BSI Standards Publication

Cleanrooms and associated controlled environments — Biocontamination control

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National foreword

This British Standard is the UK implementation of EN 17141:2020. It supersedes BS EN ISO 14698-1:2003 and BS EN ISO 14698-2:2003, which are withdrawn.

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Cleanrooms and associated controlled environments - Biocontamination control

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Reinräume und zugehörige Reinraumbereiche - Biokontaminationskontrolle

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European foreword

This document (EN 17141:2020) has been prepared by Technical Committee CEN/TC 243 “Cleanroom technology”, the secretariat of which is held by BSI.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2021, and conflicting national standards shall be withdrawn at the latest by February 2021.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 14698-1:2003, EN ISO 14698-2:2003 and EN ISO 14698-2:2003/AC:2006.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

Clean controlled environments are used to control and limit microbiological contamination where there is a risk to product quality, patient or consumer.

In this document the term “clean controlled environments” is used to cover cleanrooms, clean zones, controlled zones, clean areas and clean spaces.

This document gives guidance on best practice for establishing and demonstrating control of airborne and surface microbiological contamination in clean controlled environments. This document describes the requirements for microbiological contamination control and provides guidance on the qualification and verification of clean controlled environments.

In order to establish microbiological control, it is important to understand the risks of microbiological contamination. This is achieved by considering the sources of microbiological contamination, the associated microbiological concentrations and the likelihood of transfer and the impact on product quality, the patient or the consumer.

A formal system of microbiological control identifies, controls and monitors microbiological contamination on an ongoing basis. This is a process of continuous improvement and the principles of Plan – Do – Check – Act (PDCA) apply, as shown in Figure 1.

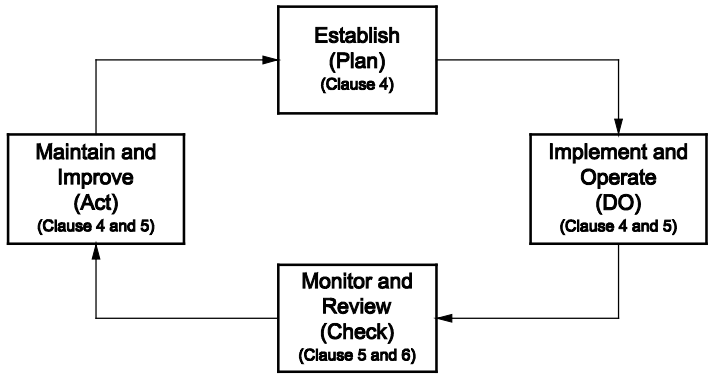


Figure 1 — Application of PDCA as the system for microbiological control

This document provides general guidance and considerations for a number of different applications. It is expected to have particular use in the Pharmaceutical, Biopharmaceutical, Medical Devices and other Life Science industries, as well as in Healthcare and Hospitals, Food, and related applications which use clean controlled environments.

In the regulated Pharmaceutical and Biopharmaceutical manufacturing sector there are already many applicable standards and regulatory guidelines. These include the EU Annex 1 GMP [31] guidance on the manufacture of Sterile Medicinal products and the FDA Aseptic Processing guidance [32]. The European and United States Pharmacopoeias also provide some guidance on certain related topics. There are numerous other documents and technical papers available from industry associations including the Parenteral Drugs Association (PDA), International Society of Pharmaceutical Engineering (ISPE) and Pharmaceutical Healthcare Sciences Society (PHSS). While there are regulations and standards on risk management of medical devices, for example EN ISO 14971 [2], there is less guidance on the microbiological control of clean controlled environments.

In the Healthcare and Hospital sector there are EU Directives, including the Tissue and Blood Directives for specialist and similar clean controlled environments. There are national standards and guidelines for specialised Operating Theatres, Isolation units, Immuno-compromised wards as part of infection

control. In addition, Hospital Pharmacy aseptic compounding units, Radiopharmacies and specialist laboratories such as Stem Cell typically refer to Life Science industry guidance documents.

In the Food and consumer related industries, while there are regulations and standards on food, beverages and cosmetics for example there is insufficient guidance regarding microbiological control in clean controlled environments.

This document includes a number of informative annexes that provide further guidance on biocontamination control in specific applications, and includes, for example:

— tables of microbiological cleanliness levels for monitoring of microbiological contamination in certain types of clean controlled environments;

— guidance in specific areas of microbiological control relating to the choice of environmental monitoring (EM) sampling methods, the management and trending of collected data and the role of alternative and real time microbiological detection systems;

— appropriate methods for establishing control, selecting appropriate alert and action levels and target levels as necessary;

— establishing a microbiological environmental monitoring plan as part of demonstrating control of the clean controlled environment.

1 Scope

This document establishes the requirements, recommendations and methodology for microbiological contamination control in clean controlled environments. It also sets out the requirements for establishing and demonstrating microbiological control in clean controlled environments.

This document is limited to viable microbiological contamination and excludes any considerations of endotoxin, prion and viral contamination.

There is specific guidance given on common applications, including Pharmaceutical and BioPharmaceutical, Medical Devices, Hospitals and Food.

2 Normative references

The following document is referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 14644-1:2015, *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration (ISO 14644-1:2015)*

3 Terms and definitions

For the purposes of this document, biocontamination control and microbiological control are synonymous, and the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia. available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
action level
level set by the user in the context of controlled environments, which, when exceeded, requires immediate intervention, including investigation of cause, and corrective action

3.2
alert level
level set by the user in the context of controlled environments, giving early warning of a drift from normal conditions, which, when exceeded, should result in increased attention to the process

3.3
clean controlled environment
defined zone in which microbiological contamination is controlled by specified means

3.4
cleanroom
room within which the number concentration of airborne particles is controlled and classified, and which is designed, constructed and operated in a manner to control the introduction, generation, and retention of particles inside the room

Note 1 to entry: The class of airborne particle concentration is specified.

Note 2 to entry: Levels of other cleanliness attributes such as chemical, viable or nanoscale concentrations in the air, and also surface cleanliness in terms of particle, nanoscale, chemical and viable concentrations may also be specified and controlled.

Note 3 to entry: Other relevant physical parameters may also be controlled as required, e.g. temperature, humidity, pressure, vibration and electrostatic.

[SOURCE: EN ISO 14644-1:2015, 3.1.1, [1]]

3.5
clean zone
defined space within which the number concentration of airborne particles is controlled and classified, and which is constructed and operated in a manner to control the introduction, generation, and retention of contaminants inside the space

Note 1 to entry: The class of airborne particle concentration is specified.

Note 2 to entry: Levels of other cleanliness attributes such as chemical, viable or nanoscale concentrations in the air, and also surface cleanliness in terms of particle, nanoscale, chemical and viable concentrations may also be specified and controlled.

Note 3 to entry: A clean zone(s) may be a defined space within a cleanroom or may be achieved by a separative device. Such a device may be located inside or outside a cleanroom.

Note 4 to entry: Other relevant physical parameters may also be controlled as required, e.g. temperature, humidity, pressure, vibration and electrostatic.

[SOURCE: EN ISO 14644-1:2015, 3.1.2, [1]]

3.6
colony forming unit
formation of a single macroscopic colony after the introduction of one or more microorganisms to microbiological growth media

Note 1 to entry: One colony forming unit is expressed as 1 cfu.

3.7
critical control point
specific point, procedure, or step in the process at which control can be exercised to reduce, eliminate, or prevent the possibility of microbiological contamination

3.8
critical zone
designated space within the clean controlled environment used to control microbiological contamination

3.9
culturable
having the ability to grow and form colony forming units (cfu), using microbiological culturing techniques

3.10
environmental monitoring
EM
measurement of specified parameters at periodic intervals within a clean controlled environment

3.11
microorganism
entity of microscopic size encompassing bacteria fungi protozoa and viruses

Note 1 to entry: Microbe is synonymous with microorganism.

Note 2 to entry: The use of the term microorganism in this standard includes bacteria, yeast and moulds only.

[SOURCE: ISO 17665-1:2006, 3.25, [50]]

3.12
microorganism of interest
microbiological contamination that has been identified as harmful to the product or the process, or the intended recipient of the product within the clean controlled environment

Note 1 to entry: This includes commonly used terms such as objectionable species, microorganism of concern or Pathogenic microorganisms or specified microorganisms.

3.13
risk assessment
actions to determine the likelihood and consequences of microbiological contamination within the clean controlled environment

3.14
sterile
free from viable microorganisms

[SOURCE: ISO/TS 11139:2018, [51]]

3.15
sterilisation
validated process used to render a product free from viable microorganisms

[SOURCE: ISO/TS 11139:2018, [51]]

3.16
target level
defined level set by the user as a goal for routine operations, for the user's own purpose

3.17
validation
confirmation, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The objective evidence needed for a validation is the result of a test or other form of determination such as performing alternative calculations or reviewing documents.

Note 2 to entry: The word "validated" is used to designate the corresponding status.

Note 3 to entry: The use conditions for validation can be real or simulated.

[SOURCE: EN ISO 9000:2015]

3.18
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

Note 1 to entry: The objective evidence needed for a verification can be the result of an inspection or of other forms of determination such as performing alternative calculations or reviewing documents.

Note 2 to entry: The activities carried out for verification are sometimes called a qualification process.

Note 3 to entry: The word "verified" is used to designate the corresponding status.

[SOURCE: ISO 9000:2015]

3.19
viable
microorganism, alive and either culturable or non culturable

3.20
viable particle
particle that contains one or more living microorganism

4 Establishment of microbiological control

4.1 General

When the clean controlled environment is classed as a cleanroom or clean zone the requirements of EN ISO 14644-1, shall be complied with.

4.2 Establishing a formal system for microbiological control

A system to maintain appropriate microbiological contamination control shall be established, implemented and maintained. The system shall identify, control and monitor factors that can affect microbiological contamination of the product. The outputs of the system shall be documented

There are a number of accepted microbiological contamination control systems that utilise a quality risk management approach [2], [3], [5], [6], [8] [9]; the selected system shall be appropriate and verified.

4.3 Microbiological contamination control system quality attributes

The microbiological contamination control system shall consider the following steps:

a) identification of all potential microbiological contamination sources and routes of contamination in the clean controlled environment, deemed microorganisms of interest;

b) assessment of the risk from these sources and routes and, where appropriate, introduce or improve microbiological contamination control methods to reduce the identified risks;

c) establishment of a monitoring schedule, with valid sampling methods, to monitor the microbiological contamination source, or their control methods or both;

d) establishment of alert and action levels, and where appropriate target levels, with measures to be taken when required, if these levels are exceeded;

e) verification on a continuing basis, that the microbiological contamination control system is effective and meeting agreed performance parameters by reviewing product contamination rates, environmental monitoring results, risk assessment methods, control methods and monitoring limits and, where appropriate, modify them accordingly;

f) establishment and maintenance of appropriate documentation;

g) education and training of all staff involved with the clean controlled environment.

