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Biotechnology — Analytical methods — Risk-based approach for method selection and validation for rapid microbial detection in bioprocesses

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Patient safety is essential in providing cell-based therapies. However, novel cell-based therapies present many challenges with respect to the timely assessment of microbial contamination. Since many cell-based therapies have short shelf lives, they are administered to patients within hours after formulation. In addition to final product testing, testing on cell banks and product intermediates is common. Microbiological testing includes bacteria, fungi, mycoplasma and viral adventitious agents. Culture-based testing methods (e.g. pharmacopeia methods) have been widely adopted by industry. However, culture-based testing methods can take days to weeks to obtain a result. More rapid methods for microbiological testing are needed to ensure patient safety prior to product administration. The development and use of rapid, validated methods that are sensitive and accurate, and that allow for the detection of a broad range of microorganisms are therefore desired and supported by this document.

Biotechnology — Analytical methods — Risk-based approach for method selection and validation for rapid microbial detection in bioprocesses

1 Scope

This document provides guidance, a framework and a risk-based approach for the selection and validation of methods for rapid microbial detection in cellular therapeutic product manufacturing.

This document provides a flexible risk-based framework for the detection of microbial contamination in cellular therapeutic products and cellular intermediates.

This document provides general requirements and risks associated with cellular therapeutic product manufacturing, with flexibility to address differences in specific manufacturing processes of each unique cellular therapeutic product.

This document primarily addresses sterility testing in cellular therapeutic product manufacturing. This document is applicable to other cell-derived therapeutic product manufacturing.

This document focuses on rapid microbial test methods (RMTMs) used for both in-process and final product testing.

Viral testing in cellular therapeutic product manufacturing is not included in this document.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

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

3.1
acceptance criteria
numerical limits, ranges, or other attributes or variables meeting predefined performance for the assays described

Note 1 to entry: Acceptance criteria are specified by the *user requirement specifications* (3.30).

3.2
accuracy
measurement accuracy
closeness of agreement between a measured quantity value and an assigned quantity value of a measurand

Note 1 to entry: The concept “measurement accuracy” is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error.

Note 2 to entry: The term “measurement accuracy” should not be used for measurement trueness and the term measurement precision should not be used for “measurement accuracy”, which, however, is related to both these concepts.

Note 3 to entry: “Measurement accuracy” is sometimes understood as closeness of agreement between measured quantity values that are being attributed to the measurand.

[SOURCE: ISO 16140-1:2016, 2.2]

3.3
analytical sensitivity
quotient of the change in measurement indication and the corresponding change in value of a quantity being measured

Note 1 to entry: Analytical sensitivity should not be used to mean *detection limit* (3.8) or quantitation limit and should not be confused with *diagnostic sensitivity* (3.9).

[SOURCE: ISO 18113-1:2022, 3.2.4, modified — Admitted term “sensitivity of a measurement procedure” deleted. Notes 1 to 3 to entry deleted. Note 4 to entry renumbered as Note 1 to entry.]

3.4
analytical specificity
capability of a measuring system, using a specified measurement procedure, to provide measurement results for one or more measurands which do not depend on each other nor on any other quantity in the system undergoing measurement

Note 1 to entry: Lack of analytical specificity is call analytical interference

Note 2 to entry: Analytical specificity should not be confused with *diagnostic specificity* (3.10)

Note 3 to entry: ISO/IEC Guide 99:2007 uses the term “selectivity” for this concept instead of “specificity”.

[SOURCE: ISO 18113-1:2022, 3.2.5, modified — Admitted term “selectivity of a measurement procedure” deleted. Notes to entry replaced.]

3.5
aseptic
conditions and procedures used to exclude the introduction of microbial contamination

[SOURCE: ISO 18362:2016, 3.3, modified — “aseptic” replaced “aseptic technique” as the term.]

3.6
cellular therapeutic product
product containing cells as the active substance

EXAMPLE Cell and gene therapy products, tissue engineered products, drug products.

Note 1 to entry: Products produced from cells for gene therapies are included in the definition of cellular therapeutic product, as cells are not necessarily the active substance for all gene therapies.

Note 2 to entry: Recombinant proteins are not included in this definition of cellular therapeutic product.

[SOURCE: ISO 20399:2022, 3.9, modified — “used for cell therapy or gene therapy” deleted from the definition.]

3.7
design qualification
DQ
process for *verification* (3.32) that the proposed specification for the facility, equipment or system of the assay meets the expectation for the *user requirement specifications (URS)* (3.30)

[SOURCE: ISO 11139:2018, 3.220.1, modified — Abbreviated term “DQ” and “of the assay” added. “user requirement specifications (URS)” replaced “intended use”.]

