates. However, it is suitable also for other emission measurements. In this study, the IAQ simulator was used for IAQ assessment in the case of hidden mould growth in the wall structure. The results presented in this paper, were from the first stage of Microdiverbuild study. IAQ simulator tests have been done also in three other setups.

Because of the limited air flows into the IAQ simulator and structural factors, microbial air samples for the cultivation or qPCR analysis were difficult. In this study, the BioVigilant® IMD-A™ was used for measuring biological and non-biological particles in the case of hidden mould growth and air leakage compared to the case with no mould growth. The real-time measurements enabled us also to follow changes in emissions during the dehy-

dration of the material inside the wall. The BioVigilant® IMD-A™ was useful in these laboratory measurements. However, the adjustable sampling intervals and another, parallel, inlet would have given more advantageous results in our study.

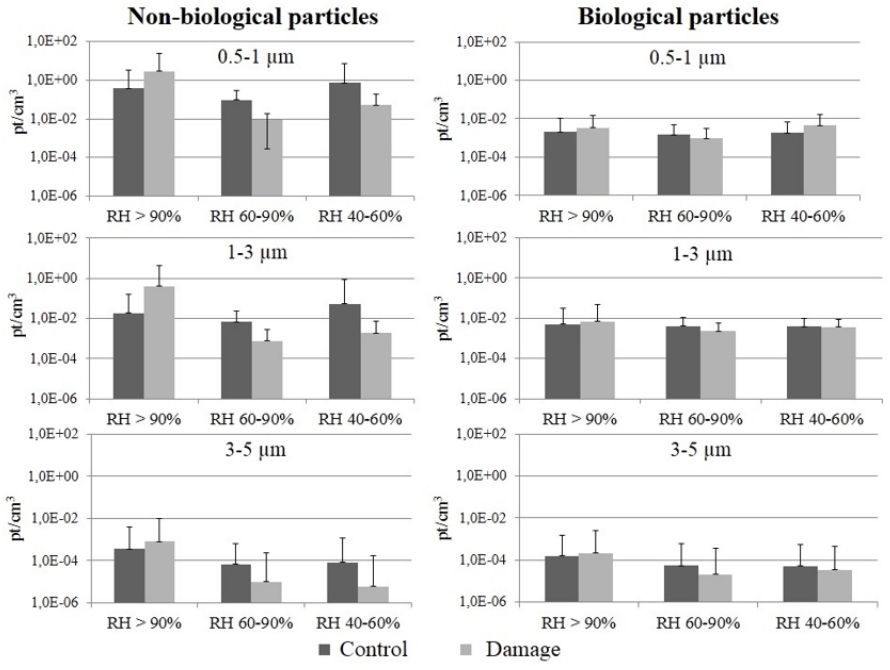


Figure 2. Particle concentrations in IAQ simulator tests.

Acknowledgements

This study was funded by Academy of Finland (Microdiverbuild 253259), VTT, Tekes (Bitefa project 40371/11) and University of Eastern Finland.

References

Airaksinen, M., Pasanen, P., Kurnitski, J. & Seppänen, O. 2004. Microbial contamination of indoor air due to leakages from crawl space: a field study. Indoor Air 14: 55–64.

ISO 16000-9. 2006. Indoor air Part 9: Determination of the emission of volatile organic compounds from building products and furnishing. Emission test chamber method.

Paavilainen, J., Järnström, H., Saarela, K. & Sarlin, T., Viitanen, H. & Airaksinen, M. 2007. Simulation of moisture and microbial problems in building. Clima 2007 – Well Being Indoors. Helsinki, Finland, 10–14 June. Abstract book: 353. CD ROM: 1436. 8 p.

Viitanen, H., Vinha, J., Salminen, K., Ojanen, T., Peuhkuri, R., Paajanen, L. & Lähdesmäki, K. 2010. Moisture and Biodeterioration Risk of Building Materials and Structures. J. Build. Phys. 33: 201–224.

