



Associazione Farmaceutici Industria  
Società Scientifica

# ***L'EVOLUZIONE DEL REGOLATORIO NELLE TERAPIE AVANZATE***

WEBINAR

**MERCOLEDÌ 9 APRILE 2025  
14:30 - 18:00**

**USP <72> e USP <73>: metodi compendiali innovativi come supporto  
per il rilascio rapido di ATMP con short shelf-life**

***Lucia Ceresa – AFI & PDA Italy Chapter***



# Disclaimer & Sources

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When the text is extracted as is from the Annex 1 or other GMP, it is reported in Italics. However, highlights & bold fonts are from the author to focus the attention on specific words and concepts.



United States  
Pharmacopeia



# Insights from Sterility guidance's

USP <72> - <73> & <1071>

Vs

Ph. Eur. 2.6.1. - USP <71> - JP 4.06



Associazione Farmaceutici Industria  
Società Scientifica

# USP <72> & <73>

United States  
Pharmacopeia



## USP-NF <72> Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

## USP-NF <73> ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

13/02/2025 15:23 USP-NF <72> Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

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USP-NF <72>

### RESPIRATION-BASED MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

#### INTRODUCTION

This chapter is intended to be used as a risk-based test for the detection of microbial contamination in short-life products and encompasses short shelf-life products and/or short manufacturing times where the product must be administered as soon as possible. (See [USP <1071> Microbiological Methods for the Detection of Contamination in Short-Life Products—A Risk-Based Approach](#) [1071] for more details.) It may also be used as an in-process control for the testing of product intermediates, cell culture media, or process solutions. Growth-based methods that use detection signals other than visible signs of microbial growth or precipitation within the culture media (i.e., turbidity, particle formation, or floccular growth) have been used to test a variety of products. Instrumentation for respiration-based methods based on online carbon dioxide (CO<sub>2</sub>) detection, colorimetric detection, or fluorescence detection have been used to detect an exponential increase in carbon dioxide as an indicator of microbial growth. The application of respiration-based methods for the detection of contamination in a variety of short-life products, such as cell therapy products, has been published in peer-reviewed literature. The primary advantages of respiration-based methodologies are 1) the broad equivalency to compendial methods and 2) the automated and periodic monitoring for earlier detection of microbial growth. Further benefits may include the direct inoculation of cultures without interference from product-related turbidity, the automation of data analysis, acquisition, reporting, and archiving, and the isolation of the organism for identification and investigation. Like all culture-based methods, disadvantages include the inability to detect microorganisms that do not grow under the culture conditions utilized. It is the end user's responsibility to select the respiration method that is the most appropriate for the product.

#### CULTURE MEDIA AND INCUBATION CONDITIONS

For recovery of aerobic microorganisms, culture media is to be incubated at 20°–25° and 30°–35°, and for the recovery of anaerobic microorganisms, culture media is to be incubated at 30°–35°. Other or additional incubation temperature(s) ranging from 20°–37° can be applied where relevant to appropriately recover potential microorganisms associated with the product/manufacturing process based on risk and a suitable justification.

The choice of culture media and incubation conditions should be risk-based in consideration of manufacturing process parameters (e.g., temperature, oxygenation level). For example, for fully aerobic processes the evaluation of anaerobic microorganisms required. Visual inspection of culture media containers may be required at the end of incubation for the detection of their presence was not detected by the system during method suitability, even when mold balls were visible.

#### Growth Promotion Test of Aerobes, Anaerobes, and Fungi

Perform growth promotion of culture media using the strains of microorganisms listed in [Table 1](#). Inoculate for not more than 3 days in the case of fungi. Seed lots are used so that the viable microorganisms used for inoculation are removed from the original master seed lot. The culture media are suitable if all microorganisms are detected.

#### METHOD SUITABILITY TEST

As a prerequisite, a primary validation of the method must be available as described in [USP <1071> \[1071\]. The method must demonstrate growth of the test microorganisms inoculated at not more than 10 CFU per test culture media container containing the product. In cases where a sample pretreatment is necessary, test microorganisms are added prior to treatment. The microorganisms used for the method suitability should include the test strains listed in \[Table 1\]\(#\) and a selection of test strains relevant to the product/manufacturing process supported by a suitable justification. Inclusion of slow growing microorganisms and/or local isolates may be included if relevant for the product risk. Use of appropriate culture collection microorganisms that are comparable to in-house isolates are acceptable.](#)

