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INTERNATIONAL STANDARD

ISO 14698-1

First edition
2003-09-01

Cleanrooms and associated controlled environments — Biocontamination control —

Part 1:
General principles and methods

Salles propres et environnements maîtrisés apparentés — Maîtrise de la biocontamination —

Partie 1: Principes généraux et méthodes

Reference number
ISO 14698-1:2003(E)

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Introduction

The principles described here are intended to promote appropriate hygienic practices. This part of ISO 14698 is one of a number of standards considering factors important for the creation of clean, controlled environments.

Hygiene has become increasingly important in many areas of modern society. In such areas, hygiene or biocontamination control methods are, or will be, used to create safe and stable products. International trade in hygiene-sensitive products has greatly increased. At the same time, the use of antimicrobial agents has been reduced or forbidden, creating a need for increased biocontamination control.

This part of ISO 14698 is the first general International Standard for biocontamination control. However, many factors besides cleanliness must be considered in the design, specification, operation and control of cleanrooms and associated controlled environments.

In some circumstances, relevant regulatory agencies could impose supplementary policies or restrictions. In such situations, appropriate adaptations of the standard testing procedures might be required.

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Cleanrooms and associated controlled environments — Biocontamination control —

Part 1: General principles and methods

1 Scope

This part of ISO 14698 establishes the principles and basic methodology of a formal system of biocontamination control (Formal System) for assessing and controlling biocontamination when cleanroom technology is applied for that purpose. This part of ISO 14698 specifies the methods required for monitoring risk zones in a consistent way and for applying control measures appropriate to the degree of risk involved. In zones where risk is low, it can be used as a source of information.

Application-specific requirements are not given. Neither are fire and safety issues addressed; for these, see regulatory requirements and other national or local documentation.

2 Normative references

The following referenced documents are indispensable for the application 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.

ISO 14644-4:2001, *Cleanrooms and associated controlled environments — Part 4: Design, construction and start-up*

ISO 14698-2:2003, *Cleanrooms and associated controlled environments — Biocontamination control — Part 2: Evaluation and interpretation of biocontamination data*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1 General

3.1.1
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

3.1.2
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

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3.1.15
qualification
process of demonstrating whether an entity — activity or process, product, organization, or any combination thereof — is capable of fulfilling specified requirements

3.1.16
risk
combination of the probability of occurrence of harm and the severity of that harm

[ISO/IEC Guide 51:1999, 3.2][2]

3.1.17
risk zone
defined and delimited space where individuals, products or materials (or any combination of these) are particularly vulnerable to contamination

3.1.18
settle plate
suitable container (e.g. a Petri dish) of appropriate size, containing an appropriate, sterile, culture medium, which is left open for a defined period to collect viable particles depositing from the air

3.1.19
swab
sterile collection device, non-toxic and non-inhibitory to the growth of the microorganisms being sampled, consisting of a specific matrix of suitable size, mounted on an applicator

3.1.20
target level
defined level set by the user as a goal for routine operations, for the user's own purpose

3.1.21
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

[ISO 9000:2000, 3.8.5][3]

3.1.22
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

[ISO 9000:2000, 3.8.4][3]

NOTE Monitoring and auditing methods, procedures and tests, including random sampling and analysis, can be used in the verification of the Formal System.

3.1.23
viable particle
particle that consists of, or supports, one or more live microorganisms

3.1.24
viable unit
VU
one or more viable particles which are enumerated as a single unit

NOTE When viable units are enumerated as colonies on agar media, it is common usage to name them colony forming units (CFU). One CFU might consist of one or more VU.

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Annex A
(informative)

Guidance on determining airborne biocontamination

A.1 Introduction

This annex provides guidance on the determination of airborne biocontamination in situations where microbial control is considered desirable or necessary. This measurement involves collection of representative samples for the detection of those viable particles that need to be controlled and monitored.

This assessment of airborne biocontamination is carried out in accordance with the basic principles of this part of ISO 14698, which require the establishment of a Formal System to assess and control biocontamination where cleanroom technology is applied.

Techniques for the validation of a sampling device are given in Annex B.

A.2 Principle

Detection and monitoring of microbial contamination of the air in a risk zone is carried out by collecting viable particles with appropriate sampling devices, according to a sampling plan, when the risk zone is in the as-built and at-rest states, as appropriate, and routinely under normal operation in the risk zone.

A.3 Sampling devices

A.3.1 General

There are a great variety of methods available for the collection and enumeration of airborne viable particles[18]. The selection of a particular method and device will depend upon the purpose for which the sample is required. The collection efficiency of samplers will vary; an appropriate method or methods and equipment should be carefully selected.

Sampling devices fall into two categories:

a) passive sampling devices, such as settle plates;

b) active sampling devices, such as impact, impingement and filtration samplers.

The manufacturer of these devices should provide instructions for their use as well as information on their limitations. The collection efficiency of active sampling devices is discussed in Annex B.

A.3.2 Selection of a sampling device

The sampling rate, duration of sampling and type of sampling device can strongly influence the viability of the microorganisms that are collected. Impingement devices may not be suitable for sampling airborne viable particles because of their low sampling volume and low rate of sampling, and their tendency to disrupt clumps of viable particles.