Modelling Bioaerosol Dispersion in Manned Spacecraft

Ilpo Kulmala, Eero Kokkonen, Tapio Kalliohaka & Matti Lehtimäki

VTT Technical Research Centre of Finland, Finland

Abstract

The crew members in a spacecraft experience exceptional working and living conditions which may adversely affect the health of the astronauts and make them more vulnerable to microbial exposures. In the BIOSMHARS project a CFD model was developed to predict airborne bioaerosol dispersion in spacecraft environment. The model was validated with air flow field and aerosol measurements. Comparisons with experiments showed reasonable agreement between calculations and laboratory measurements.

Introduction

Astronauts in a spacecraft experience exceptional working and living conditions such as high working pressure, defined died and restricted hygiene practice, microgravity and exposure to radiation. Additional stress factors are limited space of the habitats, full recirculation of air and water and abundance of high touch surfaces. These conditions may adversely affect the human immune system and make astronauts more susceptible to infections. Moreover, microbes onboard spacecraft have caused biodegradation of materials (Van Houdt et al. 2012). These risks will be more significant during the envisioned deep space missions.

In order to control biocontamination it is important to increase understanding of bioaerosol generation and dispersion and also of their survival in exceptional conditions. The BIOSMHARS (www.biosmhars.eu) project aimed to develop, to calibrate and to validate a mathematical model to predict the transportation of bioaerosols in a closed environment and the concurrent spread of biocontamination. This model is also aimed at supporting the implementation of proactive countermeasures.

The developed CFD model was verified by laboratory measurements. A mock-up of the Columbus module on the International Space Station (ISS) was constructed for experimentation with volume, geometry and ventilation system similar to its real-world counterpart.

Spacecraft indoor environment

The habitable conditions onboard manned spacecraft are created and maintained by the Environmental Control and Life Support System (ECLSS). It controls atmospheric pressure and oxygen levels, and water supply. On the ISS the indoor air composition is in general similar to that on earth except for the higher carbon dioxide levels.

In the hermetically sealed spacecraft oxygen is produced primarily by electrolysis of water. The gaseous contaminants must be continuously removed from the recirculated air to prevent the buildup of CO_2 and other contaminants from human emissions and outgassing of materials. To achieve this, the trace contaminants are removed with activated charcoal filters and catalytic converters while carbon dioxide is removed using regenerative adsorption systems (Raatschen 2009). Particulate contaminants are controlled with HEPA filtration.

Ventilation is an essential part of the spacecraft's ECLSS. Like on earth, its purpose is to provide safe and healthy indoor environment by supplying treated air, dilute contaminant concentrations and exhaust contaminants. However, there are also features which are specific to space environment. The most prominent is microgravity which means that convective currents are non-existent, resulting in that natural convection – which in terrestrial conditions is a major contributor to air mixing – does not assist in mixing of supply air. Therefore forced ventilation throughout the whole occupied area is needed to avoid poorly ventilated zones and to remove body heat. Other specific features are full recirculation of air, relatively high air exchange rates and small ventilated volumes.

Potentially harmful microbes onboard come mainly from crew members but also from animals, plants or food, resupply, or from the assembly of spacecraft components. Weightlessness causes that even large particles like skin scales with microbes attached to them do not settle but float until exhausted by the ventilation and finally removed by the HEPA filters in the recirculated air. Routine measurements of air biocontamination most commonly identified *Staphylococcus* and *Bacillus* bacterial species and *Penicillium* and *Aspergillus* fungal species. The microbial levels have been below the current threshold values aboard the ISS, which are 1000 cfu/m^3 for bacteria and 100 cfu/m^3 for fungi (Van Houdt et al. 2012).

Computational Fluid Dynamics Simulations

The modelled case was roughly similar to the Columbus laboratory module of the ISS. Like in the real module, the ventilation system consists of overhead air supply diffusers that provide the supply flow, and one large return grille located near floor. The diffusers on both sides are placed in staggered rows as shown in Figure 1. As depicted; each diffuser consists of 12 slits.