Each microorganism used for method suitability should have an associated positive control of the microorganism without product. The suitability test should also include negative controls such as uninoculated culture media with unimpaled product for each culture media type and incubation condition. Incubate until all tested microorganisms have been detected. Record the time to detection in hours. The inoculum control of each microorganism should be reported to demonstrate a level of not more than 10 CFU. Considering the variability of the microbial inoculum and assuming it follows a Poisson distribution, it is possible that a low sample size targeting not more than 10

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USP-NF <73> ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

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USP-NF <73>

### ATP BIOLUMINESCENCE-BASED MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

#### INTRODUCTION

This chapter is intended to be used as a risk-based test for the detection of microbial contamination in short-life products and encompasses short shelf-life products and/or short manufacturing times where the product must be administered as soon as possible. (See [USP <1071> Microbiological Methods for the Detection of Contamination in Short-Life Products—A Risk-Based Approach](#) [1071] for more details.) It may also be used as an in-process control for the testing of product intermediates, cell culture media, or process solutions. Growth-based methods using detection signals other than visible signs of microbial growth or precipitation within the culture media (i.e., turbidity, particle formation, or floccular growth) include adenosine triphosphate (ATP) bioluminescence measurement for the detection of growth in liquid culture media (nutrient broth) or the detection of colonies on membranes placed on solid culture media. All viable cells, including microorganisms, contain ATP at various levels. ATP bioluminescence detection of microorganisms is based on the fact that luciferase, an enzyme that catalyzes the oxidation of luciferin, is present in all living cells. Measured light is interpreted in a readout signal such as relative light units (RLU) or counts per charge coupled device (CCD) pixel. ATP bioluminescence-based methods for detecting viable microorganisms have a long history of use in the pharmaceutical, over-the-counter medicines, and cosmetic industries. The application of ATP-based methods for the detection of contamination in a variety of products has been published in peer-reviewed literature. The primary advantages of these ATP bioluminescence-based methodologies for detecting viable microorganisms are 1) the broad equivalency to compendial methods, 2) early detection of microbial growth, and 3) isolation of the organism for identification. Further benefits may include the detection of cultures without interference from product-related turbidity, the automation of data analysis, reporting, and archiving. Disadvantages of these methods include the inability to detect microorganisms that do not grow under the culture conditions utilized and products with significant ATP background that cannot be reduced.

#### CULTURE MEDIA AND INCUBATION TEMPERATURES

For recovery of aerobic microorganisms, culture media is to be incubated at 20°–25° and 30°–35° and for the recovery of anaerobic microorganisms, culture media is to be incubated at 30°–35°. Other or additional incubation temperatures ranging from 20°–37° can be applied, where relevant, to appropriately recover potential microorganisms associated with the product/manufacturing process based on risk and a suitable justification.

The choice of culture media and incubation conditions should be risk-based in consideration of manufacturing process parameters (e.g., temperature, oxygenation level). For example, for fully aerobic processes the evaluation of anaerobic microorganisms may not be required.

Culture media with relatively consistent low ATP levels should be sourced.

#### GROWTH PROMOTION TEST OF AEROBES, ANAEROBES, AND FUNGI

Perform growth promotion of culture media using the strains of microorganisms listed in [Table 1](#). Inoculate at not more than 100 colony-forming units (CFU) per test culture media container. Incubate for not more than 3 days for bacteria or not more than 5 days for fungi. Use seed lots so that the viable microorganisms used for inoculation are not more than 5 passages removed from the original master seed lot. The culture media are suitable if all microorganisms are detected.

The liquid culture media are suitable if ATP bioluminescence is detected at the predefined positive cutoff level compared to the background of the culture media without microbial inoculation. For solid culture media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum.

#### METHOD SUITABILITY TEST

As a prerequisite, a primary validation of the method must be available as described in [USP <1071> \[1071\]. The ATP bioluminescence-based method must demonstrate growth of the test microorganisms inoculated at not more than 10 CFU into the culture media containing the product. In case a sample pretreatment is necessary, test microorganisms are added prior to the treatment. The microorganisms used for the method suitability should include the test strains listed in \[Table 1\]\(#\) and a selection of test strains relevant to the product/manufacturing process supported by a suitable justification. Inclusion of slow growing microorganisms and/or local isolates may be included if relevant for the product risk. Use of appropriate culture collection microorganisms that are comparable to in-house isolates are acceptable.](#)

USP <1071>



# RAPID MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT- LIFE PRODUCTS—A RISK-BASED APPROACH

- It is widely recognized that the **current** growth-based sterility tests with an incubation period of at least 14 days **(Sterility Tests <71> are not suitable for products with a short shelf-life or for products prepared for immediate use**, which are usually infused into patients due to clinical needs before completion of the sterility test<sup>(1)</sup>.