3.8
detection limit
limit of detection
measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

Note 1 to entry: IUPAC recommends default values for α and β equal to 0,05.

Note 2 to entry: The abbreviation LOD is sometimes used.

Note 3 to entry: The term "sensitivity" is discouraged for "detection limit".

[SOURCE: ISO/IEC Guide 99:2007, 4.18]

3.9
diagnostic sensitivity
ability of an *in vitro* diagnostic examination procedure to identify the presence of a target marker associated with a particular disease or condition

Note 1 to entry: Also defined as percent positivity in samples where the target marker is known to be present.

Note 2 to entry: Diagnostic sensitivity is expressed as a percentage (number fraction multiplied by 100), calculated as 100 × the number of true positive values (TP) divided by the sum of the number of true positive values (TP) plus the number of false negative values (FN), or 100 × TP/(TP + FN). This calculation is based on a study design where only one sample is taken from each subject.

Note 3 to entry: For microbial detection, diagnostic sensitivity represents the fraction of target organisms that were detected correctly.

[SOURCE: ISO 18113-1:2022, 3.2.17, modified — "identify the presence of a target marker" replaced "have positive results". Second sentence of Note 1 to entry deleted. Note 3 to entry replaced.]

3.10
diagnostic specificity
ability of an *in vitro* diagnostic examination procedure to recognise the absence of a target marker associated with a particular disease or condition

Note 1 to entry: Also defined as percent negativity in samples where the target marker is known to be absent.

Note 2 to entry: Diagnostic specificity is expressed as a percentage (number fraction multiplied by 100), calculated as 100 × the number of true negative values (TN) divided by the sum of the number of true negative values (TN) plus the number of false positive values (FP), or 100 × TN/(TN+FP). This calculation is based on a study design where only one sample is taken from each subject.

[SOURCE: ISO 18113-1:2022, 3.2.18, modified — "recognise the absence of a target marker" replaced "have negative results". Second sentence of Note 1 to entry deleted. Note 3 to entry deleted.]

3.11
false negative
result indicated by the test method to be *negative* (3.15) which has subsequently been shown to contain the target microorganisms

[SOURCE: ISO 13843:2017, 3.14, modified — "microorganisms" replaced "organism".]

3.12
false positive
result indicated by the test method to be *positive* (3.19) which was subsequently shown not to contain the target microorganisms

[SOURCE: ISO 13843:2017, 3.15, modified — "microorganisms" replaced "organism".]

3.13
fit for purpose
in line with prearranged requirements for an intended use

[SOURCE: ISO 20387:2018, 3.24, modified — Admitted term "fitness for the intended purpose" and Note 1 to entry deleted.]

3.14
installation qualification
IQ
process of establishing by objective evidence that all key aspects of the process equipment and ancillary system for the assay instrument installation comply with the approved *user requirement specifications (URS)* (3.30)

[SOURCE: ISO 11139:2018, 3.220.2, modified — "for the assay instrument" added and "user requirement specifications (URS)" replaced "specification".]

3.15
negative
test result indicating the absence of the analyte in a given test portion as defined by the procedure of the method

[SOURCE: ISO 16140-1:2016, 2.43, modified — "negative" replaced "negative test result" as the term. "the absence of the analyte" replaced "the analyte was not detected" and "qualitative" deleted before "method".]

3.16
nucleic acid amplification techniques
NAT
biochemistry and molecular biology methods that involve the *in vitro* synthesis of many copies of DNA or RNA from one original template

Note 1 to entry: NAT is characterized by existence of reverse transcription, amplification method and type of determination (qualitative or quantitative)

Note 2 to entry: Examples of amplification methods are PCR and iso thermal amplification (NEAR, TMA, LAMP, HAD, CRISPER, SDA)

3.17
operational qualification
OQ
process of obtaining and documenting evidence that installed equipment operates within predetermined limits when used in accordance with its operational procedures

[SOURCE: ISO 11139:2018, 3.220.3]

3.18
performance qualification
PQ
process of establishing by objective evidence that the assay process, under anticipated conditions, consistently produces a result which meets all predetermined *user requirement specifications (URS)* (3.30)

[SOURCE: ISO 11139:2018, 3.220.4, modified — "assay" added before "process", "result" replaced "product" and "user requirement specifications (URS)" replaced "requirements".]

3.19
positive
test result indicating the presence of the analyte in a given test portion as defined by the procedure of the method

Note 1 to entry: When the reference method or alternative method provides a preliminary positive test result requiring further testing to confirm this result, this test result can be considered as a presumptive positive test result. If the further testing specified by the method's procedure confirms that the test result can indeed be considered as being positive, the test result can be considered as a confirmed positive test result.