The simulation of particulate contaminant dispersion with CFD method is typically performed either with Eulerian or Lagrangian method as presented in Figure 2. The Eu-

lerian method treats particles as a continuum and solves the conservation equations for particle phase and is widely used to predict particle concentration distribution. The particle properties are approximated through a diffusion coefficient which includes all the physics regarding the particle dynamics. In more detail, the concentration simulation is performed by solving an additional passive scalar transport equation in addition to the Navier—Stokes equations describing the air flow field.

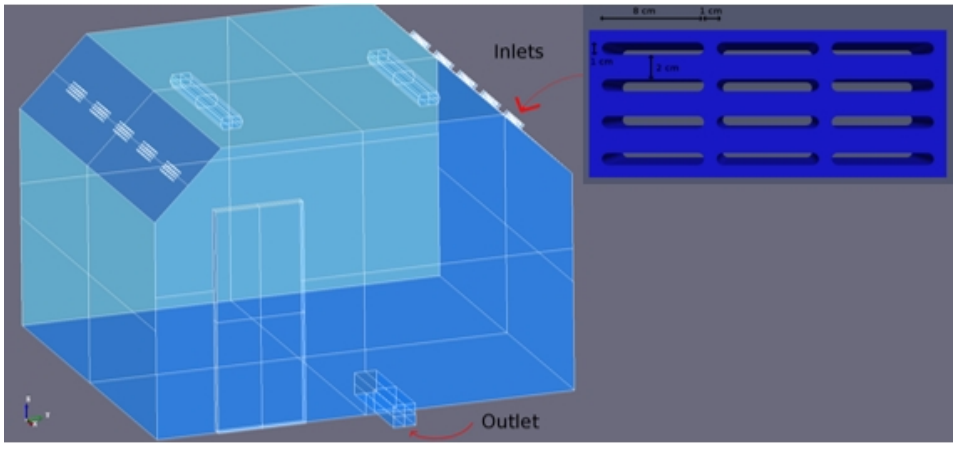


Figure 1. Geometry of test room at VTT used in the CFD model. The geometry, inlet characteristics (shape, dimensions, locations) and ventilation properties are roughly similar to the Columbus module at the ISS.

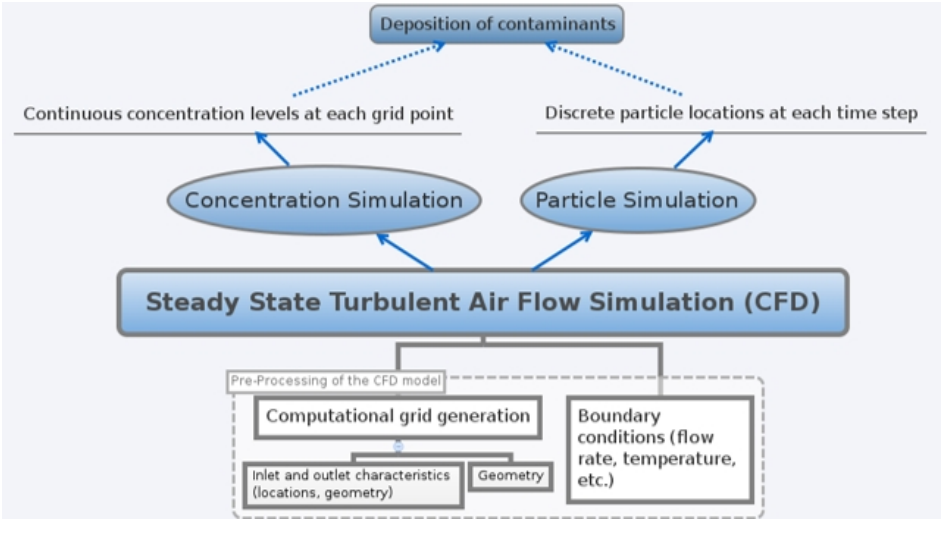


Figure 2. The biocontamination modelling overview by using computational fluid dynamics (CFD) approach.

The solved equation for scalar φ can be written as:

$$\frac{\partial \rho \varphi}{\partial t} + \frac{\partial}{\partial x_i}\left(\rho U_i \varphi - \Gamma \frac{\partial \varphi}{\partial x_i}\right) = S_\varphi, \quad (1)$$

where Γ is the diffusion coefficient – defined as the sum of the laminar and turbulent coefficients – and S_φ the scalar source term. The Eulerian method is computationally cheaper than the Lagrangian method and can be solved in steady state or transient formalism.