(see Sterility Tests {71}; Sterility Assurance {1211}; Validation of Alternative Microbiological Methods {1223}; Microbiological Chapters—Glossary {1117.1})

## Change to read:

(1071)\*RAPID MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS—A RISK-BASED APPROACH

## INTRODUCTION

It is widely recognized that the current growth-based sterility tests with an incubation period of at least 14 days (see [Sterility Testing \(71\)](#)) are not suitable for products with a short shelf life or for products prepared for immediate use, which are usually infused into patients due to clinical needs before completion of the sterility test (72). These products may include (but is not exhaustive) compounded sterile preparations (CSPs), nuclear medicine products, and Advanced Therapy Medicinal Products (ATMPs). For a general discussion on the factors influencing sterility testing that contribute to sterility assurance, see [Sterility Assurance \(73\)](#). If a microbiological test is conducted, performed several days before the completion of a test that detects microbial contamination prior to product use.

The utilization of rapid microbiological methods (RMMs) to detect microbial contamination should be risk-based; select the preferred technology for the intended use and product balancing user requirement specifications (URS) + specifically, limit of detection (LOD), sample size, and critical attributes. For example, many radiopharmaceuticals use a real-time microbiological test due to the short half-life of radiotracers, while (SDS) and from an overnight test in one that is completed within, e.g., 48 h due to their short beyond use date (BUD). The choice of the RMM should factor in assay sensitivity and reliability versus sensitivity to detect the presence of a low-level contaminant and should go so far as to conduct administration. Contacting the regulatory authorities for further guidance.

# CONTAMINANT

Selecting an appropriate technology for the testing is a decision. Whereas a rapid time to result and the ability to detect a large amount and wide range of drugs may be necessary and other URP-aligned, not all user requirement specifications. Different technologies to be considered may include

- A rapid result time, ideally in real time
- Ability to detect a low quantity of the product to be administered
- Ability to detect a wide range of microorganisms. Although all the analytical platforms should have the ability to detect a wide range of microorganisms, it is important to demonstrate that the ISMM is capable of detecting common outbreaks, facility isolates, and product recalls associated with the manufacturer.
- A small sample size, ideally less than 100 µl. Articles tested and quantity per container tested that does not consume a large proportion of the container. Where possible, manufacturers should consider available sampling points that would provide the most representative sample during process design.
- The ability to collect multiple samples at once from the same or different batches.
- The ability to handle, e.g., closed systems to reduce inadvertent contamination during testing.
- The use of appropriate instruments and reagents.
- The use of reference material and controls appropriate for the technology.
- The clarity of use/complexity of test and data interpretation.
- Low rates of false positive and false negative results.
- Improved patient safety arising from:
  - Completion of the test prior to administration
  - Tests that provide progressive monitoring and reports of contamination detection
- Ability to identify the detected microorganism to help guide clinical therapy and for investigating a contamination source.
- Robustness and reliability of equipment and reagents used in the testing.
- Sample preparation suitable for both manual and automated methods.
- Automated and automated continuous/periodic monitoring.

# Compendial & Harmonized Sterility ...

- Growth-based, **14-day** incubation but often longer up to **21-day**!
- Based on **visual, human analysis**
- Four-eyes principle requires additional personnel and still **based on human decision**
- Prone to **interpretation** and **transcription error**



*Could we **eliminate** this subjectivity?*

# USP <1071> & <1211>

For a general discussion on the factors (other than sterility testing) that contribute to **sterility assurance** (see Sterility Assurance <1211>).