4.4 Identification of all potential sources and routes of microbiological contamination

4.4.1 General

Before the risk assessment process can start the nature of the process should be investigated and understood.

All potential microbiological contaminants, and their routes of transfer, that pose a risk to the product, patient or consumer shall be identified.

Microbiological contamination can come from people and what they wear, materials, equipment, services and processes, the physical condition of the facility and surrounding environment as well as the supply air, airflow patterns and movement within the clean controlled environment, and ongoing cleaning. When there is a risk of product or process contamination from particular types of microorganisms these can be considered as microorganisms of interest.

Microorganisms of interest shall be identified during the risk assessment process.

The following factors should be considered as part of the risk assessment:

a) clean controlled environment application, (e.g. pharmaceutical, medical device, food, cosmetics);

b) microbiological species, (e.g. survival possibility, or associated toxins);

c) potential for causing microbiological contamination of the product and/or harm to the intended recipient, (e.g. spoilage of product prior to end of shelf life in food);

d) product form (e.g. does the product contain preservatives, or any potential growth substrates that may prevent growth);

e) intended product target population (e.g. patient, infant, immuno-compromised recipient);

The presence of moulds and other microbiological contamination, including microorganisms of interest, can be indicators of poor cleaning or poor design and increase the risk of product and/or process contamination.

Arising from a risk assessment, action and alert, and if appropriate target levels for routine monitoring can be set for total microbiological concentrations without reference to the microorganisms of interest or by consideration of both.

When the initial qualification of a new non-operating premises or where the activity is not yet representative of normal operation, microbiological contamination may not be sufficiently representative. It may therefore be necessary to re-evaluate the risk in normal operation.

4.4.2 Sources of microbiological contamination

4.4.2.1 General

Sources of microbiological contamination can be prime and derived or associated.

4.4.2.2 Prime sources

The following are examples of prime sources:

— People - A major source of contamination;

— Supply Air - Air supplied into clean controlled environments. (re-circulated or fresh make up);

— Product Materials - Product in solid or liquid form, containers and packaging;

— Utilities - Compressed air, nitrogen, propane, oxygen, WFI;

— Machines - Processing and packaging equipment.

4.4.2.3 Derived or associated sources

The following are examples of derived or associated sources:

— Air Within - Air within clean controlled environments;

— Contact Parts- Product contacting parts such as pipework and closure hoppers;

— Surfaces - Clean controlled environment floors, walls, workstation surfaces, barrier gauntlets, trolleys, buckets, balances, disinfectant containers, monitoring devices;

— Adjacent Areas - Change rooms, corridors, pass through transfer hatches.

4.4.3 Routes of transfer of microbiological contamination

There are 3 routes of transfer of microbiological contamination to the product or critical zone in a clean controlled environment:

— airborne deposition;

— surface contact;

— liquid.

NOTE The transfer of potential sources of microbiological contamination via the liquid route is not part of the scope of this document.

4.5 Risk assessment

A fundamental part of quality risk management is risk assessment. There are a number of ISO standards that address the subject of quality risk management and risk assessment in different applications. IEC 31010 [28] gives a list of verified risk assessment methodologies.

The hazard analysis critical control point (HACCP) system [4], [5], [6], [7], fault tree analysis (FTA) [8], failure mode and effect analysis (FMEA) system [9], or any other verified system can be used. IEC 31010:2019 [28] provides more information on risk assessment methods

Risk assessment shall be carried out to identify, assess, eliminate, where possible and control microbiological contamination risks that have a detrimental impact on product quality, patient or consumer. Risk assessment shall identify the variables in the clean controlled environment that have to be monitored and what microbiological contamination needs to be measured, see 4.3, Microbiological contamination control system quality attributes.

The results of the risk assessment shall be documented and include a scientific rationale for decisions taken in relation to mitigating risks and residual risk. The results of the risk assessment shall be reviewed regularly as part of on-going quality management, during change control and during the periodic product quality review of the microbiological control programme.

4.6 Establishment of microbiological environmental monitoring plan

4.6.1 General

A microbiological environmental monitoring plan shall be established that specifies the types of measurements to be taken as well as the location and frequency, specify appropriate measured levels that should not be exceeded, and specify the actions to be taken when the levels are exceeded.

The microbiological control system shall specify the associated measurement methods.

There are a number of industry guidelines and ISO standards that address the subject of microbiological environmental monitoring in different applications. Refer to informative Annexes A, B, C and D as well as the Bibliography.

4.6.2 Monitoring locations

The monitoring locations in the clean controlled environment and the associated number shall be determined as part of the risk assessment and related to the degree of risk.

Personnel gowning and material transfer airlocks as well as product pass through hatches shall be carefully considered and may require proportionally more monitoring because of the higher risk associated with the materials and personnel that are moving into the clean controlled environment.

The risk assessment should take into consideration the type of product manufactured and the nature of the manufacturing process and/or activity undertaken in the clean controlled environment e.g. a terminally sterilised product or a process with a low contamination risk to the patient or consumer will require less microbiological environmental monitoring than an aseptically prepared product or process.

The application of the Plan-Do-Check-Act continuous improvement process may revise the selection of the optimum microbiological monitoring locations and associated number, as a result of the microbiological control information gathered.

4.6.3 Monitoring frequencies

The frequency of sampling shall be related to the degree of risk and shall be specified in the microbiological environmental monitoring plan as either continuous or periodic, daily, weekly, monthly or another agreed period. The frequency of sampling shall be specified for each source, or its control method, or both.

In setting the frequency of sampling it should be recognised that too frequent sampling could potentially introduce further risk due to possible intrusion of sampling personnel into a critical zone. It is necessary to strike a balance between sufficient sampling and the potential introduction of contamination and/or taking the necessary control steps to reduce contamination risks.

4.7 Establishment of alert and action limits

The microbiological environmental monitoring plan shall specify the levels of measured concentrations of microorganisms of interest in air and on surfaces that should not be exceeded. Alert and action limits shall be set with the alert levels set lower values than the action level. Target levels can also be established as appropriate. An alert level shall be used to indicate that the microbiological contamination is higher than expected and give an early warning of potential loss of control.

The microbiological environmental monitoring plan shall specify the alert conditions under which action is to be taken.

When an action level is exceeded immediate investigation is required to identify the cause and, if necessary, corrective action. The microbiological environmental monitoring plan shall specify what action is to be taken as a result in order to regain microbiological control.

It is necessary to carry out microbiological monitoring for a period of time in order to set alert and action levels that are not continuously exceeded.

In some highly contained clean controlled environments the microbiological contamination can be very low and may not conform to normal distributions. In such cases parameters such as average or standard deviations may not be suitable to establish action and alert levels. Instead levels that are likely to be exceeded at defined frequencies should be considered more appropriate for setting action and alert levels.

4.8 Establishment of documentation system

The microbiological control system and associated environmental monitoring plan as well as the reporting requirements shall be documented, regularly reviewed and updated, as necessary to incorporate any changes implemented.

Reports shall provide a review and analysis of the microbiological environmental monitoring results and any deviations from the expected results. When action levels are exceeded these shall be reported, as well as the actions taken to correct the deviations, or the explanations as to why no action was necessary.

NOTE In some cases, alert levels can also be reported, particularly those associated with a multiple or unusual occurrence.

4.9 Personnel education and training

Personnel shall be competent and have the necessary education, experience, skills and training to ensure performance of their assigned functions. Personnel shall perform only those activities for which they are qualified and authorised to carry out.

All personnel shall be trained and retrained as specified in order to perform their assigned responsibilities adequately.

Training records shall be maintained.

Guidance on cleanroom operations, including personnel gowning and behaviour is given in ISO 14644-5 [26].

5 Demonstration of microbiological control

5.1 Trending

The results of microbiological environmental monitoring shall be analysed and the data trended, in particular to identify any adverse trend.

Resulting trends should be analysed at intervals that are frequent enough to identify any adverse trends that might need corrective or preventive action.

In a number of applications, including non-sterile manufacturing and food an adverse trend is considered more important than an individual result.

Although statistical analysis of data (line graphs, Pareto charts) is important, there is no one algorithm that will identify all adverse trends. A suitably competent microbiologist should review the data to identify any possible issues, for example an unexpected quantitative or qualitative shift in the types of microorganisms recovered due to changes such as seasons, disinfection programs, operator population, training, raw materials and/or facility controls.

5.2 Verification of the formal microbiological control system

5.2.1 General

The results of microbiological environmental monitoring shall be examined periodically in order to confirm that the microbiological control system chosen is functioning in accordance with the established procedures and the specified requirements have been fulfilled, see 4.3 e).

During the initial implementation of the microbiological environmental monitoring plan, the original limits may change as the data from routine monitoring becomes available for review.

If verification indicates deviations from the established limits or a change in the microbiological control of the clean controlled environment, corrective action shall be initiated. If appropriate, the formal microbiological control system shall be modified.

NOTE Corrective action could require use of microbiological environmental monitoring and auditing methods, procedures and tests, including random sampling and analysis. It could also require the systematic verification of all working steps and equipment to ensure the proper functioning of the formal system.

5.2.2 Out of specification (OOS) investigation

The objective of investigating an OOS result is to determine if there has been a real change in the occurrence of microbiological contamination.

To maintain control over the performance of the microbiological control system prompt investigation of out-of-specification results shall be carried.

Each occurrence of an out-of-specification (OOS) test result requires evaluation to decide if it was a true result. It is essential that any out-of-specification result that cannot be confirmed as a testing error should be investigated to determine the cause and appropriate corrective action.

5.2.3 Records

All regular and periodical checks of methods, instruments and internal audits, as well as records of original observations, calculations, derived data and final reports should be appropriately filed and retained for an agreed period. It is essential that the records include the identity of personnel involved in the monitoring, preparation, testing, evaluation and reporting. It should be possible to conduct an audit trail to show the details of how and when any results have been changed. Records of signatures, initials or signs should be maintained and updated as appropriate.

It is essential that appropriate protection of data and records, including any electronic records held on a computer, be provided.

5.2.4 Sample tracking

The sample analysis laboratory should have suitable and dependable procedures that allow for the clear identification and handling of samples from microbiological environmental monitoring. This shall cover their reception and progress through the entire analytical process to the final results and their correct identification with the original monitoring location.

5.2.5 Integrity of results

In order, to avoid collection of erroneous results, the following factors shall be considered as part of collection of results:

a) application;

b) identification of application-specific parameters;

c) data collection locations;

d) limit of detection and sensitivity of test measurement system;

e) data collection.