The Lagrangian particle tracking method emphasizes the individual behaviour of each particle and determines particle trajectories based on the equation of motion. The Lagrangian method is always transient, even though it can be solved on top of the steady state air flow field in such a way that the flow field affects to the particles, but the particles do not affect to the flow field (one way coupling). This specific equation, which stems directly from Newton's second law, corresponds to a force balance on the particle equating particle inertia with the forces acting on the particle, and can be written in the Lagrangian reference frame and in Cartesian coordinates as:

$$\frac{du_{p,i}}{dt} = \frac{1}{\tau}\frac{C_D\, Re_P}{24}\left(u_i - u_{P,i}\right) + g_i + \frac{F_C}{m} + \frac{F}{m}, \quad (2)$$

where u_p and u_i are the particle velocity and the fluid velocity, respectively. The first term on the right hand side presents drag force and the second gravitational force. In the model, the value for g_i can be set at will: on earth it is 9.81 m/s^2 while under microgravity it is close to zero. F_C is the Coulomb force due to electrical field and F represents the components of all other additional external forces acting on the particle such as thermophoretic force, Brownian force and lifting force. In addition to equation 2, a dispersion model can be used to model the turbulence effects on particles dynamics.

The CFD calculations were performed by using an open source CFD toolbox OpenFOAM (www.openfoam.org). The computational grid was generated with the OpenFOAM native mesher SnappyHexMesh. The air flow inside the cabin was simulated by solving the steady-state Navier—Stokes equations with a Reynolds-averaged (time-averaged) enclosure. The turbulence was modelled using the high-Re RNG k—ε and low-Re Launder—Sharma k—ε turbulence models. A fully convergent solution was not reached until one of the standard parameters in k—ε turbulence models C_μ· was changed from 0.09 to 0.12 according to similar findings of (Smirnov et al. 2006) Columbus module air flow simulations. Increasing the C_μ parameter increases the turbulent viscosity which in turn increases the dissipation of the turbulence. The boundary conditions for the simulations are shown in Table 1. The used grid for high Reynolds and low Reynolds number turbulence models had 5.6 and 21.2 million cells, respectively.

The contaminant concentration dispersion was modelled by solving the equation 2 on top of the presolved air flow field calculated with the Launder—Sharma k—ε turbulence model. The particles used for calculations had a polydisperse size distribution with a count median diameter of 1 micrometer to describe the DEHS particles used in the experiments.

Table 1. The boundary conditions of the air flow simulation. The inlet velocity corresponds to approximately 15 changes per hour ventilation rate.

Inlet boundary condition	Value (uniform over the inlet surface)
U	1.47 m/s
p	zeroGradient
k	0.0324 m^2/s^2
ε	0.754 m^2/s^3

Measurements

A mock-up approximating the Columbus module mock was built at VTT for validation measurements. The volume of the test room is 33 m^3 and it is equipped with similar supply and exhaust distribution than the module. During the experiments the isothermal air flow rates were adjusted to 420 m^3/h corresponding to the values used in the ISS. Like in reality, the supply air was filtered with a HEPA H13 grade filter.

The velocities were measured with an air velocity transducer (TSI model 8465). The transducer was connected to a laptop for data logging via a 16 bit A/D converter (NI 6008). The recorded values were then used for calculating the average velocity. To achieve high position accuracy and to facilitate automated collection of large quantities of data a computer controlled xy-table was used for traversing the velocity transducer. Velocities were measured at different distances downstream the supply air diffuser to cover the region of influence.

In addition to velocity measurements, experiments were made with test particles to study the particle dispersion inside the test room. DEHS particles were generated with a nebulizer and detected using a particle counter (Met One) connected to a PC for data collection. The test particles were fed and measured at different locations in the test room. The settling velocity of one micrometer size particle is 0.035 mm/s (Hinds 1999), while the measured velocities in the test room were at least one thousand times higher. The gravitational effects were therefore insignificant compared to the velocities created by the ventilation, corresponding thus to the situation onboard spacecraft.