[SOURCE: ISO 16140-1:2016, 2.50, modified — "positive" replaced "positive test result" as the term.]

3.20
precision
closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 2 to entry: The "specified conditions" can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-1).

[SOURCE: ISO/IEC Guide 99:2007, 2.15, modified — "precision" replaced "measurement precision" as the term. Notes 3 and 4 to entry deleted.]

3.21
qualification
activities undertaken to demonstrate that utilities, equipment and methods are suitable for their intended use and perform properly

Note 1 to entry: Qualification of equipment and/or processes generally includes *installation qualification* (3.14), *operational qualification* (3.17) and *performance qualification* (3.18).

[SOURCE: ISO 11139:2018, 3.220, modified — "or modes" deleted after "methods".]

3.22
rapid microbial test method
RMTM
analytical method that allows the user to get microbiology test results faster compared with traditional visual observation methods using direct inoculation and culture-plating

Note 1 to entry: Generally, this means in a significantly reduced time as compared with the traditional method (e.g. hours or days).

3.23
reference material
material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties

[SOURCE: ISO/IEC Guide 99:2007, 5.13, modified — Abbreviated term "RM", notes to entry and examples deleted.]

3.24
risk assessment
overall process of risk identification, risk analysis and risk evaluation

[SOURCE: ISO Guide 73:2009, 3.4.1]

3.25
risk control
process in which decisions are made and measures implemented by which risks are reduced to, or maintained within, specified levels

[SOURCE: ISO 14971:2019, 3.21]

3.26
risk-based approach
methodology that allows the prioritization of activities based on a previous analysis of data and according to the biosafety level

3.27
robustness
measure of a test method's capacity to remain unaffected by small, but deliberate, variations in method parameters and to provide an indication of its reliability during normal usage

[SOURCE: ICH Q2(R1)[11]]

3.28
shelf life
period of time after production during which a product that is kept under specified conditions retains its specified properties

[SOURCE: ISO 1382:2020, 3.485, modified — The term "storage life" deleted. "material or" deleted before "product" and "that is" added.]

3.29
sterility
state of being free from *viable microorganisms* (3.33)

Note 1 to entry: In practice, no such absolute statement regarding the absence of microorganisms can be proven.

[SOURCE: ISO 11139:2018, 3.274]

3.30
user requirement specifications
URS
requirements specific to a user or requirements that are not covered in general requirements

3.31
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.13, modified — Notes to entry deleted.]

3.32
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.12, modified — Notes to entry deleted.]

3.33
viable microorganism
microorganism within a sample that has at least one attribute of being alive (e.g. metabolically active, capable of reproduction, possession of an intact cell membrane, with the capacity to resume these functions) defined based on the intended measurement purpose

4 General considerations

Prior to patient administration, cellular therapeutic products should be tested for microbial contamination. Many of these products rely on the activity of viable cells for a therapeutic effect. Viable cells cannot be terminally sterilized and rely on a combination of aseptic techniques and closed-system manufacturing to ensure sterility of the final product. These products typically have a relatively short shelf life and are manufactured as single lots or small lots, presenting challenges for utilizing compendial or culture-based methods for detecting microbial contamination.[35][36][37][38]

When selecting a rapid method, the following should be taken into account:

a) the shelf life of the sample;

b) the volume of sample available for testing;

c) the number of samples to be tested;

d) the manufacturing step from which the sample will be collected;

e) the time to result;

f) the microorganisms to be detected;

g) how to distinguish viable from non-viable microorganisms;

h) the ability to speciate microbes that are identified in the sample.

In addition, it is important to take the availability of resources to conduct the tests into account such as trained personnel and required instrumentation.

NOTE 1 It is important to note that sampling can introduce microbial contamination into the manufacturing process.

NOTE 2 The amount of material available for testing can be limited, especially for autologous cellular therapeutic products. In some cases, parallel cellular therapeutic products can be manufactured to assess microbial contamination.

NOTE 3 Test methods can require specific training and experience to be conducted and analysed.

It is recommended to consider adding cell supernatants (e.g. culture solution, washing solution, frozen stock solution) instead of cell-containing terminations for sterility testing, to solve the problem of small samples of cell products that cannot be sampled for testing.