5.2.6 Data recording

To ensure that all information is readily available, clear procedures for data recording and handling shall be developed and implemented, and the following aspects should be considered:

a) raw data;

b) list of types of information held in the records;

c) identification and location of laboratory documents, or computerised records;

d) use of workbooks, worksheets or computers or other appropriate means to record the various types of observations, calculations and other relevant information;

e) procedures to be followed for recording, checking, correcting, signing and countersigning of observations, calculations and reports;

f) recommendations for consistent interpretation;

g) specific, legal or regulatory requirements.

5.2.7 Data evaluation

Data evaluation shall be carried out in an agreed manner.

Before statistical calculations can be performed on results, where many observations have been recorded, consideration should be given to condensing and grouping the data; this may be done in a qualitative way by grouping the measurements to form frequency tables and charts or by using descriptive statistics.

The data to which statistical methods can be applied may be individual measurements or counts of the number of elements that possess specific attributes.

For each evaluation a test procedure is required that describes the approach taken to develop the test method and the statistical techniques used to verify the test procedure. The agreed test procedure should be verified by either being in a standard or published in a peer-reviewed scientific journal or book.

The application of any statistical technique involves the extrapolation from the sample to the microbiological population of the risk zone from which the sample is drawn. Such extrapolations involve risks, since the sample may not accurately reflect the microbiologically contaminating population. This risk shall be quantified and reduced to an acceptable level by using probability sampling and application of statistics. [2] [3]

Interpretation and evaluation of results should be based on more than one statistical method.

The selection and use of statistical methods for monitoring and verification are not described in this standard and are referred to in the Bibliography.

5.2.8 Trend analysis

Data coming from a single sample are often not significant; furthermore, microbiological monitoring techniques can have a degree of variability. Therefore, graphic presentation of the results collected over a period of time should be considered in distinguishing sampling variation from trends, or in indicating that a significant change has occurred, even though the results fall within the specified limits.

Control chart methods may be applied to provide an objective and statistically valid means [2] [3] [4] [5] [6] [8] [9], to assess the quality of the risk zones and are particularly applicable to monitoring. In the verification step sampling for batch acceptance purposes can be applied as another quality control technique. Charting by means of Shewhart control charts [4], control charts 'based on range' or 'cumulative sum charts' should be considered [5] to measure the deviation from usual random spread and to highlight out-of-specification results.

6 Microbiological measurement methods

6.1 General

As part of the microbiological contamination control process, measurement of concentrations of microorganism on surfaces and in air, and their identification, shall be carried out to enable assessment of the effectiveness of the control system.

There are numerous methods available for the collection, enumeration and identification of airborne and surface microbiological contamination. For the purposes of this standard, the methods are limited to established culture based procedures.

Refer to informative Annex E for further information on these culture based methods.

This does not preclude the additional use of alternative methods that may enhance the understanding of the state of control of the clean controlled environment or provide other advantages for certain applications. Such methods may require further technical development and validation to facilitate their acceptance for use. It should be noted that some of these alternative methods do not offer a means of culturing the microorganisms collected to enable identification and some only provide identification without the ability to enumerate.

Refer to informative Annex F for further information on alternative methods.

6.2 Choice of sampling method

The sampling method selected shall be appropriate for the clean controlled environment to be monitored and shall take into consideration the following:

a) time and duration of the clean controlled environment activities;

b) accessibility into the clean controlled environment for the sampling device;

c) effect of the sampling device on the process or environment to be monitored;

d) efficiency and precision of the sampling method.

6.3 Volumetric air samplers

A suitable sampling device shall be selected based on user requirements and verified against the manufacturer's specifications,

The supplier of the sampler shall demonstrate the collection efficiency of the sampler.

The sampling techniques shall be validated.

The volumetric air flow of the sampler shall be periodically calibrated, see E.6.

6.4 Culture media and incubation

The quality of the media shall be subject to a suitable verification programme and shall take into consideration the following:

a) justification of the selected media and associated incubation conditions and validation to demonstrate detection of low levels of microbiological contamination, the indigenous microbiological contamination and any microorganisms of interest;

b) establishment of controls to ensure media will not contaminate the clean controlled environment or give false counts;

c) media suitability (media sterility and ability to support growth);

d) use of neutralisers in media used for surface sampling to ensure that disinfectant residues on surfaces do not suppress the growth of the microorganisms sampled;

e) validation of the actual condition of the media used for the sampling of air to ensure that-dehydration, due to the air that flows over them, does not influence the growth promoting properties of the culture medium;

f) ability of the media to detect fungi as well as bacteria.

6.5 Incubators

The incubator shall be appropriately qualified before use and operation. Requalification should be carried out periodically or following service or other changes to the incubator. The temperature range in the incubator chamber shall be mapped to ensure that all locations attain and maintain the required incubation temperatures to ± 2,5 ^{0}C of the target temperature. This qualification should be carried out at least once every three years.

During routine use the temperature within the incubator shall be continuously monitored throughout the incubation period from a representative location and an alarm issued if there is any deviation from the required incubation temperatures.

Annex A
(informative)

Guidance for life science pharmaceutical and biopharmaceutical applications

A.1 Introduction

There are many regulations applicable to the Life Science, Pharmaceutical and Biopharmaceutical industry.

There are multiple guidance documents and regulations that address microbiological control in clean controlled environments, including the following non-exhaustive list:

1) European Commission EudraLex "The Rules Governing Medicinal Products in the European Union" Volume 4, EU Guidelines for Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use, Part II: Basic Requirements for Active Substances used as Starting Materials. Brussels [30].

2) European Commission EudraLex "The Rules Governing Medicinal Products in the European Union" Volume 4, EU Guidelines for Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use, Annex 1 - Manufacture of Sterile Medicinal Products [31].

3) FDA Guidance for Industry - Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice [32].

4) Parenteral Drug Association (PDA) Technical Report (TR) No. 13 - Fundamentals of an Environmental Monitoring Program [33]

5) PDA Technical Report No. 69 - Bioburden and Biofilm Management in Pharmaceutical Manufacturing Operations [34]

6) PDA Technical Report No. 70 - Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities [35]

7) Pharmaceutical Microbiology Manual (PMM) Pharmaceutical Inspection Convention (PIC/S) PI 007-6 "Recommendation on the Validation of Aseptic Processes"[36]

8) United States Pharmacopeia (USP) <1116> Microbiological Control and Monitoring of Aseptic Processing Environments [37]

9) USP <1072> Disinfectants and Antiseptics [38]

10) Code of Federal Regulations (CFR) Title 21, Volume 8. Cite 21 CFR 820.70 - Production and Process Controls:

    a) 21 CFR 211.42 [39]

    b) 21 CFR 211.25 [40]

    c) 21 CFR 211.28 [41]

    d) 21 CFR 211.113 [42]

This document is intended to serve as a supplement to the body of existing guidance documents. This standard is intended to offer good science in microbiological contamination control and encourage regulators to refer to the new standard for viable microorganisms and microbiological contamination control.

The normative section of this standard gives clear guidance and resolve conflicts and confusion in specific areas of microbiological contamination control such as choice of environmental monitoring (EM) sampling methods, how to use the data collected for trending.

Annex F gives a summary of the evolving technologies in microbiological contamination control and in particular gives guidance on the appropriate use and role of rapid microbiological measurement (RMM) or Instantaneous Microbiological Detection (IMD).

There are several reference guidance documents from industry associations including:

1. PDA Technical Report No. 13 [33] - Fundamentals of an Environmental Monitoring Program;
2. ISPE Baselines Guides:
   - Sterile Product Manufacturing Facilities [52];
   - Risk-Based Manufacture of Pharmaceutical Products (Risk-MaPP) [53];
   - Oral Solid Dosage Forms [54];
   - Biopharmaceutical Manufacturing Facilities [55];
3. PHSS Technical Monograph
   - No 20 Bio-contamination [47].

A.2 Risk/impact assessment

For risk/impact assessment see 4.5.

Risk/impact assessment of the pharmaceutical application is given in EU GMP Annex 1 [31] guidance. Critical zones, referred to as Grade A, B, C and D are given for every stage of the manufacture of Sterile Medicinal Products.

Should the results of any OOS investigation indicate that a root cause analysis is required a Corrective Action Preventative Action (CAPA) should be used. Such CAPA should be defined in terms of microbiological impact to the safety and efficacy of the product. The efficacy of any corrective/preventive actions should be verified.

A.3 Demonstrating control

For demonstrating control see Clause 5.

The limits for the different zones or grades are given in EU GMP Annex 1 [31].

Limits and measuring results can be expressed as:

- air samples: cfu/m^3;
- settle plates: cfu/Ø 90 mm/4 hr;
- contact plates: Ø 55 mm and cfu/plate.

Annex B
(informative)

Guidance for life science medical device applications

B.1 Introduction

This Annex provides guidance on the microbiological environmental monitoring in production areas for the manufacture of medical devices. EU Medical Devices Directives and associated regulations require a risk assessment of an infection in the recipient by the medical device and that risk should be eliminated or minimised as much as possible.

The requirements for patient safety and the confidence of the patients and healthcare professionals determines the extent of microbiological contamination control in the clean controlled production environment. The manner in which the medical device is used determines the accepted level of microbiological contamination and the type(s) of microorganism of interest. And because most medical devices are terminally sterilised careful consideration should be given to the risks from certain microorganisms, for example the presence of endotoxins or microorganism sporulation.

In addition, the use of preventive antibiotics can be reduced with better microbiological contamination control of medical devices up to the point of use.

While there is no specific guidance on microbiological environmental monitoring in existing medical devices standards and regulations there is good reference guidance in Life Science Pharmaceutical and Biopharmaceutical regulations, including:

— FDA guidance - aseptic processing [32];

— EMA/PIC/s Annex 1 – sterile medicines [31];

— EN ISO 13408-7 – aseptic processing of healthcare devices [46].

This Annex addresses the gap in guidance for terminal sterilised medical devices.

Endotoxins and pyrogens are not within the scope of this document.

B.2 Risk assessment

B.2.1 General

EN ISO 14971 [2], dealing with medical device risk assessment and EN ISO 13485 [16], dealing with medical device quality management are relevant documents to use as part of microbiological control.

In order to determine the different areas in the manufacturing facility which have, either a direct impact or an indirect impact from surrounding areas, it is important to analyse the process flow and the production steps. Refer to 4.5 for requirements for risk assessment.

The following factors should be considered when assessing the impact of microbiological contamination:

— intended use/ how to be used;

— patient population (microorganisms of interest);

— implanted and the location of implantation (microorganisms of interest);

— supplied sterile;

— sterilised by the user and what is the method of sterilisation;

— type of disinfection/agents;

— other applicable microbiological contamination controls;

— time, size and area of the medical device that is exposed to the environment, in air and on surfaces.

