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Cleanrooms and associated controlled environments — Biocontamination control —

Part 2: Evaluation and interpretation of biocontamination data

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

Partie 2: Évaluation et interprétation des données de biocontamination

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this part of ISO 14698 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14698-2 was prepared by Technical Committee ISO/TC 209, *Cleanrooms and associated controlled environments*.

ISO 14698 consists of the following parts, under the general title *Cleanrooms and associated controlled environments — Biocontamination control*:

— *Part 1: General principles and methods*

— *Part 2: Evaluation and interpretation of biocontamination data*

Introduction

This part of ISO 14698 presents a framework for the evaluation of biocontamination data collected following the principles and methods given in ISO 14698-1. It may also be applied to biocontamination data collected by other systems.

Cleanrooms and associated controlled environments — Biocontamination control —

Part 2: Evaluation and interpretation of biocontamination data

1 Scope

This part of ISO 14698 gives guidance on methods for the evaluation of microbiological data and the estimation of results obtained from sampling for viable particles in risk zones for biocontamination control. It should be used, where appropriate, in conjunction with ISO 14698-1.

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 14698-1:2003, *Cleanrooms and associated controlled environments — Biocontamination control — Part 1: General principles and methods*

3 Terms and definitions

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

3.1
action level
microbiological 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
microbiological level set by the user for controlled environments, giving early warning of a potential drift from normal conditions

NOTE When alert levels are exceeded, this should result in increased attention to the process.

3.3
audit trail
chain of related documents, or entries within records, that allows related information to be traced

3.4
biocontamination
contamination of materials, devices, individuals, surfaces, liquids, gases or air with viable particles

3.5
cleanroom
room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room, and in which other relevant parameters, e.g. temperature, humidity and pressure, are controlled as necessary

[ISO 14644-1:1999, 2.1.1] [1]

3.6
data stratification
regrouping of data so that important trends and deviations can be more easily seen and understood

3.7
estimate
value of an estimator obtained as a result of an estimation

[ISO 3534-1:1993, 2.51] [2]

3.8
estimation
operation of assigning, from the observations in a sample, numerical values to the parameters of a distribution chosen as the statistical model for the population from which this sample is taken

[ISO 3534-1:1993, 2.49] [2]

3.9
estimator
statistic used to estimate a population parameter

[ISO 3534-1:1993, 2.50] [2]

3.10
hazard
biological, chemical or physical element or factor that adversely affects individuals, the environment, process or product

3.11
risk
combination of the probability of the occurrence of harm and the severity of that harm

[ISO/IEC Guide 51:1999, 3.2] [8]

3.12
risk zone
defined and delimited space where individuals, products or materials (or any combination of the above) are particularly vulnerable to biocontamination

3.13
target level
defined microbiological level set by the user, for its own purpose

3.14
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]

3.15
viable particle
particle that consists of, or supports, one or more live microorganisms

3.16
viable unit
VU
one or more viable particles that are enumerated as a single unit

NOTE When VU are enumerated as colonies on agar media, it is common usage to name them colony-forming units (CFU).

4 Evaluation and interpretation of biocontamination data

4.1 General

Information on the setting of action, alert and, where appropriate, target levels, the validation of counting methods and the collection of biocontamination data are discussed in ISO 14698-1. This part of ISO 14698 discusses the evaluation and interpretation of the data collected.

Management of microbiological results from risk zones should take the following factors into account:

— types of result to be collected;

— necessary information;

— methods to process the collected results (e.g. statistical procedures, correlation analysis, artificial intelligence, etc.);

— grouping of results to focus on important trends and deviations, i.e. data stratification;

— method by which the results will be expressed (e.g. qualitatively, quantitatively, graphically, numerically) and the units of measurement that will be used;

— robustness of, and potential problems posed by, the analytical methods;

— trend analysis;

— control charting;

— estimation, interpretation and reporting of results.

It is recommended that the evaluation of results be performed in two stages: during the initial monitoring (set-up procedure) phase and during the routine monitoring phase.

4.2 Estimation and evaluation of data from the initial monitoring phase (set-up procedure — see Figure 1)

4.2.1 Significance of biocontamination

To obtain reliable estimates of biocontamination gathered according to ISO 14698-1, it is necessary to consider the following variables:

— sampling-adequate number and homogeneity of the sample material and accuracy of dilution of the samples, if appropriate;

— composition of the viable particle spectrum involved; its variability with time and the effect of stress and injury on survival and recovery;

— results originating from different sampling sites in risk zones and other controlled environments;

— culturing technique and the methodology of counting;

— selection of method of analysis and relationship between direct and indirect testing.

4.2.2 Corrective action

To maintain control over the performance of the testing laboratory, it is very important to identify and eliminate the cause of any errors that may occur. Prompt investigation of out-of-specification results should include attention to the possibility of testing error.

The investigation should include:

— a standard method for highlighting abnormal results;

— elimination of gross or systematic errors;

— evaluation of change;

— establishment of the recovery efficiency of the revised method;

— verification of equipment;

— justification and documentation;

— clear rules to decide how the final result is derived when an analysis has been repeated.