... The sterility of a lot purported to be sterile is therefore defined in **probabilistic terms**, where the likelihood of a contaminated unit or article is acceptably remote. Such a state of sterility assurance **can be established only** through the use of adequate sterilization cycles and subsequent aseptic processing, if any, under appropriate current good manufacturing practice, and **not by reliance solely on sterility testing.**

USP <1211> STERILIZATION AND STERILITY ASSURANCE OF COMPENDIAL ARTICLES





# STERILITY TEST: last in a Series of Critical Controls !



- The sterility test applied to the finished product should only **be regarded as the last in a series of critical control measures** by which sterility is assured.<sup>(1)</sup>
- **It cannot be used to assure sterility of a product that does not meet its design, procedural or validation parameters.**<sup>(1)</sup>
- ... **quality cannot be tested into products; it should be built-in or should be by design.**<sup>(2,3)</sup>

<sup>(1)</sup> Annex 1 2022, 10.5

<sup>(2)</sup> PAT - Process analytical technology CGMPs September, FDA 2004:  
<https://www.fda.gov/media/71012/download>

<sup>(3)</sup> PAT - Process analytical technology (Ph. Eur. 5.25) Effective from 1 Jan. 2020



## Products (list is not exhaustive):

- **compounded sterile preparations** (CSPs),
  - **nuclear medicine products**,
  - **Advanced Therapy Medicinal Products (ATMPs)**.
- The utilization of rapid microbiological methods (**RMMs**) to detect microbial contamination should be **risk-based**...
  - When considering the risk to the patient, the choice of the RMM should factor in assay sensitivity and reliability versus the time to detection.
  - The risk assessment should contain a **justification** as to why the RMM is considered **appropriate** for the product or material being tested and state the **risk/benefit** to applying the RMM.
  - ... on the factors (other than sterility testing) that contribute to sterility assurance, see Sterility Assurance <1211>.

(see Sterility Tests <71>; Sterility Assurance <1211>; Validation of Alternative Microbiological Methods <1223>; Microbiological Chapters—Glossary <1117.1>)

Change to read:

## <1071>\*RAPID MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS—A RISK-BASED APPROACH

### INTRODUCTION

It is widely recognized that the current growth-based sterility tests with an incubation period of at least 14 days (see [Sterility Tests <71>](#)) are not suitable for products with a short shelf life or for products prepared for immediate use, which are usually infused into patients due to clinical needs before completion of the sterility test (2). These products may include (but is not exhaustive) compounded sterile preparations (CSPs), nuclear medicine products, and Advanced Therapy Medicinal Products (ATMPs). For a general discussion on the factors (other than sterility testing) that contribute to sterility assurance, see [Sterility Assurance <1211>](#). If a microbiological test is conducted, patient safety is best served through the completion of a test that detects microbial contamination prior to product use.

The utilization of rapid microbiological methods (RMMs) to detect microbial contamination should be risk-based, so the stakeholder can select the preferred technology for the intended use whilst balancing user requirement specifications (URS) that include time to result, specificity, limit of detection (LOD), sample size, and product attributes. For example, many radiopharmaceuticals would benefit most from the use of a real-time microbiological test due to the short half-life of radionuclides, while CSPs and autologous cell therapies would benefit from an overnight test or one that is completed within, e.g., 48 h due to their short beyond-use dating. When considering the risk to the patient, the choice of the RMM should factor in assay sensitivity and reliability versus the time to detection. Assays should be reasonably sensitive to detect the presence of a low-level contaminant and should do so in a time frame that allows results to be available before product administration. Contacting the regulatory authority for further guidance is recommended.

### USER REQUIREMENT SPECIFICATIONS FOR A RAPID MICROBIOLOGICAL METHOD FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

Selecting an appropriate technology for the testing of a short-life product should be a risk-based decision. Whereas a rapid time to result and the ability to detect a low amount and wide range of microorganisms reliably should be targeted, not all user requirement specifications should be met. The following are key considerations for the selection of a RMM for short-life products.

- A rapid result time, ideally in real time or less than 24 h, preferably before the product is administered
- Ability to detect a low quantity of microorganisms in the test sample
- Ability to detect a wide range of viable microorganisms in a product. Although all the analytical platforms should have the ability to detect a wide range of bacteria, yeasts, and molds, it is important to demonstrate that the RMM is capable of detecting microorganisms implicated in sterility test failures, infection outbreaks, facility isolates, and product recalls associated with the manufacturing process risk.
- Sample quantity, i.e., minimum number of articles tested and quantity per container tested that does not consume a large proportion of the available product; whenever feasible, manufacturers should consider available sampling points that would provide the most meaningful results, along with assay requirements during process design
- Ability of the method to test multiple samples at once from the same or different batches
- Aseptic test material handling, e.g., closed systems to reduce inadvertent contamination during testing
- Availability of instruments and reagents
- Availability of reference material and controls appropriate for the technology
- Ease of use/simplicity of test and data interpretation
- Low rates of false positive and false negative results
- Improved patient safety arising from:
  - Completion of the test prior to administration
  - Tests that provide progressive monitoring and reports of contamination detection
- Ability to identify the detected microorganisms to help guide clinical therapy and for investigating a contamination source
- Robustness and reliability of equipment and reagents used in the testing
- Sample preparation suitable for both manual and automated methods
- Automation and automated continuous/periodic monitoring