Based on an understanding of the nature and impact of microbiological contaminants on the medical device the following steps should be carried out:

a) document the manufacturing process steps and locations where microbiological contamination can occur;

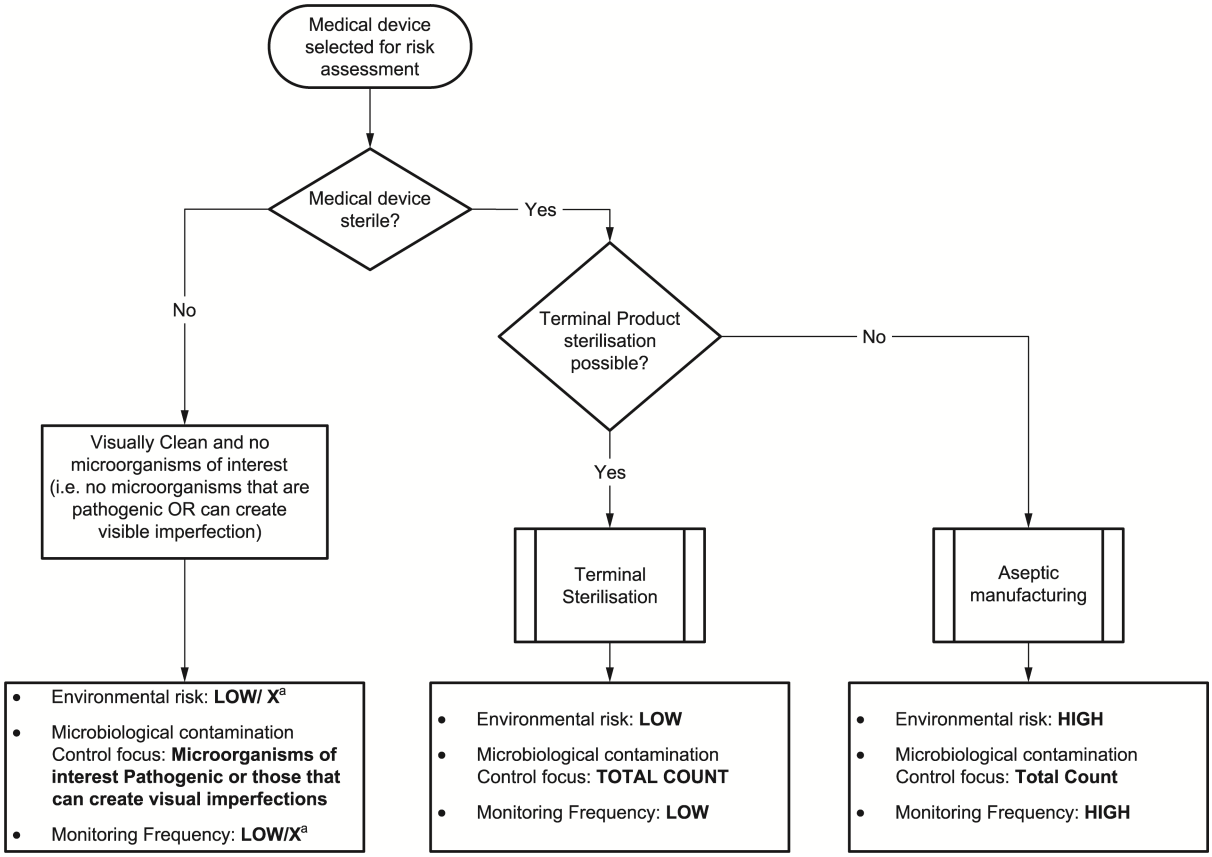
b) identify the risks likelihood of occurrence and impacts, based on the risk assessment requirements in EN ISO 14971 [2];

c) identify the appropriate microbiological controls to eliminate, reduce or minimise the impact to the medical device;

d) design an appropriate microbiological environmental monitoring (EM) plan.

The overall objective is to minimise as much as possible microbiological contamination or "bioburden" on the product.

See Figure B.1 for an example of risk assessment considerations for sterile and non-sterile products.



The factor "X[a]" depends on the origin of the microorganism of interest, (human or environmental) and the relationship to the type of production (automatic or manual); the risk may be different. Total count is a secondary consideration.

Figure B.1 — Example of a microbiological risk assessment for a medical device product

While detailed examples, which cover all types of medical devices and manufacturing methods are difficult to give the following three examples are provided as guidance:

1) sterile - terminal sterilisation after the manufacturing process is complete, see B.2.2;

2) sterile – terminal sterilisation is not possible B.2.3;

3) non-sterile B.2.4.

B.2.2 Example 1: Sterile - terminal sterilisation is possible from a packaged product

B.2.2.1 General

For this example, the product is a sterile device for subcutaneous or intravenous use.

B.2.2.2 Risk Assessment

The sterilisation process gives a minimum 6 log reduction in microorganisms and spores.

Endotoxins may be present in sterilised products and can cause fever in the recipient of the product. Endotoxins are related to the cell structure of Gram negative rods and are an important factor in personal hygiene.

B.2.2.3 Microbiological control method

Maintaining control of the total microbiological count in the production environment is sufficient and is based on the correct technical performance of the cleanroom facility.

In addition, personnel hygiene control, during operations prevents the transmission of Gram negative rods.

B.2.2.4 Suggested microbiological monitoring plan

Suggested microbiological monitoring plan should cover total count using active air sampling and surface sampling using settle plates should be carried out at a low frequency, for example monthly, quarterly or twice per year, depending on the production activity.

B.2.3 Example 2: Sterile – No terminal sterilisation is possible due to product properties

B.2.3.1 General

For this example, the product contains active ingredients or skin tissue for transplantation using manual methods.

B.2.3.2 Risk assessment

Every microorganism is a potential risk.

B.2.3.3 Microbiological control method

Besides the control of the environment (air, surfaces) all actions in the room should be seen as a potential risk for microbiological contamination. This also includes people and materials coming into the clean controlled environment during production.

B.2.3.4 Suggested monitoring plan

Suggested microbiological monitoring plan should cover the total count using both active air sampling, and surface sampling using settle plates and as necessary contact plates. And because personnel are considered a high risk factor, fingertips and personnel gowning should be part of the microbiological environmental monitoring plan (refer to Table B.1 and Table B.4) using contact plates and swabs.

In addition, microbiological contamination of incoming materials should also be considered, for example sampling every production shift.

Microbiological monitoring should be carried out at a high frequency, for example per shift or daily, depending on the production activity.

B.2.4 Example 3: Non-sterile products

B.2.4.1 General

Examples include skin products such as dressings or plasters or an oral medication.

B.2.4.2 Risk assessment

Type of pathogens depends on intended use on the human body.

Skin products: microorganisms causing skin infections are a risk: for example, mould or Staphylococcus aureus. The skin-microbiome will protect the receiver for most other microorganisms.

For oral medication, food pathogens are a risk. The microbiome in the mouth and the digestion will protect the receiver for most other microorganisms.

B.2.4.3 Microbiological control method

Normal hygienic procedures control the total count. Monitoring on total count (high levels) will control the implementation of the procedures.

Baseline study for individual microorganisms in the rooms will give guidance for the presence of specific microorganisms of interest. When followed by root cause research the hazard/risk for the presence of specific microorganism of interests can be implemented.

B.2.4.4 Suggested microbiological environmental monitoring (EM) plan

Control the total count with regular sampling: air, surfaces and settle plates on monthly basic. If the risk for individual microorganisms of interest cannot be reduced or controlled in the production process, then sampling with selective methods is recommended to control those microorganisms, for example mould Staphylococcus aureus or Coliforms. The Frequency depends on the risk factor, for example the origin of the microorganism of interest, human or environmental has an influence.

B.3 Establishing Microbiological Control

B.3.1 Microbiological contamination limits

There are no microbiological limits given in EU Directives for the environmental monitoring of contamination during manufacturing of medical devices.

Table B.1 can be used for microbiological monitoring of medical devices in clean controlled environments, during operations.

Table B.1 — Recommended limits for microbiological contamination monitoring for clean controlled environments during medical devices manufacture

Category	**Air sample** cfu/m^3	**Settle plates (diameter 90 mm)** cfu/4h [a]	**Contact plates (diameter 55 mm)** cfu/plate	**Glove print 5 fingers** cfu/glove
1 [b]	< 1	< 1	< 1	< 1
2 [b]	10	5	5	5
3	100	50	25	N/A
4	200	100	50	N/A

Appropriate alert and action levels should be set for microbiological monitoring based on a risk assessment and environmental monitoring results over time. If these levels are exceeded operating procedures should specify the corrective action required.

Endotoxins and Pyrogens, while not in the scope of this standard, should be considered during risk assessment of certain medical devices such as sterilised implantable devices.

NOTE These are average values of culturable viable microorganisms.

[a] Individual settle plates may be exposed for less than 4 h, based on a risk assessment.

[b] Limits, for this category are relevant to aseptic processing of sterile medical devices like tissue products. Refer to EN ISO 13408-7 [46].

Baseline microbiological contamination levels in the “At Rest” state and then “Operational state” should be established in order to set alert and action levels. The microbiological environmental monitoring plan should specify what objectionable species should be monitored and/or the total cfu count as well as how the results should be evaluated, particularly adverse trends and corrective action required.

B.3.2 Additional microbiological control considerations

Facilities used for the production, assembly and packaging of medical devices differ considerably in size and configuration, depending on the nature of the medical device.

In addition to the Normative part of this standard particular consideration should be given to:

— Floors, walls & ceiling finishes and access to for cleaning and maintenance; both above and at working height level. As it is common to have different working height levels in one cleanroom, e.g. platforms, special consideration should be given to this;

— HVAC facilities for clean air, differential pressure, temperature and relative humidity control;

— Identification of isolates in objectionable species of interest.

B.4 Demonstrating microbiological control

B.4.1 Enumeration as part of measurement methods (Clause 6)

It is generally accepted that, as with other traditional indirect measurement methods, the estimation of microbiological contamination levels can be influenced by instruments and procedures used to perform these counts. Therefore, the enumeration of viable particles from the samples should be performed only by appropriately validated methods. Refer to the EN ISO 18593 [17] for further guidance and how to handle overgrown plates and swarming colonies.

B.4.2 Methods for sampling

The following methods can be used for sampling.

— Active air samplers - to sample air for airborne microorganisms, the sample volume of an active measurement should be at least 100 l (or more, depending on the level), see Table B.3 for recommended sampling frequencies for routine monitoring.

— Settle plates - the manufacturer‘s recommended maximum exposure time should be observed, typically 4 h as otherwise the agar will dry out.

— Surfaces are sampled using contact plates or sterile swabs, secondary or cross contamination should be avoided when sampling.