5 Risk management for microbiological contamination

5.1 Risk management in manufacturing process

A risk-based approach for determining methods to detect microbial contamination in cellular therapeutic product manufacturing should be used. It should take into account the source and method used for the collection of the cellular starting material.[1][2][3][4]

Potential sources of microbial contamination of cellular therapeutic products include, but are not limited to, cellular starting material, raw materials and consumables, and the manufacturing environment.[5]

Apheresis products are the most common source of cellular starting materials. Sources of microbial contamination can be associated with the incomplete disinfection of the skin, sterility failures in kits and bags used to collect and store the apheresis products, and technician/operator error. Donor bacteraemia can also be a source of contamination for the apheresis product.

Microbial contamination and infectious viruses in reagents, ancillary (raw) materials and recommendations for ancillary materials are described in ISO 20399. Consumables should be pre-sterilized and single use to reduce the risk of microbial contamination.[6]

Cell processing/manufacturing should be performed in a closed system or an appropriate clean room (e.g. ISO 6 to ISO 7) to prevent microbial contamination.

NOTE Open or benchtop processes increase the risk of contamination from the air and surfaces that possibly have not been adequately cleaned or disinfected.

Some general factors to be taken into account in a risk assessment for RMTMs are outlined in Annex A. Detailed points to take into account in some critical decisions for the use of RMTMs in cellular therapeutic product manufacturing that require a risk assessment can be found in Annexes B, C, D and E.[6][7]

Environmental controls can minimize the risk of microbial contamination. Examples of environmental controls are:

— sanitization procedures;

— high efficiency particulate air (HEPA) filtration and air flow;

— gowning procedures;

— aseptic technique;

— clean-room procedures and classifications (in accordance with ISO 14644-1).

Points to take into account when developing risk control can include, but are not limited to:

a) input materials:
   1) collection process and donor selection (see Annex B);
   2) autologous or allogenic, fresh or frozen;
   3) conditions;

b) ancillary materials in accordance with ISO 20399 and consumables (pre-sterilized, single use, etc.);

c) environmental factors;

d) equipment;

e) process steps:
   1) closed or open process steps;
   2) cell banking;
   3) culture or expansion;
   4) purification;
   5) final product;

f) containment strategy (see Annex C);

g) monitoring (see Annex C);

h) storage, packaging and administration (see Annex D);

i) in-process and final-release testing (see Annex E).

5.2 Risk management in microbial testing

The use of a risk assessment approach to rapid microbial testing in cellular therapeutic product manufacturing can limit the risk of validation of a rapid microbial testing system.[8][9][10][11] A risk-based approach can be used to establish the most appropriate rapid microbial testing mechanism for intended use. This often focuses on determining the user requirement specifications (URS) as a foundation.

NOTE The following documents discuss risk assessment and give general guidance on how to implement it in manufacturing processes:

— ISO 31000;

— ISO 13022;

— ISO Guide 73.

6 Selection of a fit-for-purpose assay

6.1 General

Well-defined user requirements and a clear understanding of an assay's intended use(s) guide the design of assays with biological relevance and sufficient performance (e.g. selectivity, analytical sensitivity, analytical specificity, precision, accuracy, robustness) to enable subsequent decision-making (fit for the intended purpose or fit for purpose).

To identify or develop an appropriate assay for detecting microbial contamination in cellular therapeutic products, the goal of testing should be established and documented. For example, detection versus quantification. If a test is needed to detect and identify "every" bacterial or fungal contaminant, then a sequencing approach should be used. If a test is needed to determine taxonomic or quantity resolution of only certain reference microorganisms or a limited list of compendial microorganisms,[35][36][37][38] then multiplexed PCR or a similar targeted approach is most suitable.

Appropriate assay design shall include specifications for the test method and strategies to ensure measurement quality and reproducibility of results. This can include incorporating replicate measurements, using sample randomization to reduce biases, and the inclusion of appropriate measurement controls.

Appropriate assay design shall also include approaches to ensure an appropriate analytical sensitivity and an established and documented detection limit. The uncertainty of the measurement should also be established and documented.

The assay shall have a high selectivity for the measurement target without significant interference from other components in the cell preparation.

The intended use of the assay should guide the fit-for-purpose requirements of the measurement. The uncertainty of measurement should be taken into account.

To determine the appropriate assays, users shall assess the issues of the number and types of microorganisms required for testing. The extent necessary for identification shall be determined. There shall be an assessment made as to whether a determination between viable and non-viable microbial cells is needed.

The assay should be sufficiently robust so that the results are not significantly affected by small changes in the measurement process (e.g. temperature fluctuations, minor sample handling fluctuations) as defined by the user for the intended purpose.

The assay should be sufficiently robust for the measurement target so that the results are not significantly affected by small changes in other components of the cell preparation (e.g. serum