- ...so the stakeholder can select the **preferred technology** for the **intended use** whilst balancing user requirement specifications (**URS**) that include :
  - time to result,
  - specificity,
  - limit of detection (LOD),
  - sample size,
  - product attributes.
- ... for example... **CSPs** and **autologous cell therapies** would benefit from an overnight test or one that is completed within, e.g., 48 h due to their short beyond-use dating.

## Assays:

- reasonably sensitive to detect the presence of a **low-level contaminant** and should do so **in a time frame** that allows results to be available **before product administration**.

Contacting the regulatory authority for further guidance is recommended.

Change to read:

## <1071>\*RAPID MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS—A RISK-BASED APPROACH

### INTRODUCTION

It is widely recognized that the current growth-based sterility tests with an incubation period of at least 14 days (see [Quality Tests/211](#)) are not suitable for products with a short shelf life or for products prepared for immediate use, which are usually infused into patients due to clinical needs before completion of the sterility test (2). These products may include (but is not exhaustive) compounded sterile preparations (CSPs), nuclear medicine products, and Advanced Therapy Medicinal Products (ATMPs). For a general discussion on the factors (other than sterility testing) that contribute to sterility assurance, see [Quality Assurance/1211](#). If a microbiological test is conducted, patient safety is best served through the completion of a test that detects microbial contamination prior to product use.

The utilization of rapid microbiological methods (RMMs) to detect microbial contamination should be risk-based, so the stakeholder can select the preferred technology for the intended use whilst balancing user requirement specifications (URS) that include time to result, specificity, limit of detection (LOD), sample size, and product attributes. For example, many radiopharmaceuticals would benefit most from the use of a real-time microbiological test due to the short half-life of radionuclides, while CSPs and autologous cell therapies would benefit from an overnight test or one that is completed within, e.g., 48 h due to their short beyond-use dating. When considering the risk to the patient, the choice of the RMM should factor in assay sensitivity and reliability versus the time to detection. Assays should be reasonably sensitive to detect the presence of a low-level contaminant and should do so in a time frame that allows results to be available before product administration. Contacting the regulatory authority for further guidance is recommended.

### USER REQUIREMENT SPECIFICATIONS FOR A RAPID MICROBIOLOGICAL METHOD FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

Selecting an appropriate technology for the testing of a short-life product should be a risk-based decision. Whereas a rapid time to result and the ability to detect a low amount and wide range of microorganisms reliably should be targeted, not all user requirement specifications listed below may be necessary and other URS may be additionally considered. URS of different technologies to be considered may include:

- A rapid result time, ideally in real time or less than 24 h, preferably before the product is administered
- Ability to detect a low quantity of microorganisms in the test sample
- Ability to detect a wide range of viable microorganisms in a product. Although all the analytical platforms should have the ability to detect a wide range of bacteria, yeasts, and molds, it is important to demonstrate that the RMM is capable of detecting microorganisms implicated in sterility test failures, infection outbreaks, facility isolates, and product recalls associated with the manufacturing process risk.
- Sample quantity, i.e., minimum number of articles tested and quantity per container tested that does not consume a large proportion of the available product; whenever feasible, manufacturers should consider available sampling points that would provide the most meaningful results, along with assay requirements during process design
- Ability of the method to test multiple samples at once from the same or different batches
- Aseptic test material handling, e.g., closed systems to reduce inadvertent contamination during testing
- Availability of instruments and reagents
- Availability of reference material and controls appropriate for the technology
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  - Completion of the test prior to administration
  - Tests that provide progressive monitoring and reports of contamination detection
- Ability to identify the detected microorganisms to help guide clinical therapy and for investigating a contamination source
- Robustness and reliability of equipment and reagents used in the testing
- Sample preparation suitable for both manual and automated methods
- Automatic and automated continuous/periodic monitoring

## USER REQUIREMENT SPECIFICATIONS FOR A RAPID MICROBIOLOGICAL METHOD FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

- A rapid result time
- Ability to detect, a low quantity of microorganisms in the test sample
- Ability to detect a wide range of viable microorganisms in a product.
  - ... ability to detect a wide range of bacteria, yeasts, and molds, it is important to demonstrate that the RMM is capable of detecting microorganisms implicated in ***sterility test failures***, ***infection outbreaks***, ***facility isolates***, and ***product recalls*** associated with the ***manufacturing process risk***.