Necessary controls should be included to ensure the viability of the collected microorganisms during transport and storage.

B.4.3 Microbiological Environmental Monitoring (EM) plan

B.4.3.1 Number of measuring locations

The monitoring locations should be determined based on risk assessment and as per 4.6.2. For each clean controlled environment locations both active air and surface sampling should be considered.

If there are no other criteria in the risk analysis defined, then the number of the measuring locations should be:

— Active air samples: N/3 with a minimum of one, see Table B.2;

— Surface samples: 3 +N/3 on each work area.

NOTE N is the minimum number of determined locations according to EN ISO 14644-1:2015, Table A.1 [1].

Table B.2 — Minimum number of sample locations for active air monitoring in cleanrooms

Cleanroom area m^2	Minimum number of locations for active air measurement
≤ 8	1
> 8 ≤ 28	2
> 28 ≤ 52	3
>52 ≤ 68	4
>68 ≤ 104	5
>104 ≤ 148	6
>148 ≤ 232	7
>232 ≤ 436	8
>436 ≤ 1 000	9
> 1 000	Determine using Formula B.1

$$N_L = \frac{\left(27 \times \left(\frac{A}{1\,000}\right)\right)}{3} \qquad \text{(B.1)}$$

where

N_L is the minimum number of sampling locations to be evaluated, rounded up to the next whole number;

A is the area of the cleanroom in m^2

NOTE This is a modified formula from EN ISO 14644-1 [1].

B.4.3.2 Sampling frequencies for active air sampling

Table B.2 Sampling frequencies for routine active air sampling based on the classification of cleanrooms per EN ISO 14644-1

Table B.3 — Sampling frequencies for routine active air sampling based on the classification of cleanrooms per EN ISO 14644-1

ISO Class according EN ISO 14644-1	Monitoring (routine)
5	By shift
6	Working day
7	Weekly
8	Monthly

B.4.3.3 Monitoring of personnel

Table B.4 is an example of how to monitor the microbiological control of a clean zone for a product with a high-risk potential and where the person has a significant influence on the quality of the product.

It focuses on the person, the working space and the air around this specific clean zone. The person's hairline should be used as a positive control point, for the media control. There should be at least one control point location in every cleanroom or in each clean zone. This type of monitoring would provide particularly important feedback as part of the training of new cleanroom staff.

Table B.4 — Example of microbiological sampling of a person working in cleanroom

Microbiological examination of a work area:		**cfu / plate**	**cfu / dm²**	**Result**
Environment:	Central area: Right hand work area: Left hand work area:			
Person / Hands:	Palm right: Thumb / index finger right: Palm left: Thumb / index finger left:			
Person / Clothing:	Chest area:			
Floor of work area:				
Inner surface of container (work area) used:				
Positive control sample (outside of cleanroom)				
Person / Forehead:	Hairline:			
Sampling of hairline should be done outside of the cleanroom and used as a positive control				
Microbiological examination of air (active air sampling):				
cfu / plate:	**cfu /[m³]:**	**Result:**		
Microbiological examination of air via sedimentation (passive air sampling):				
cfu / plate:	**Result:**			

B.5 Other informative annexes for Medical Device applications

The borderline between a medicinal product and a medical device is sometimes difficult to determine. Therefore, Annex A can be useful for medical devices where there is a high risk and impact of microbiological contamination.

In addition, Annex D can also be very useful because of the microbiological control methods in food manufacturing for microorganisms of interest.

Annex C
(informative)

Guidance for healthcare/hospital applications

C.1 Introduction

Clean controlled environments are very important in healthcare and hospitals within high risk areas such as, but not limited to operating rooms, immuno-compromised wards, isolation areas, prenatal wards, biosafety laboratories, and other laboratories including tissue and blood laboratories.

Microbiological control in operating rooms for infection-prone surgery has long been established. Lidwell et al. (1983) [29], recommended that that air in the wound area, on average, should contain no more than 10 cfu/m^3 during ongoing surgery. Today this value is internationally accepted for surgery susceptible to infections, e.g. orthopaedic and trauma surgery. The recommendations for general operating rooms during ongoing surgery are mostly less than 100 cfu/m^3.

When critical process steps are carried out, following the Tissue Directive, for example bone marrow work, this could be performed in a microbiological safety cabinet (MBSC) within the operating theatre room.

Specific relevant reference documents to healthcare and hospital applications include:

— EN ISO 14644 series on cleanrooms and associated controlled environments [1], [23], [24], [25], [26], [27];

— EU Annex 1 GMP guidance documents on the manufacture of sterile medicinal products [31];

— WHO Guidance on Surgical Site infections 2016 [48];

— European Directives and Standards, including Tissue and Blood Directives [49];

— Centre for Disease Control (CDC) Guidance documents.

C.2 Establishing control in a healthcare/hospital application

It is important that careful consideration is given to monitoring cleaning and decontamination processes to ensure all surfaces are adequately cleaned and disinfected, in particular where there are multi-resistant microorganism species present. Infection control surveillance and monitoring programs, as per national and WHO guidelines should be implemented to establish and demonstrate control.

C.3 Risk assessment for operating room hospital applications

It is important to consider the following factors in high risk operations which have an impact on patient safety, outcome and wellbeing:

— location of the operating area within the human body relative to the central nervous system;

— duration of the handling and manual intervention;

— exposure time of the open wound to the operating room surroundings;

— type of manipulations in the operating technique;

— patient native microbiological population;

— presence of identified, colonized and infected patients with resistant pathogens to antibiotics, in the clean controlled environment.

Annex D
(informative)

Guidance for food applications

D.1 Introduction

Clean controlled environments are used in the food industry to reduce the risk of dangerous microorganisms affecting the quality of food products as well as the visual presentation of food products to the consumer. Clean controlled environments and microbiological control can improve the organoleptic quality of products, extend the shelf life and reduce the use of artificial preservatives. Microbiological environmental monitoring in critical food manufacturing areas can reduce microbiological contamination and food spoilage risks and demonstrate control of the food manufacturing process. There is an absence of practical guidance in existing standards for clean controlled environments in these areas.

Examples of critical food manufacturing areas include:

a) meat or fish cutting and packaging, (meat, ham, salami, fish);

b) production and packaging of baby food, dairy products and cheese;

c) packaging fresh fruit, fresh juice and vegetables;

d) ready to use and eat cooked foods.

This document provides specific guidance on the establishment of microbiological control requirements for clean controlled environments in food industries. This document gives guidance on methods to identify potentially dangerous and difficult to remove microorganisms.

It describes the principles of microbiological control and gives guidance with examples on microbiological verification of the clean controlled environments in order to establish control and then a process for maintenance and demonstration of control.

This Annex gives specific application guidance to achieve the desired purpose of a high quality and consequently a healthier food product for consumption:

— lower costs of production (higher yield);

— low microbiological content;

— no dangerous microbiological contamination;

— increased shelf life;

— improved visual presentation.

D.2 Establishment of microbiological control

The hazard analysis critical control point (HACCP) system is the recommended formal system of microbiological control established, implemented and maintained commonly in food industries.

In establishing microbiological control, it is important to identify the microorganisms of interest, (i.e. objectionable species of interest) for each critical area in the clean controlled environment.

There is no prescriptive list of these microorganisms of interest, rather the facility should utilise the risk assessment process to identify any microorganisms of interest based on consumer population, safety and product quality characteristics.

However, risk assessments in food manufacture should pay particular attention to:

— pathogens in food that will cause illness or even death;

— food quality in terms of visual presentation.

Examples of microorganisms of interest include yeasts and moulds in yoghurt packaging or lactobacilli and pathogenic microorganisms, such as Listeria monocytogenes and Salmonella, in ham cutting and packaging.

The design of environmental monitoring (EM) plan should contain justification for choices regarding:

a) monitoring locations including those identified as 'critical' to the manufacturing process, both in air and on surfaces;

b) controls in critical areas of the clean controlled environment after cleaning and disinfection;

c) monitoring frequencies: the minimum recommendation is at least once per month.

See Tables D.1 and D.2 for guidance on microbial cleanliness for air and surface monitoring (numerical designations and/or incidence rates).

See Annex E for monitoring methods of air and surfaces, for guidance on culture based microbiological measurement methods for reporting cfu per unit of air or unit of surface area sampled.

See Annex F for a review of rapid microbiological measurement methods (RMMs) and alternative real time microbiological detection methods (AMMs);

For microbiology of the food chain, EN ISO 18593 [17] gives guidance on horizontal methods for sampling techniques from surfaces using contact plates and swabs.

For Lactobacilli, ISO 15214 [18] gives a horizontal method for the enumeration of mesophilic lactic acid bacteria.

For Salmonella spp, EN ISO 6579-1 [21] gives a horizontal method for the detection.

The use of alternate methods should be verified to establish relative performance and any potential effects on trend data.

Selected methods should be consistent to ensure comparable data.

D.3 Microbiological cleanliness levels for monitoring

The following Tables D.1 and D.2 give guidance on microbiological monitoring of cleanliness levels in food applications, based on culture microbiological measurement methods.

Table D.1 — Airborne microbiological cleanliness levels for monitoring by culture based methods

Airborne Contamination Level (ACL)	Airborne viable count limit in operation [a] cfu/m³
ACLx 1	≤1
ACLx 2	< 10
ACLx 3	< 100
AClx 4	< 1 000
ACLx 5	< 10 000
Intermediate levels are permitted, for example ACLx 3,5. NOTE "x" is the microorganism of interest. [a] Combined with an appropriate incidence rate.	

Table D.2 — Surface microbiological cleanliness levels for monitoring by culture based methods

Surface Contamination Level (SCL)	Surface viable count limit [a] cfu	Sampled area
SCLx 1	absent	1 m²
SCLx 2	< 4	1 dm²
SCLx 3	< 40	1 dm²
SCLx 4	< 300	1 dm²
SCLx 5	> 300 [b]	1 dm²
Intermediate levels are permitted, for example SCLx 3,5. NOTE 1 "x" is the microorganism of interest. NOTE 2 SCL is 1 m² all other SCL are 1 dm² [a] Combined with an appropriate incidence rate. [b] High counts may be found in certain food applications such as dairy.		

D.4 Demonstration of microbiological control

Table D.1 and Table D.2 can be used as a flexible tool as part of demonstrating microbiological control during environmental monitoring (EM).

Microbiological EM results should be carefully evaluated for trends, in particular out of specification results when further investigation is required.

In accordance with the principles of Plan–Do–Check–Act process of continuous improvement the Microbiological EM Plan should be reviewed on an agreed basis and revised, if necessary based on the results of the Microbiological EM data.