Results and discussion

The resulting air flow velocity field for the Launder—Sharma k—ε turbulence model is presented in Figure 3. The calculated and measured velocity profiles downstream the supply air diffuser are presented Figure 4. It is seen that there are fluctuations in the measured velocities but overall the flow patterns are well predicted. The individual jets from the slots merge downstream and this phenomenon is reproduced by the simulations. The location and value of the supply jet velocity maximum is also well predicted.

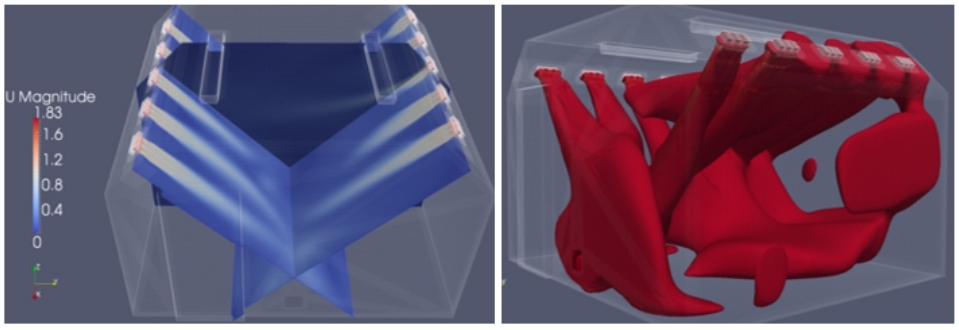


Figure 3. On the left the simulated air flow field is represented by the velocity magnitude cut planes inside the test room at VTT. On the right the simulated velocity magnitude is represented by a contour of 0.12 m/s showing an upward tilt of the jets.

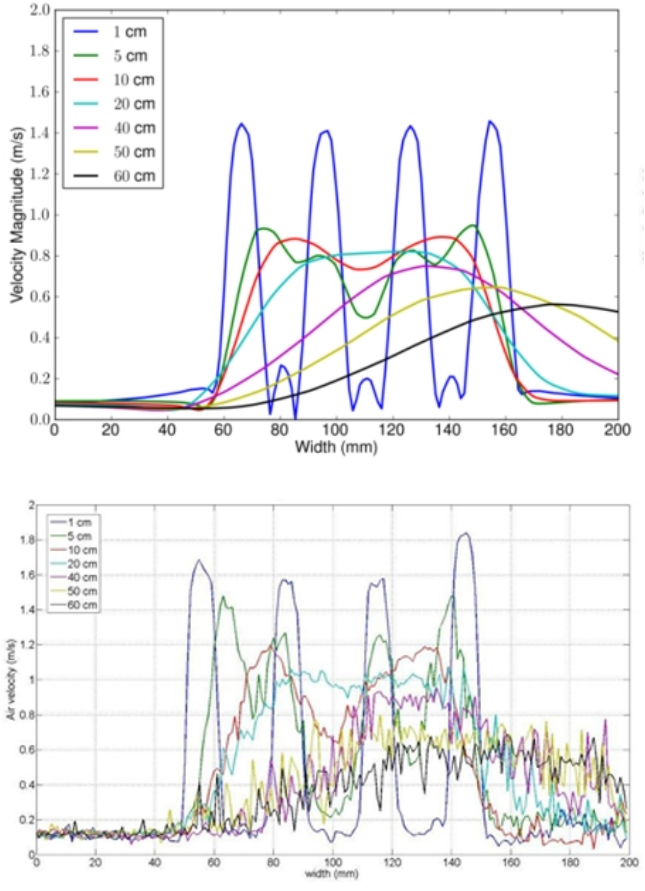


Figure 4. Simulated (upper) and measured (lower) velocities across the supply air diffuser at different distances from the inlet.