4.2.3 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. It is essential that the records include the identity of personnel involved in sampling, 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. Reports should be distributed as required. This may include mail, facsimile transmission and electronic data transfer.

It is essential that appropriate protection of data and records, including those held in the computer, be provided.

1	**Establish sampling points and sampling plan — Identification of data to be collected** (as described in ISO 14698-1)
	• establish data collection parameters • establish sampling plan • establish/prepare data collection/recording sheets • set preliminary limits
	↓
2	**Preliminary measurement phase** (as described in ISO 14698-1)
	• determine the presence of biocontamination • select methods • conduct sampling and microbiological examinations
	↓
3	**Analysis and monitoring of data**
	• tabulate data, e.g. in evaluation table/matrix • stratify (sort/steamline) preliminary data • express results • apply statistical evaluation procedures with the determination of precision and accuracy • establish control charts
	↓
4	**Data evaluation**
	• evaluate initial limits at control points • assign target/alert/action levels from preliminary measurements • consider out-of-specification results
	↓
5	**Standardization and validation**
	• sampling, measuring methods • electronic data processing • personnel training
	↓
6	**Data storage/Documentation/Archive**

Figure 1 — Estimation and evaluation of data from initial monitoring phase

4.3 Estimation and evaluation of biocontamination data resulting from the routine monitoring phase (see Figure 2)

4.3.1 Sampling and sample tracking

Information on the most important step for valid results, i.e. sampling, is given in ISO 14698-1. In addition, the laboratory should have suitable and dependable procedures that allow for the clear identification and handling of samples from their reception and progress through the entire analytical process to the final results and their correct identification with the original sample.

4.3.2 Collection of results

The general guidelines for a sampling plan, outlined in ISO 14698-1, should be followed. In addition, to avoid collection of erroneous results, the following factors should be considered:

— particular application;

— identification of application-specific parameters;

— data collection points in the process/system;

— limit of detection and sensitivity of test system;

— operation and operational data collection.

4.3.3 Data recording

To ensure that all information of practical relevance to the tests performed is readily available during a defined period of time, clear procedures for data recording and handling should be developed and implemented, and should cover the following aspects:

— raw data;

— list of types of information held in the records;

— identification and location of laboratory documents, or computerized records;

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

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

— recommendations for consistent interpretation;

— specific, legal or regulatory requirements;

— requirements appropriate to the field of application with effect on action, alert and target levels.

1	**Monitoring of control points in risk zones**
	• examine samples taken according to sampling plan • enumerate cfu/VU • record cfu/VU data
	↓
2	**Analysis and monitoring of data**
	• data stratification-group measurements: tabulate data or use descriptive statistics • express results • examine time trends • establish control charts
	↓
3	**Data evaluation and interpretation**
	• statistics/correlation analysis • control analysis/artificial intelligence
	↓
4	**Verification of data**
	• calculations • electronic data processing of results • microbiological classification of control points • microbiological quality level of risk zones
	↓
5	**Results evaluation and conclusions from data estimation**
	• consider out-of-specification results • apply corrective action(s), if appropriate • adjust target, action and alert levels if necessary • adjust quality level decisions in risk zones
	↓
6	**Data storage/Documentation/Archive**

Figure 2 — Estimation and evaluation of data from the routine monitoring phase

4.3.4 Data evaluation

Before statistical calculations can be performed on results, especially when many observations have been recorded, it is necessary to condense and group the data in such a way that the main features are clear. 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 measurement:

a) a document is needed that describes the approach taken to develop a method and the statistical techniques used to validate the method;

b) typically, the method should have been published in a peer-reviewed scientific journal or in a book;

c) the way in which improved measurement methods are introduced should be described.

4.3.5 Application of statistics to results

The essence of any statistical technique is the extrapolation from the sample to the microbial population of the risk zone from which the sample is drawn. Such extrapolations involve risks, since the sample may not accurately reflect the contaminating population. When monitoring and evaluation are done properly, this risk may be quantified and reduced to an acceptable level by using probability sampling and application of statistics [4], [5].

It is recommended that interpretation and evaluation of results be based on more than one statistical method. Due to the complexity of statistical evaluations and available literature, the selection and use of statistical methods for monitoring and verification are not described in this part of ISO 14698.

4.3.6 Trend analysis and control chart presentations

Data coming from a single sample are often not significant; furthermore, microbiological monitoring techniques may have serious shortcomings that result in a high degree of variability. Therefore, graphic presentation of the results collected over a period of time may be useful 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 [4], [5], to assess the quality of the risk zones and are particularly applicable to monitoring. In the verification step [ISO 14698-1:2003, principle 4.2 f)], sampling for batch acceptance purposes can be applied as another quality control technique. Charting by means of Shewhart control charts [6], control charts “based on range” or “cumulative sum charts” may also be appropriate [7] to measure the deviation from usual random spread and to highlight out-of-specification results.