D.5 Example for food manufacture

Table D.3 is an example to illustrate the steps in establishing and demonstrating microbiological control of the food manufacturing environment relating to Ham cutting and packaging.

Pathogens in food can cause illness or even death.

Many microorganisms, even non-pathogenic (such as, for example, Lactobacilli, yeasts, moulds, Clostridium resistant to heat treatments, etc.), can change the visual presentation and the organoleptic quality of the ham.

The prerequisite requirement is that the source Ham is of the correct product quality, ie pasteurised and contains the appropriate level of preservatives.

Table D.3 — Steps to demonstrate microbiological control in ham cutting and packaging

Establish control (Risk issue/subject)	**Demonstrate control (Risk mitigation/Recommendation)**
Product quality (Safety): Pathogens (can cause illness or even death) Visual presentation of the Ham product	Ensure: absence of *Listeria monocytogenes end* per 25 g; absence of *Salmonella spp* per 25 g; <100 cfu/g of Lactobacilli
Level of control required by Facility	Cleaning and disinfection of specified internal surfaces of facility, equipment and surfaces; Verify cleaning completed at the end of each shift /batch processing cycle
Personnel intervention, contact and handling	All personnel trained according to the appropriate internal training program; Refer to Clause 4.9
Selection of the sampling locations	cutting area; conveyor belt; packaging area
Cleanliness levels for Microbiological EM (from Tables D.1 and D.2)	**Lactobacilli:** AIR < 1 cfu/m^3 class ACL1, SURFACES < 4 cfu/ dm^2 SCL2 ***Listeria monocytogenes* and Salmonella spp:** AIR < 1 cfu/m^3 class ACL, Salmonella spp SURFACES Absent per m^2 SCL1, Salmonella spp
Microbiological EM – ACTION level	**Lactobacilli:** AIR 100 cfu/ m^3 SURFACES 500 cfu/dm^2 ***Listeria monocytogenes* and Salmonella spp:** AIR 1 cfu/m^3 SURFACES Absent per m^2
Microbiological EM – ALERT level	**Lactobacilli:** AIR 10 cfu/ m^3 SURFACES 100 cfu/ dm^2 ***Listeria monocytogenes* and Salmonella spp:** AIR 1 cfu/m^3 SURFACES Absent per m^2
Microbiological EM – TARGET level	**Lactobacilli:** AIR 5 cfu/ m^3 SURFACES 50 cfu/dm^2 ***Listeria monocytogenes* and Salmonella spp:** AIR ≤ 1 cfu/m^3 SURFACES Absent per m^2

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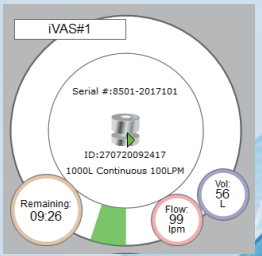
- 带电子签名的批次报告
- 数据导出 XML Microsoft Excel
- 连接 3rd 方系统或者平台
- 远程网络客户端



Annex E
(informative)

Guidance on culture based microbiological measurement methods and sampler verification

E.1 General

Measurement of microbiological contamination consists of some form of sampling and then an assessment of the number of microorganisms present in the sample. There are a variety of sampling methods that can be used which should efficiently collect and then count microorganisms. An understanding of the limitations of the chosen system is of fundamental importance for the correct evaluation and interpretation of the results. It is important to understand that the number of microorganisms detected by one method cannot be directly compared with the results from a different method and a clean controlled environment can appear to have different microbiological concentrations depending on the system used. The efficiencies of some sampling methods can be very low, with some surface sampling methods substantially less than 50 %, and some air sampling methods less than 10 %. However, efficiencies in the region of 50 % may be acceptable if the actual efficiency is known and the variation is low. A sampling method with low efficiency and poor variation is not acceptable.

Established sampling methods typically utilise defined nutrient media on which the contamination is recovered and can be cultured to quantify the level of microbiological contamination present in the sample. Growth Promotion Testing (GPT) of the nutrient media should be carried out to verify the ability of the media to support microbiological contamination growth. The media is incubated and under favourable conditions, microorganisms will grow on the media to produce a visible cluster known as a colony forming unit (cfu).

The number of visible cfu are counted to provide an estimate of the number of microorganisms present on the incubated media and so in the sample. It is recognised that the cfu may have originated from more than one microorganism and that not all the microorganisms on the media may be able to grow sufficiently to produce a visible cfu. The limitations of this type of quantification need to be understood as well as recognising that a single microorganism deposited into, or onto, a product or a work surface may be able to proliferate to levels that may have harmful effects.

E.2 Air sampling

E.2.1 Volumetric air samplers

E.2.1.1 General

A specific volume of air is sampled and microorganisms (present as microbe-carrying particles) are removed from the air and transferred to a suitable nutrient media (agar) surface. This method is often referred to as volumetric (or active) air sampling. After the specified sampling time, the media is either directly incubated or processed and examined and the number of cfu and types of microorganisms are recorded. From the total number of recovered microorganisms, the number of microorganisms per unit volume can be determined. Results are expressed as the number of cfu per sampling volume e.g. cfu/m^3.

E.2.1.2 Direct impaction air sampling

E.2.1.2.1 General

Direct impaction samplers employ either inertial impaction or centrifugal forces to remove microbe-carrying particles from the air and impact onto a nutrient media surface. Several types are available.

E.2.1.2.2 Cascade sampler (multiple sieve sampler)

The air sample is drawn through perforations onto media plates. The sampling rate is typically 28,3 l/min. A stack of media plates is separated by perforated plates, the perforations having decreasing diameter from the top to the bottom of the stack. Microbe-carrying particles impact on the top media plate, and the smaller particles cascade over, through the perforations, until they impact on a media plate lower down in the stack. After the specified sampling time, the plates are incubated and examined and the number of cfu and types of microorganisms are recorded.

E.2.1.2.3 Single sieve to agar sampler

The air sample is drawn through a perforated disc and typically impinges on a 90 mm diameter media settle plate. After the specified sampling time, the plate is incubated and examined and the number of cfu and types of microorganisms are recorded. The sampling rate is typically 100 l/min and the volume can be set between 33 and 2 000 l.

E.2.1.2.4 Slit to agar sampler

The air is drawn through a slit and impinges on a rotating media plate. The dimension of the slit, the distance from the slit to the media surface and the airflow are controlled to give maximum impingement of the microbe-carrying particles onto the media plate. After the specified sampling time, the plates are incubated and examined and the number of cfu and types of microorganisms are recorded. The sampling rate is typically 28,3 l/min.

E.2.1.2.5 Centrifugal sampler

The air is drawn into the sampling head by means of an impeller. The impeller then directs the air onto a media strip fitted around the circumference of the sampling head. After the specified sampling time, the strip is removed, incubated and examined and the number of cfu and types of microorganisms are recorded. The sampling rate is typically 100 l/min and some models can be set to sample fixed volumes from 1 l to 2 000 l at the specified sampling rate.

E.2.1.3 Indirect impaction samplers

E.2.1.3.1 General

Indirect air sampling involves the filtration of air onto a filter or into a liquid for subsequent transfer onto a media plate which is then incubated and examined and the number of cfu and types of microorganisms are recorded. Results are expressed as the number of cfu per sampling volume e.g. cfu/m^3.

E.2.1.3.2 Membrane filter

The air is drawn through a membrane filter mounted in a sampling head. The filter is transferred to a media plate which is then incubated and examined and the number of cfu and types of microorganisms are recorded. Filters made from gelatine can be used as this material retains moisture and this may help in preventing the death of the microorganisms by desiccation.

E.2.1.3.3 Liquid impingement

The air is drawn through an air intake and microorganisms' impact in a liquid which is a buffer solution or other suitable medium. The sampling rate can be between a few l/min and several hundred litres/minute for extended periods, into hours. After the specified sampling time, the solution is examined for the presence of growth by either filtration and addition of the filter to media or direct addition to a media plate. The liquid sample can also be analysed using AMM or RMM.

E.2.1.4 Remote air sampling

To sample air at the required location, as it may not be possible to get an air sampler close due to issues with access, contamination or disturbance of the surrounding air, sampling may use some type of extension tube (often plastic). This creates low collection efficiency, due to deposition of microbe-carrying particles in the tubing, by gravitational settling or impaction, and these losses may be significant, with approximately 50 % not uncommon. To determine the proportion lost, the number of microbe-carrying particles obtained from a sampler without an extension tube can be compared, over several experiments, in an occupied environment with a sampler and extension tube. When sufficient results have been obtained, a continuous cumulative average can be used to assess the data and determine the degree of loss. The remote air sampling system should be designed and validated for the intended use.

E.2.2 Settle plates

Settle plates are a method of determining the number of microorganisms (present as microbe-carrying particles) that may deposit onto, or into, product and surfaces. Circular plates, approximately 9 cm diameter (surface area is approximately 58 cm^2 to 64 cm^2) or 14 cm diameter (surface area approximately 154 cm^2), containing a defined nutrient agar, are exposed for a specified time in the environment allowing microbe-carrying particles to deposit onto them, mimicking the microbial deposition mechanism of contamination, whilst not disrupting the flow of air. Plates are then incubated under specified conditions, examined and the number of cfu and types of microorganisms are recorded. From the total number of recovered microorganisms, the number of microorganisms deposited per unit area, per time can then be determined and provides a more direct means of measuring potential product contamination than the measurement of the concentration of microorganisms in the air. Settle plate sampling has the additional attribute that it can be used to continually sample at the monitoring location with typically exposure times of a maximum of 4 h to avoid the media drying out which will affect the recovery of microorganisms (note: this time may have to be reduced in locations where the media may dry out quickly, e.g. where the plates are exposed to high air flow rates). Results are semi-quantitative and can be expressed as the number of cfu per plate per time e.g. cfu/settle plate/4 h.

E.3 Surface sampling

E.3.1 General

For cleanroom surfaces that are relatively flat and accessible, contact plates and strips can be used. For uneven and difficult to access surfaces, swabbing is typically utilised. After sampling, the sampling site surface should be appropriately cleaned to remove any residue of the sampling medium.

E.3.2 Contact plates and strips

Contact plates are typically circular 55 mm diameter contact plates containing nutrient agar poured to give a convex meniscus. The nutrient agar can be rolled over the surface to be sampled or can be applied to the surface for a few seconds with uniform and steady pressure ensuring no movement. The viable particles will adhere to the agar surface. Contact strips can be similarly utilised, removing them from their container and applying to the surface to be sampled. The lidded plates and resealed strips are then incubated and examined and the number of cfu and types of microorganisms recovered are

recorded. Results are expressed as the number of cfu per plate or per surface area e.g. cfu/contact plate or cfu/dm^2.