Onboard spacecraft the air velocities created by the mechanical ventilation must be high enough to compensate the lack of natural convection from human body in order to maintain thermal comfort. On the other hand, the velocities must not be too high to avoid draught. Therefore the air velocities should be in the range from 7.6 to 20 cm/s in the habitable area (Raatschen 2009). Based on the simulation results shown in Figure 3, this requirement is fairly well met.

The resulting simulated concentration distributions are shown in Figure 5 by relative concentrations (normed to the outlet concentration) contours and cut planes. The relative concentration value “Rel. Concentration = 1” corresponds to the situation where the particles would be uniformly dispersed. Except for the regions near the source, the contaminant concentrations showed relatively small differences as a function of location and the system can be described as well mixed system.

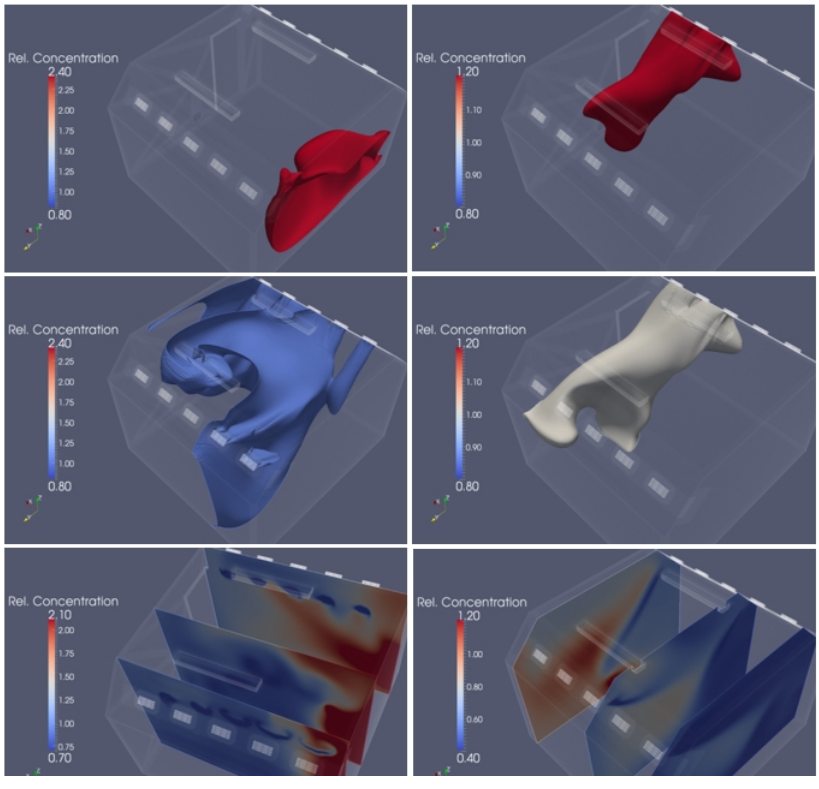


Figure 5. The simulated concentration dispersion from two injection points: near the rear wall (left) and near the side wall (right). The concentration is scaled by the average concentration of the outlet surface so that the value of “Rel. Concentration = 1” corresponds to the uniformly dispersed concentration.

The accurate prediction of mean air flows is a prerequisite for meaningful particle dispersion modelling. Another important factor is the modelling of dispersion due to turbulence. This is very complex phenomena and all the models based on the solving of Reynolds-

averaged Navier Stokes (RANS) equations approximate the turbulent stresses using simplifications. An indication of the turbulence model performance is how well the calculated particle concentrations agree with the predicted ones. However, the simulated and measured steady state concentration levels were fairly uniform across the whole test room so that these measurements do not provide very much information for the validation of the model and other measurements are needed

Another way to assess the performance of the model is to study the dynamic behaviour of particle dispersion. This idea is illustrated in Figure 6 in which a large number of particles are released simultaneously and their tracks are followed. The measured time dependent concentrations at two different measurement points are presented in Figure 7. It is clearly seen how the particles first reach the lower measurement point in the path of average air flow trajectories and then after some time delay the upper measurement point near the supply air diffuser. As seen from the figure, the final concentrations are close to each other within measurement accuracy, suggesting that the air in the test room is well mixed.