Change to read:

### (1071)\*RAPID MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS—A RISK-BASED APPROACH

#### INTRODUCTION

It is widely recognized that the current growth-based sterility tests with an incubation period of at least 14 days (see [Sterility Tests/21](#)) are not suitable for products with a short shelf life or for products prepared for immediate use, which are usually infused into patients due to clinical needs before completion of the sterility test (2). These products may include (but is not exhaustive) compounded sterile preparations (CSPs), nuclear medicine products, and Advanced Therapy Medicinal Products (ATMPs). For a general discussion on the factors (other than sterility testing) that contribute to sterility assurance, see [Quality Assurance/1211](#). If a microbiological test is conducted, patient safety is best served through the completion of a test that detects microbial contamination prior to product use.

The utilization of rapid microbiological methods (RMMs) to detect microbial contamination should be risk-based, so the stakeholder can select the preferred technology for the intended use whilst balancing user requirement specifications (URS) that include time to result, specificity, limit of detection (LOD), sample size, and product attributes. For example, many radiopharmaceuticals would benefit most from the use of a real-time microbiological test due to the short half-life of radionuclides, while CSPs and autologous cell therapies would benefit from an overnight test or one that is completed within, e.g., 48 h due to their short beyond-use dating. When considering the risk to the patient, the choice of the RMM should factor in assay sensitivity and reliability versus the time to detection. Assays should be reasonably sensitive to detect the presence of a low-level contamination and should do so in a time frame that allows results to be available before product administration. Consulting the regulatory authority for further guidance is recommended.

#### USER REQUIREMENT SPECIFICATIONS FOR A RAPID MICROBIOLOGICAL METHOD FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

Selecting an appropriate technology for the testing of a short-life product should be a risk-based decision. Whereas a rapid time to result and the ability to detect a low amount and wide range of microorganisms reliably should be targeted, not all user requirement specifications listed below may be necessary and other URS may be additionally considered. URS of different technologies to be considered may include:

- A rapid result time, ideally in real time or less than 24 h, preferably before the product is administered
- Ability to detect, a low quantity of microorganisms in the test sample
- Ability to detect a wide range of viable microorganisms in a product. Although all the analytical platforms should have the ability to detect a wide range of bacteria, yeasts, and molds, it is important to demonstrate that the RMM is capable of detecting microorganisms implicated in sterility test failures, infection outbreaks, facility isolates, and product recalls associated with the manufacturing process risk.
- Sample quantity, i.e., minimum number of articles tested and quantity per container tested that does not consume a large proportion of the available product; whenever feasible, manufacturers should consider available sampling points that would provide the most meaningful results, along with decay requirements during process design
- Ability of the method to test multiple samples at once from the same or different batches
- Aseptic test material handling, e.g., closed systems to reduce inadvertent contamination during testing
- Availability of instruments and reagents
- Availability of reference material and controls appropriate for the technology
- Ease of use/ simplicity of test and data interpretation
- Low rates of false positive and false negative results
- Improved patient safety arising from:
  - Completion of the test prior to administration
  - Tests that provide progressive monitoring and reports of contamination detection
- Ability to identify the detected microorganisms to help guide clinical therapy and for investigating a contamination source
- Robustness and reliability of equipment and reagents used in the testing
- Sample preparation suitable for both manual and automated methods
- Automation and automated continuous/periodic monitoring



## CRITICAL OPERATING PARAMETERS TO BE USED IN DETERMINING A RISK-BASED RAPID MICROBIOLOGICAL METHOD FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

- The estimated operating parameters:
  - LOD,
  - time to results,
  - sample size

for the candidate technologies **suitable for an RMM** can be obtained from various sources: they may be shared by system vendors, found in literature reports, or **determined by proof-of-concept studies executed by either the stakeholder or a contract laboratory.**

## EXAMPLE TECHNOLOGIES FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

- Adenosine triphosphate (ATP) bioluminescence (USP <73>)
- Nucleic acid amplification
- Respiration (USP <72>)
- Solid phase cytometry

others may apply based on the stakeholder's URS evaluation.