E.3.3 Swabs and sponges

Sterile swabs are typically used for sampling recessed surfaces where the use of a contact plate is not possible. Both direct plating and membrane filtration methods can be used. In food applications where there are large surfaces used in food preparation and manufacturing sponges or large sterile wipes are used to sample these very large surfaces (1 m^2 or more) to detect the presence or absence of microorganisms.

The swab is moistened in a sterile buffer liquid or similar agent to improve the transfer efficiency and drawn across a defined surface area whilst slowly rotating it.

For direct plating methods the moistened swab is inoculated onto the surface of an agar plate using a rolling action. This swab is then reintroduced into the tube containing the buffer solution. A second, un-moistened swab is used to take a sample on the same surface as the previous but perpendicular to what has already been done. The second swab is then introduced into a tube containing sterile buffer. A fraction of each of the two buffers is then inoculated into agar to allow the growth of the colonies present. The plate is then incubated and examined and the number of cfu and types of microorganisms is related to the area that has been sampled.

For membrane filtration methods the swab can then be placed in a specified amount of rinse liquid, agitated and the rinse liquid poured onto a media plate or the liquid filtered through a membrane and the membrane transferred onto a media plate. The plate is then incubated and examined and the number of cfu and types of microorganisms is related to the area that has been sampled.

A count of viable microorganisms on the suspension is then completed. Results are expressed as the number of cfu per swabbed area per sampling area e.g. cfu/sample area.

In Life Science Grade A GMP applications, where no growth at all would be the expected outcome the result can be expressed as either “Growth” or “No growth”.

NOTE For more information, refer to ISO 18593 [17].

E.4 Microbiological growth media

E.4.1 General

A number of different culture media are available and where appropriate, may be required to detect fungi as well as bacteria. Tryptone Soya agar (TSA) is a medium with low selectivity suitable for the cultivation of many environmental bacteria and is also suitable for the evaluation of fungi, although more selective media such as Sabouraud Dextrose agar (SDA) may be used if appropriate. When necessary to detect or search for a microorganism of interest, a selective culture medium is required. Appropriate additives should be included to overcome, or minimize, the effects of residual antimicrobial activity at the sampling surface including personnel gloves. The quality of the media is subject to a suitable verification programme prior to use. False counts, possibly leading to unjustified investigation and action, may arise from the use of inadequately controlled microbiological test materials and the environment may also be at risk of contamination. The storage conditions and expiry date, handling and transfer of the microbiological test materials are other important considerations required to manage the risk of environmental contamination. The ability of culture media to recover low levels of inoculated test cultures needs to be established.

E.4.2 Media suitability (media sterility and ability to support growth)

Media is required to be sterile and terminal irradiation, within secure wrappings is recommended to negate the need for some level of pre-incubation, under the established conditions, and inspection prior to use. The fertility is determined by adding a small quantity of test microorganisms appropriate to the

media, such as those listed in the European and United States Pharmacopoeias (typically 0,1 ml of 10^3 microbes/ml) and incubating at a suitable temperature. Following incubation counts of microorganisms are compared to those of other media of the same type and inoculated in a similar way. Typically, if 50 % to 200 % of each test microorganism grows, the medium is considered to support growth sufficiently. This type of testing may be completed on samples removed from each lot of manufactured media or on a less frequent schedule dependent upon demonstrated media performance.

E.4.3 Media dehydration

Settle plates in high airflow areas, or those in air samplers where large quantities of high flows over them, can become dehydrated. The dehydration can be reduced if greater media volumes are used in the plates. The actual plate sampling conditions need to be determined to ensure the recovery is satisfactory. Adding a suspension of microorganisms to media that have been subjected to dehydration is not an appropriate method as the water added with the test microorganisms may help rehydration and affect the result.

E.4.4 Media disinfectant inhibition

Disinfectant residues present on surfaces may inhibit the growth of any microorganisms transferred to the microbial growth media used for sampling. Such microbial growth media incorporates inactivators) to neutralise any surfaces disinfectants (e.g. Lecithin and Tween). The effectiveness of these neutralisers can be examined by either dispensing the disinfectant onto test surfaces and then sampling the surface or using surface sampling media plates that have been used to sample surfaces within the environment and after incubation found to be sterile. To these media plates, a microbiological growth promotion suspension is added. A set of control plates are also inoculated with the same microbial suspensions and the resultant counts are compared to ensure that growth is not inhibited or show inactivation of known levels of a range of disinfectants.

E.4.5 Plate incubation

Incubation conditions may vary depending on the microorganism of interest that is being enumerated.

In general, total count incubation at 30 ^{0}C to 35 ^{0}C for a minimum of 3 days is suitable for the growth of many bacteria and 20 ^{0}C to 25 ^{0}C for a minimum of 4 days is suitable for the growth of some fungi. Incubation times and temperatures should be justified. Incubation temperatures are maintained in the range 20 ^{0}C to 35 ^{0}C and within 2,5 ^{0}C of the target temperature.

E.5 Validation of air samplers

E.5.1 General

The suitability of air samplers to recover airborne microbiological contamination can be determined by consideration of physical and biological collection efficiencies [12] [15]. Physical efficiency relates to the ability of the sampler to collect particles of various sizes. The biological efficiency assesses the ability of a sampler to collect viable microbe-carrying particles which can form cfu. It includes the losses caused by both the physical efficiency and the effect that the sampling has on the viability of the microorganisms due to stressing during collection and dehydration of the media. Due to the complex and specialized activities required, the validation of a sampler is likely to be carried out by the manufacturer. The values of the collection efficiency and the associated certificates will be retained by the user, see 6.3.

E.5.2 Physical collection efficiency

The physical collection efficiency is the cut-off size (d_{50} value) which defines the aerodynamic equivalent particle diameter size at which the sampler collects 50 % of the particles in the air [12] [13]. The average equivalent diameters of microbe-carrying particles (MCPs) that form the cfu are generally larger than 1 µm [15] and a d_{50} value smaller than 2 µm is considered appropriate. The d_{50} value can be

calculated for impaction samplers with multiple holes or those using rectangular slits. For active air samplers based on impingement and or cyclonic operational principles, no d_{50} value can be calculated. For all active microbiological air samplers, the effects of impact stress and the effect of the media dehydration during the sampling period are further considerations. [24] [11] [12] [15] [56].

The physical collection efficiency is influenced by both inlet or extraction efficiency and by separation efficiency. Inlet or extraction efficiency is a function of the inlet design of the sampler and its ability to collect particles from the air in a representative way and transport the particles to the impaction nozzle or the filter. Separation efficiency is the ability of the sampling device to separate and collect particles of different sizes from the air stream by impaction onto the collection medium or into the filter medium. The physical collection efficiency is based on the physical characteristics of the sampling device such as airflow, orifice shape, orifice size and the number of orifices. A simplified formula to calculate the d_{50}-value [12] in µm is shown in Formula (E.1):

$$d_{50} = \sqrt{\frac{40 \times Dh}{U}} \qquad \text{(E.1)}$$

where

40 is the constant factor for air viscosity (°C);

Dh is the equivalent hydraulic diameter of the air inlet nozzle(s) (mm);

U is the impact velocity (m/s).

NOTE For a circular opening, the equivalent hydraulic diameter is the hole diameter. For a rectangular slit, the equivalent hydraulic diameter will be approximately twice the slit width.

Computational fluid dynamics (CFD) has also been shown to be an effective method for the determination of d_{50} values [15].

Experimental work to determine the physical collection efficiency is given in (E.6.1.2.1).

E.5.3 Biological collection efficiency

The biological efficiency assesses the ability of a sampler to collect viable microbe-carrying particles and includes the losses caused by both the physical collection efficiency and the effect that the sampling has on the viability of the microorganisms. Experimental work to determine the biological collection efficiency is given in E.6.1 and E.6.2.

E.6 Experimental method

E.6.1 Aerosol chamber method

E.6.1.1 General

The aerosol chamber method is conducted in a controlled and closed space that ensures no variations in concentration of the aerosol to be tested.

The aerosol concentration within the test chamber should be homogenous.

A particle counter and method of sampling air within the chamber should be provided to ensure the aerosol is well mixed and to check on its concentration.

Temperature and relative humidity should be maintained at (22 ± 2) °C and (50 ± 10) % RH, respectively. The apparatus within the test area should be able to be operated without influencing the test conditions.

Data demonstrating the following tests should be provided by the supplier/manufacturer of the equipment.

E.6.1.2 Test strains of microorganisms

E.6.1.2.1 Strains for testing physical collection efficiency

A suitable test strain which survives well under the collection conditions should be used e.g. *Bacillus atrophaeus* NCTC 10073 or ATCC 9372. The test strain should be prepared in a culture medium meeting the nutritional requirements of the test strain and used as a washed spore suspension.

Alternately, polystyrene spheres and other types of non-viable particles can be used to determine the physical efficiency of air samplers [13]. The results obtained are similar to those produced by microbiological particles. However, in some samplers it is impossible to count all the non-viable particles whereas if spores are used, they will grow into cfu which are easily enumerated.

E.6.1.2.2 Strains for testing biological collection efficiency

A suitable test strain with a survival rate susceptible to the collection conditions should be used e.g. *Staphylococcus epidermidis* (NCTC 11047; ATCC 14990) can be used and great care has to be taken to assure uniform conditions throughout all tests.

E.6.1.3 Generation of microbe-carrying particles

Aerosols of a controlled particle size are produced by an apparatus such as the spinning-top or spinning disc aerosol generator [10] or other appropriate instruments. The wet particle diameter can be determined by use of an equation that relates it to the density and surface tension of the liquid and the rotational speed and diameter of the spinning disc or it can be measured microscopically.

After formation, the wet particles will be reduced by evaporation to a size that depends upon their solid content. Care should be taken to ensure that only dry particles reach the testing chamber, e.g. by separating the aerosol generator sufficiently far from the chamber.

The diameter of the dry particle can be calculated using the following formulae or determined microscopically by sampling the air in the test chamber on a filter membrane.

The radius (r) of any sphere is related to its volume (V) by Formula (E.2):

$$r = \left(\frac{3}{4} V + \pi \right)^{1/3} \tag{E.2}$$

where

r is the radius;

V is the volume.

In the case of a dry particle, its size will be determined by both the amount of solid material contained within the wet particle and the spore, for the radius of the dry particle see Formula (E.3).

$$Radius\ of\ dry\ particle = \left[\frac{3}{4} \left(V_p + V_s \right) / \pi \right]^{1/3} \tag{E.3}$$

where

V_s is the volume of spore (approximately 0,5 μm^3);

V_p is the volume of particle after evaporation.