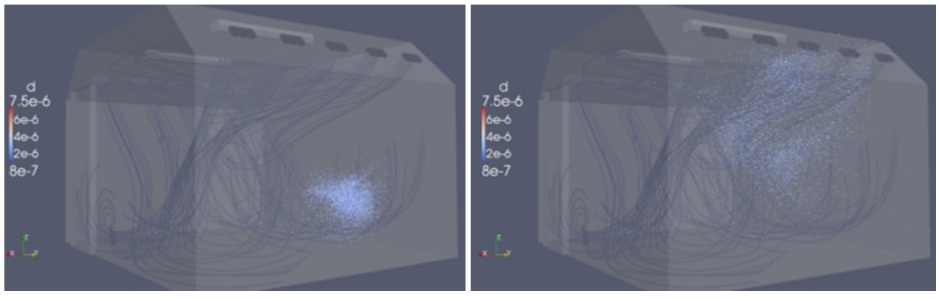


Figure 6. The predicted particle transport in VTT test room represented by snapshots at 20 s and 48 s time points. A number of 10 000 particles were injected from a point in the middle of the rear wall. The full animation can be seen from the project website.

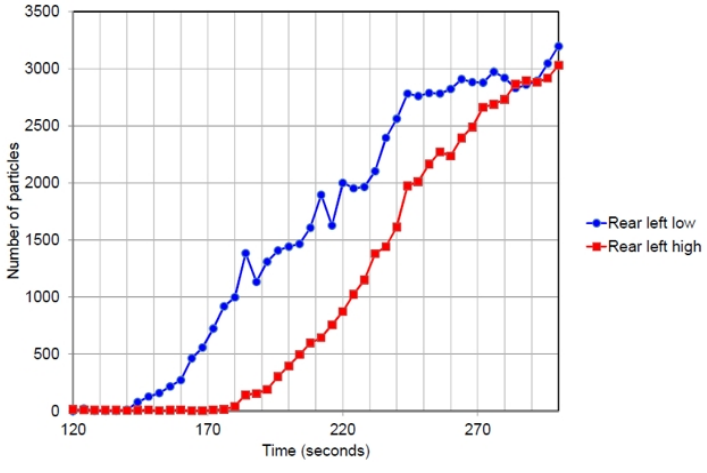


Figure 7. Measured particle concentrations as a function of time at two different measurement points. The particles were injected (started at 120s time point) and measured at rear left low and rear left high positions.

Conclusions

Humans are the main source of microbes isolated from manned space stations. To estimate the risks caused by exposure to potential pathogens and to study the efficiency of countermeasures the dispersion of bioaerosols should be reliably predicted. The results show that the developed CFD model can satisfactorily simulate air movements and particle dispersion in a spacecraft-like confined environment. A reliable model requires, however, accurate and experimentally verified boundary and initial conditions to ensure that the assumptions used in the calculations are valid. The predictions must also be based on sound models that can describe the underlying physical phenomena adequately.

Acknowledgements

The research leading to these results has received funding from the European Union Seventh Framework Programme ([FP7/2007-2013]) under grant agreement n° 263076 within the BIOSMHARS Project (BIOcontamination Specific Modelling in Habitats Related to Space).

The authors would like to thank all the partners of the BIOSMHARS consortium (http://www.biosmhars.eu) including MEDES (the French Institute for Space Physiology and Medicine), the Belgium Nuclear Research Centre SCK-CEN, the Institute of Biophysics of the Siberian Branch of the Russian Academy of Sciences, the Institute of Biomedical Problems (IBMP) of the Russian Academy of Sciences, and the University of Eastern Finland and BioMérieux.

References

Hinds, W. 1999. Aerosol Technology Properties, Behavior, and Measurement of Airborne Particles. Wiley, New York. ISBN 0-471-19410-7.

Raatschen, W. 2009. Life support systems. In: Ley, W., Wittmann, K., Hallmann, W. (eds) Handbook of space technology. John Wiley & Sons, Chichester. Pp. 418–442.