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Because of the number and variety of microbial air sampling systems commercially available, the selection for a particular application should consider, as a minimum, the following factors:

a) type and size of viable particles to be sampled;

b) sensitivity of the viable particles to the sampling procedure;

c) expected concentration of viable particles;

d) ability to detect high or low levels of biocontamination;

e) appropriate culture media (see 5.5.2)[19];

f) time and duration of sampling;

g) ambient conditions in the environment being sampled;

h) disturbance of unidirectional airflow by the sampling apparatus;

i) sampler properties such as

   1) appropriate suction flow rate for low levels of viable airborne particles,

   2) appropriate impact/airflow velocity,

   3) collection accuracy and efficacy,

   4) ease of handling (weight, size) and operation (ease of use, auxiliary equipment, dependence on vacuum pumps, water, electricity, etc.),

   5) ease of cleaning and disinfection or sterilization, and

   6) possible intrinsic addition of viable particles to the biocontamination to be measured.

The exhaust air from the sampling apparatus should not contaminate the environment being sampled or be reaspirated by the sampling device.

A.3.3 Passive microbial sampling devices (sedimentation sampling devices)

Passive microbial air sampling devices such as settle plates do not measure the total number of viable particles in the air; they measure the rate at which viable particles settle on surfaces. Settle plates may therefore be used for the qualitative and quantitative evaluation of airborne contamination of products. This can be done by determining the settle plate count per time; then, by relating both the area and time of exposure of the product to that of the settle plate, the possible contamination of the product can be calculated[20], [21].

A.3.4 Active microbial sampling devices

A.3.4.1 General

The use of active air sampling devices in risk zones is essential for the assessment of the microbial quality of air. There are several types of active devices commercially available, each having its own limitations.

Based on the principles of sampling, the two main types of apparatus considered suitable for risk zones with normal (low level) biocontamination are impact samplers and filtration samplers.

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Annex C
(informative)

Guidance on determining biocontamination of surfaces

C.1 Introduction

This annex provides guidance on the determination of biocontamination of surfaces in situations, particularly risk zones, where biocontamination control is considered desirable or necessary. This measurement involves the collection of representative samples for the detection of viable particles that are present and that may need to be controlled or monitored. These methods might not give the total number of viable microorganisms present but, under controlled conditions, can give relevant and comparable results. These methods are applied routinely in the operational condition and, if appropriate, in as-built and at-rest conditions.

This assessment of airborne biocontamination is carried out in accordance with the basic principles of this part of ISO 14698, which require the establishment of a Formal System to assess and control biocontamination where cleanroom technology is applied.

C.2 Principles

A count of microorganisms on a surface at a point in time is obtained by a contact device or a swab. A contact device can apply a solid nutrient medium of known area to the surface, which is then incubated. The resultant colonies give a mirror-image "map" of the original viable units. A swab can be used to wipe a surface and the number of microorganisms removed by the swab can be counted.

A count of the rate at which microorganisms are falling on the surface is obtained by exposing, for a known period, a nutrient surface of known area, which is then incubated. The resultant colonies give a rate of deposition per area per period.

C.3 Sampling devices

C.3.1 Contact sampling devices

Contact plates or other devices can be used that allow a nutrient medium, held in a suitable flexible or rigid container, to make contact with the surface to be sampled. The accessible contact surface should be $\geqslant 20\ cm^2$.

The nutrient medium should be applied to the surface for a few seconds with a uniform and steady pressure to the whole area, without allowing any circular or linear movement. The device is then returned to its container and the sampled surface is cleaned to remove any nutrient residues.

C.3.2 Swabs

Collection of viable units may also be achieved by appropriate application of a swabbing technique. The use of sterile moistened swabs, sponges or wipes is particularly convenient for sampling large, non-absorbent, irregular or recessed surfaces not accessible to contact devices.

The swab should be pre-moistened with a sterile rinse medium. The swab should be stroked in close parallel sweeps over the defined sampling area, while being slowly rotated. Sampling of the same area should be repeated, stroking the same swab perpendicular to the initial sweep. The swab should then be placed in a

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specified amount of rinse liquid and agitated. The rinse liquid should be assayed for viable units. After sampling, the sample site surface should be cleaned to remove any residue of the rinse medium.

C.3.3 Settle plates

Settle plates are suitable for the qualitative and quantitative evaluation of possible surface contamination by airborne viable particles depositing from the air.

Where appropriate, the number of microorganism-carrying particles depositing from the air onto surfaces in a given time can be determined by settle plates containing a suitable culture medium; the plates are then incubated. This technique does not measure the total number of microorganisms present in the air; it measures the number that have settled onto a surface during the sampling period. The sensitivity of this method may be enhanced by using large-diameter Petri dishes (i.e. 14 cm diameter) and extending exposure time, while taking care to avoid dehydration of the culture medium[24].

C.4 Expression of results

The number of viable particles on surfaces should be expressed in viable units per 1 dm^2, or in the case of settle plates, per 1 dm^2 per hour (1 dm^2 = 100 cm^2).

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