The calculation of the volume of the particle after evaporation is in Formula (E.4)

$$V_p = \frac{volume\ of\ wet\ particle\left(m^3\right) \times concentration\ of\ solid\ in\ particle\left(g / m^3\right)}{density\ of\ the\ solid\ material\ in\ solution\left(g / m^3\right)} \quad \text{(E.4)}$$

Having determined the radius of the dry particle the diameter is easily ascertained.

The aerodynamic behaviour of a particle will vary according to its density. Therefore, it is necessary to calculate the equivalent particle diameter of the dry particle, i.e. the size the dry particle would be if it were of a density of 1g/cm^3 = 1 000kg/m^3, see Formula (E.5).

$$Equivalent\ particle\ diameter = d\left(p\right)\frac{1}{2} \quad \text{(E.5)}$$

where

d is the diameter of the dry particle;

ρ is the density of incorporated solid material.

For physical efficiency testing, different concentrations of solids should be dispersed in the solutions to provide a range of particle sizes when sprayed. The concentrations of solids required can be calculated using the equations given in E.6.1.3. Five solutions should be prepared to provide particle sizes over an appropriate range of equivalent particle diameters. For each particle size a statistically relevant number (at least 10) experiments should be carried out.

The solid used to generate particles should not inhibit tested organism growth in the concentration range necessary for the experiment.

E.6.1.4 Testing

The tests take place inside the chamber. The sampler to be tested and a 0,45 µm membrane filter, should be placed close to one another. A particle counter should be used to check that the particle concentration is the same at the sampler and membrane-filter positions.

The membrane sampler, operating at a flow rate of approximately 5 l/min, should not face upward but should face to the side or downward, preventing deposition by gravity of particles onto the membrane. Both samplers should be switched on together. The sampling time should be chosen such that a statistically relevant number of colonies are formed and will depend on the concentration of microbe-carrying particles in the air. After the test, place the membrane on a Petri dish containing a suitable growth medium and incubate the filter plate and the plate from the sampler before counting the resulting colonies.

For biological efficiency testing, an already qualified alternative to membrane sampler can be used as reference.

E.6.2 Simplified laboratory method

E.6.2.1 General

As an alternative to the aerosol chamber method the laboratory method could be carried out in various premises with differing levels of airborne microbiological cleanliness under conditions related to daily practice [14].

This method has the advantage that the tests are performed with naturally occurring microorganisms and not with artificial aerosols. The tests have to be performed in a minimum of two different locations with sufficiently high airborne microbiological concentrations (above 80 cfu/m^3) so that the

corresponding number of cfu captured on the growth media is between 80 to 150. This number ensures sufficient physical separation between colonies whilst remaining statistically relevant. The sampling time may need to be adjusted so that the required number of colonies captured are within this range. When the required sampling time has been established, it should remain constant throughout all of the testing.

The equipment to be qualified, is compared to a qualified reference method such as membrane filtration, or an already qualified impaction method.

E.6.2.2 Testing

The air samplers to be compared are positioned in the room as close to each other as possible (within 1 m) making sure that there is no mutual disturbance or interference during sampling; the sample intake should be at the same height (e.g. 80 cm to 120 cm above floor).

It should be made sure that both instruments are calibrated on volume or mass flow. If the instruments do not run at the same air flow rate they should run in parallel during the same sampling time [14]. The sampled volumes should be used to calculate the cfu/m^3 after appropriate incubation.

To guarantee comparable conditions the testing should be distributed over the day and the position of the two instruments should be alternated after each test.

Since the distribution of microorganisms (cfu) in any facility is never completely homogenous a statistically significant number of tests have to be performed to reduce the effects of this variation.

E.6.3 Incubation

Incubate all collected samples from both air samplers at appropriate incubation conditions to ensure growth for enumeration of counts. For example:

— membrane filters on an agar Petri dish

— directly impacted agar plates

Suitable incubation conditions will vary depending on the microorganism of interest that is being enumerated, see E.4. for more information on growth media

To ensure comparability of incubated samples it is good practice to use the same media reference. If the same media reference is not used, growth promotion properties of the different media should be assessed to ensure consistent test results.

E.6.4 Collection efficiency calculations from testing results

The reference concentration obtained from a membrane filter inside the chamber over 5 different particle sizes in the appropriate range (e.g. 0,8 µm to 15 µm) [10]. The biological efficiency of the sampler can be determined from Formula (E.6):

$$\text{Efficiency of sampler}(\%) = \frac{\text{test sampler count}}{\text{total count}(\text{from reference sampler})} \times 100 \qquad \text{(E.6)}$$

Acceptable limits are: (100 ± 50) %.

The results may be plotted as particle size against efficiency, with all points plotted as means with standard deviations of efficiencies.

E.6.5 Air sampler revalidation

Because of the complex and specialised activities required, sampler validation is likely to be completed by a suitably competent external body. The collection efficiency values and associated certificates would be retained by the user.

It is not the expectation that the validation work would be periodically repeated. The sampler would be subject to scheduled maintenance and calibration of the critical operating parameters associated with the rate of flow of air through the sampler and the associated time control.

Annex F
(informative)

Rapid microbiological methods (RMM) and alternative real time microbiological detection methods (AMMs)

F.1 General

The established culture based methods involve capturing microorganisms in, or on, a growth medium, providing permissive conditions for growth and subsequent visual detection of the microorganisms after a suitable period of time. These methods suffer from two major limitations; first, proliferation is always selective and variable and limited to culturable microorganisms; second, adequate growth for visual detection takes significant time.

Numerous new instruments and methods, developed over the past 20 years, have been designed to improve the detection of microbiological contamination by mitigating some of these limitations. These methods endeavour to reduce time-to-results, increase sensitivity, accuracy, precision and reproducibility compared to the established culture based method.

All of these methods have taken one of two approaches to achieve this. Some utilise the established culture based sample collection methods, but use technologies that decrease the time at which actively growing microorganisms can be detected. These methods are called Rapid Microbiological Methods (RMMs) and as they are growth dependent, are limited to culturable microorganisms.

Alternative Microbiological Methods (AMMs) do not rely on growth and proliferation of microorganisms to facilitate detection. Such a technology is the measurement of real time viable particles using counters that incorporate fluorescence based optical spectroscopy. This method detects microbiological particles, with no discrimination as to whether they are viable, culturable or non-culturable, in almost real time, without having to locate and subsequently remove growth media into and out of the clean controlled environment.

These methods can be used to enhance the understanding of the state of control of the clean controlled environment and may provide considerable advantages for certain applications.

Both RMMs and AMMs are rapidly evolving and any reference in this informative annex would be overtaken quickly and therefore of no real value in future reading. However there are numerous web resources on both subjects.

NOTE One current weblink example on RMMs is http://rapidmicromethods.com/files/matrix.php [45] and gives a significant level of detail on methods and applications. However, if this weblink is no longer supported, the latest information can be searched for.

F.2 Implementation of RMMs and AMMs

The implementation of RMMs and AMMs should be actively considered if they can provide advantages for microbiological control and measurement.

RMMs rely on proliferation and therefore retain all the advantages and the limitations of microbiological culture based sampling methods. Consequently, much of the methodology is familiar to users and therefore more readily understandable and easy to implement. In addition, many of these methods can be automated in order to minimise method errors and the potential for sample contamination.

AMMs use a variety of technologies to detect microorganisms without employing a proliferation step to increase their concentration and will therefore be less familiar to the user and more difficult to implement.

It should be noted that the metrics used to measure microbiological contamination may be in units applicable for the technology used and not directly comparable to growth culture based measurements, reported as cfu. It is important to establish and understand the relationship of the results with those reported by the established culture based methods. All RMMs and AMMs need to be appropriately verified and it is recommended that dialogue with any regulatory or controlling authorities is initiated to define the testing program to be completed in order to evaluate the technology and to compare results with those established for the culture based methods [44].

F.3 Validation of RMMs and AMMs

F.3.1 General

RMMs and AMMs that are to be used should be validated. The intention is to demonstrate that the selected RMM or AMM is suitable for its intended use and provides results that are equivalent or superior to the established culture based method. See Table F1 for qualitative and quantitative indications of the recommended validation parameters for consideration (refer to PDA TR33 [44], EP 5.1.6 [43] and USP <1223> [44])

NOTE Consult the manufacturer for the validation of the particular microbiological detection method. This is due to the fact that validation is dependent on the nature of the RMMs or AMMs and the intended use for that method [45].

Table F.1 — Qualitative and quantitative indication of Validation Parameters for RMMs and AMMs

Verification parameter	Qualitative test	Quantitative test
Accuracy	X	√
Precision	X	√
Specificity	√	√
Limit of detection	√	√
Limit of quantification	X	√
Linearity	X	√
Operational range	X	√
Robustness	√	√
Repeatability	√	√
Ruggedness	√	√
Equivalency	√	√

F.3.2 Acceptance criteria considerations

The results obtained using the established culture based methods and those obtained using the RMM may be well correlated because both methods are based upon the growth of microorganisms. It is unlikely that such a strong correlation will exist with those obtained using an AMM. All microbiological detection methods, including the established culture based methods, can only provide an estimate of the actual number of microorganisms present in a sample and will vary according to the method used. This is one of many factors that must be considered when determining the applicable acceptance criteria for verification testing. Additional factors that should be considered include:

— sampling error: all samples are independent and the concentration of airborne or surface microorganisms are not homogenous;

— lack of accurate concentration standards: there is no method to determine the true concentration of a sample;

— inherent variability: microbiological variability and sample perturbation (e.g. inoculation on a substrate or aerosol generation) may differentially affect detection by various methods;

— sample generation: airborne and surface inoculations are highly variable due to limitations of current technology.

F.3.3 Verification test execution considerations

Verification testing requires the creation of a microbiological aerosol with minimal variation. This requires specialised equipment and therefore most Users will rely on the testing performed by the manufacturer or third-party testing organization. Users should focus on testing to demonstrate the method is fit for use in their application. If the manufacturer testing is not adequate to demonstrate the remaining validation parameters have been addressed, a third-party testing organisation should be utilised, or a sound scientific justification prepared to detail why testing was not performed.

F.4 Action and alert levels

F.4.1 Setting action and alert levels

Alert and action levels based on established culture based methods may not be applicable when using a RMM or AMM. For example, if the new method has improved sensitivity, results that exceed the alert or action level may occur and may not be indicative of a change in the state of control. Additionally, as AMM produce results reported in some other unit than cfu, new levels applicable to the method used must be set and a new risk assessment completed.

F.4.2 Result outside of action and alert levels

The established culture based methods provide time delayed results which generally only allow for reactive actions to be taken to try to address any adverse microbiological contamination and return to a state of control. The use of RMM or AMM that provides results in real, or reduced time, provides the opportunity to take proactive actions to return to a state of control. This may include immediate steps that can be taken to remove or segregate any implicated product whilst ensuring the remainder of the product can be appropriately secured.

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