Table 1. Typical Operational Parameters for Examples of Candidate Rapid Microbiological Technologies

Technology	LOD (CFU)	Time to Result	Sample Size Range <sup>b</sup> (mL)
Chapter <71> (for comparative purposes only)	Theoretical LOD of 1–3 CFU based on a Poisson distribution	14 days	Refer to <71>, Table 2 and Table 3
Adenosine triphosphate (ATP) bioluminescence	1–10 <sup>a</sup>	2–7 days (including pre-enrichment)	0.1–1000
Nucleic acid-based amplification	10–100	2–4 h	0.2–2
Respiration	1–10 <sup>a</sup>	Overnight to 7 days	Up to 10 per bottle
Solid phase cytometry	1–10	2–8 h	1–1000

<sup>a</sup> LOD for growth-based methods are achievable considering the enrichment of the sample.

<sup>b</sup> Quantity of product that can be tested for the given technology

# How much is the “compendial” sterility test sensitivity?



**$10^6$  CFU**

Human eyes sensitivity !

The smallest little “dot” on the agar plate!



Limit of “detection” = 1-10 CFU

**$10^{-17}$  ATP M**

Instrument/reagents sensitivity !

## METHOD VALIDATION AND SUITABILITY TESTING

- Primary validation should demonstrate that the RMM system is adequate in detecting the intended target and must characterize the principle of detection.
- If the RMM **is not** described in a USP general method chapter, then a validation is required according to <1223> (Ph.Eur. 5.1.6)
- If the RMM **is** described in a USP general method chapter, in addition to the primary validation, the respective USP chapter would apply.
- For release sterility testing, regulators may request additional validation data and contacting the regulatory agency for further guidance is recommended.
- **The method suitability must be demonstrated for each product being tested.**
- The detection of the challenge organisms in the presence of product must be demonstrated. It is recommended that an appropriate number of lots of product be determined prospectively for suitable testing to enable an assessment for the potential of lot-to-lot variability and justified in a risk assessment.
- With signals other than the colony-forming unit derived from laboratory culture ... method suitability testing should also verify if the sample **interferes** with the assay.

# Ph. Eur. 5.1.6, **draft** April 2025

## 5.1.6. ALTERNATIVE METHODS FOR CONTROL OF MICROBIOLOGICAL QUALITY

► This general chapter is published for information. ◄

DRAFT

### § 3-9. ROUTINE APPLICATION OF THE ALTERNATIVE MICROBIOLOGICAL METHOD

- The user must ensure that the alternative method is fit for the intended purpose during routine use throughout its lifecycle.
- Suitability tests are designed to control critical parameters that may have an impact on the method (including reagent quality, consumable quality and test environment).
- They depend on the purpose of the method and are based on an understanding of the alternative method (including risk assessment and prior knowledge).
- Suitability testing is typically conducted with one or more predefined materials (including positive and/or negative controls)

Pharmeuropa 37.2



# Ph. Eur. 5.1.6, draft 2025

Table 5.1.6.-2 – Performance characteristics criteria for qualitative, quantitative and identification tests

Performance characteristics	Qualitative test	Quantitative test	Identification test
Accuracy	○ <sup>(1)</sup>	●	●
Precision (including repeatability and intermediate precision)	○	●	○
Specificity	●	●	●
Detection limit	●	●	○
Quantitation limit	○	●	○
Linearity	○	●	○
Range	○	●	○
Robustness	●	●	●

ion

• (1) An accuracy study of the alternative microbiological method with respect to the pharmacopoeial method can be performed instead of the validation of the detection limit. •

• ● – this characteristic is normally tested •

• ● – this characteristic may be needed in some cases •

• ○ – this characteristic is normally not relevant •



Associazione Farmaceutici Industria  
Società Scientifica

# USP <72> & <73>

United States  
Pharmacopeia



## USP-NF <72> Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

## USP-NF <73> ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

13/02/2025 15:23 USP-NF <72> Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

Revised on: Mon Feb 03 2025 13:02:18 pm  
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USP-NF <72>

### USP-NF <72> RESPIRATION-BASED MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

#### INTRODUCTION

This chapter is intended to be used as a risk-based test for the detection of microbial contamination in short-life products and encompasses short shelf-life products and/or short manufacturing times where the product must be administered as soon as possible. (See [USP-NF <73> ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products](#) for more details.) It may also be used as an in-process control for the testing of product intermediates, cell culture media, or process solutions. Growth-based methods that use detection signals other than visible signs of microbial growth or precipitation within the culture media (i.e., turbidity, particle formation, or floccular growth) have been used to test a variety of products. Instrumentation for respiration-based methods, such as those using optical sensors to detect changes in oxygen consumption or carbon dioxide production, have been used for the detection of contamination in a variety of short-life products, such as cell therapy products, has been published in peer-reviewed literature.