Smirnov, E., Ivanov, N., Telnov, D. & Son, C. 2006. CFD Modelling of Cabin Air Ventilation in the International Space Station: A Comparison of RANS and LES Data with Test Measurements for the Columbus Module. Int. J. Vent. 5(2): 219–227.

Van Houdt, R., Mijnendonckx, K. & Leys, N. (2012). Microbial contamination monitoring and control during human space missions. Planetary Space Sci. 60: 115–120.

http://www.biosmhars.eu/.

Series title and number

VTT Technology 168

Title	**45th R3Nordic Symposium** **Cleanroom Technology, Contamination Control and Cleaning**
Author(s)	Gun Wirtanen & Satu Salo (Eds.)
Abstract	R3-Nordic, the Nordic Society of Cleanroom Technology, is a non-profit, independent association for the promotion of new technologies in cleanroom technology and contamination control in the Nordic countries. The aim of the annual R3Nordic Symposium is to provide knowledge within the pharmaceutical, food and electronic industries as well as hospitals and hospital pharmacies. This year the sessions at the 45th R3Nordic Symposium are Pharma, Hospital and General Sessions and the presentations deal with construction and design, planning, auditing, contamination control, cleanroom technology and management, sterilization techniques, cleaning of clean rooms, protective clothing, monitoring techniques, rapid test methods and regulations in clean and controlled rooms. The venue of the annual symposium 2014 is Naantali Spa in Naantali. The persons involved in the Programme Committee are Satu Salo, Kari Leonsaari, Leila Kakko, Sirkka Malmioja, Antti Mikkola, Raimo Pärssinen and Gun Wirtanen. The editors of the proceedings would like to express their gratitude to the speakers for preparing the abstracts published in the journal Renhetsteknik 1/14 as well as the extended abstracts or full papers published in these electronic proceedings.
ISBN, ISSN	ISBN 978-951-38-8142-9 (USB flash drive) ISBN 978-951-38-8253-2 (URL: http://www.vtt.fi/publications/index.jsp) ISSN-L 2242-1211 ISSN 2242-122X (Online)
Date	May 2014
Language	English
Pages	185 p.
Name of the project	45th R3Nordic Symposium
Commissioned by	R3Nordic – Nordiska föreningen för Renhets-teknik och Rena Rum
Keywords	Contamination control, clean room, clean-room, air, water, hygiene, cleaning, disinfection, cleanroom clothing, body-box test, air-borne contamination, airborne particles, particle measuring techniques, hospital infections, microbiological methods, hygienic design, pharmaceutical industry, hospital, hospital pharmacy
Publisher	VTT PL 1000, 02044 VTT, Puh. 020 722 111

45th R3Nordic Symposium

Cleanroom technology, contamination control and cleaning

R3-Nordic, the Nordic Society of Cleanroom Technology, is a non-profit, independent association for the promotion of new technologies in cleanroom technology and contamination control in the Nordic countries. The aim of the annual R3Nordic Symposium is to provide knowledge within the pharmaceutical, food and electronic industries as well as hospitals and hospital pharmacies. This year the sessions at the 45th R3Nordic Symposium are Pharma, Hospital and General Sessions and the presentations deal with construction and design, planning, auditing, contamination control, cleanroom technology and management, sterilization techniques, cleaning of clean rooms, protective clothing, monitoring techniques, rapid test methods and regulations in clean and controlled rooms. The venue of the annual symposium 2014 is Naantali Spa in Naantali. The persons involved in the Programme Committee are Satu Salo, Kari Leonsaari, Leila Kakko, Sirkka Malmioja, Antti Mikkola, Raimo Pärssinen and Gun Wirtanen. The editors of the proceedings would like to express their gratitude to the speakers for preparing the abstracts published in the journal Renhetsteknik 1/14 as well as the extended abstracts or full papers published in these electronic proceedings.

ISBN 978-951-38-8142-9 (USB flash drive)
ISBN 978-951-38-8253-2 (URL: http://www.vtt.fi/publications/index.jsp)
ISSN-L 2242-1211
ISSN 2242-1211 (Print)
ISSN 2242-122X (Online)

r3 nordic
Nordiska föreningen för Renhetsteknik och Rena Rum