4.4 Verification

To determine the continuing efficiency of monitoring and analytical methods, a review of the results should be conducted periodically. From this, the microbiological classification of risk zones can be verified by reviewing the assigned alert and action levels or, where appropriate, target levels (see also ISO 14698-1).

4.5 Out-of-specification results

Each occurrence of an out-of-specification test result requires evaluation to decide if it was a true result. Information as to how this might be done is given in the flow chart in Figure 3. It is essential that any out-of-specification result that cannot be confirmed as laboratory error be investigated to determine the cause and appropriate corrective action.

During the set-up procedures, the limits are tentative and may change as the routine monitoring evolves. A result outside these tentative limits may be considered to be a true result reflecting a real change in the occurrence of biocontamination, prompting re-evaluation of a tentative specification. In that case, no formal verification may be required but the decision should be justified and documented.

4.6 Validation of results

There shall be defined procedures for the validation of results prior to reporting; these should include the following:

— written procedure for the acceptance of results by the appropriate, trained person;

— if results are entered into a computer system, procedure for the checking of hard copy against the database;

— system for the reporting and presentation of results;

— clearly defined procedure for the release of reports of results.

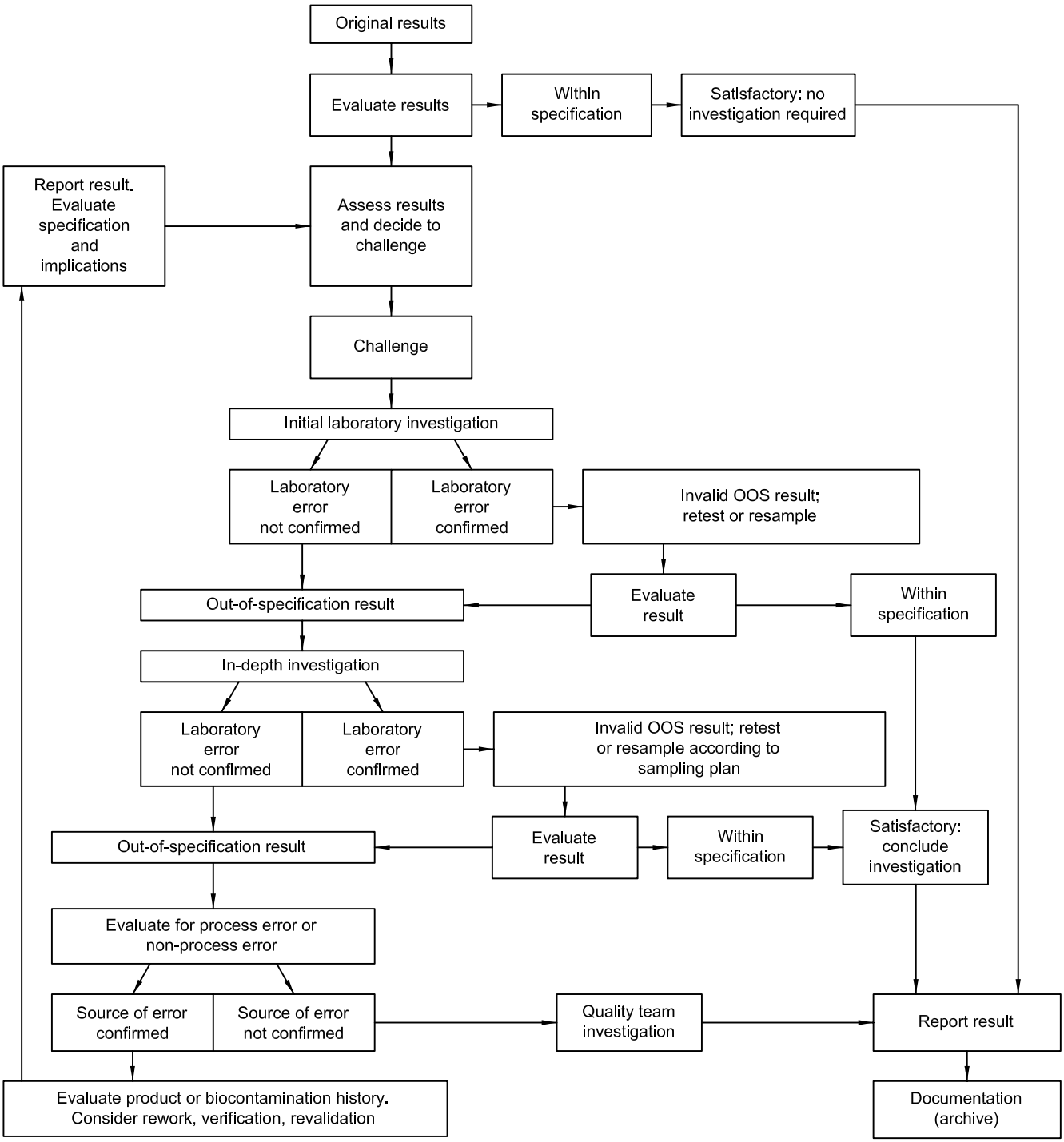

Figure 3 — Investigation of out-of-specification (OOS) result

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