The primary advantages of respiration-based methodologies are 1) the broad equivalency to compendial methods and 2) the automated and periodic monitoring for earlier detection of microbial growth. Further benefits may include the direct inoculation of cultures without interference from product-related turbidity, the automation of data analysis, acquisition, reporting, and archiving, and the isolation of the organism for identification and investigation. Like all culture-based methods, disadvantages include the inability to detect microorganisms that do not grow under the culture conditions utilized. It is the end user's responsibility to select the respiration method that is the most appropriate for the product.

#### CULTURE MEDIA AND INCUBATION CONDITIONS

For recovery of aerobic microorganisms, culture media is to be incubated at 20°–25° and 30°–35°, and for the recovery of anaerobic microorganisms, culture media is to be incubated at 30°–35°. Other or additional incubation temperature(s) ranging from 20°–37° can be applied where relevant to appropriately recover potential microorganisms associated with the product/manufacturing process based on risk and a suitable justification.

The choice of culture media and incubation conditions should be risk-based in consideration of manufacturing process parameters (e.g., temperature, oxygenation level). For example, for fully aerobic processes the evaluation of anaerobic microorganisms required. Visual inspection of culture media containers may be required at the end of incubation for the detection of their presence was not detected by the system during method suitability, even when mold balls were used.

#### Growth Promotion Test of Aerobes, Anaerobes, and Fungi

Perform growth promotion of culture media using the strains of microorganisms listed in [Table 1](#). Incubate for not more than 3 days in the case of fungi. Seed lots are used so that the viable microorganisms used for inoculation are removed from the original master seed lot. The culture media are suitable if all microorganisms

#### METHOD SUITABILITY TEST

As a prerequisite, a primary validation of the method must be available as described in [USP <1071> 13301463\\_2.en.pdf](#). The method must demonstrate growth of the test microorganisms inoculated at not more than 10 CFU per test culture media. The method must be suitable for the product. In cases where a sample pretreatment is necessary, test microorganisms are added prior to treatment. The microorganisms used for the method suitability should include the test strains listed in [Table 1](#) and a selection of test strains relevant to the product/manufacturing process supported by a suitable justification. Inclusion of slow growing microorganisms and/or local isolates may be included if relevant for the product risk. Use of appropriate culture collection microorganisms that are comparable to in-house isolates are acceptable.

Each microorganism used for method suitability should have an associated positive control of the microorganism without product. The suitability test should also include negative controls such as uninoculated culture media with unimpaled product for each culture media type and incubation condition. Incubate until all tested microorganisms have been detected. Record the time to detection in hours. The inoculum control of each microorganism should be reported to demonstrate a level of not more than 10 CFU. Considering the variability of the microbial inoculum and assuming it follows a Poisson distribution, it is possible that a low sample size targeting not more than 10

04/02/2025 23:02

USP-NF <73> ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

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DOI Ref: uspnc  
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USP-NF <73>

### USP-NF <73> BIOLUMINESCENCE-BASED MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

#### INTRODUCTION

This chapter is intended to be used as a risk-based test for the detection of microbial contamination in short-life products and encompasses short shelf-life products and/or short manufacturing times where the product must be administered as soon as possible. (See [USP-NF <72> Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products](#) for more details.) It may also be used as an in-process control for the testing of product intermediates, cell culture media, or process solutions. Growth-based methods using detection signals other than visible signs of microbial growth or precipitation within the culture media (i.e., turbidity, particle formation, or floccular growth) include adenosine triphosphate (ATP) bioluminescence measurement for the detection of growth in liquid culture media (nutrient broth) or the detection of colonies on membranes placed on solid culture media. All viable cells, including microorganisms, contain ATP at various levels. ATP bioluminescence detection of microorganisms is based on the fact that luciferase, an enzyme that catalyzes the oxidation of luciferin, is present in all cells. Measured light is interpreted in a readout signal such as relative light units (RLU) or counts per charge coupled device (CCD) pixel.

ATP bioluminescence-based methods for detecting viable microorganisms have a long history of use in the pharmaceutical, over-the-counter medicines, and cosmetic industries. The application of ATP-based methods for the detection of contamination in a variety of products has been published in peer-reviewed literature.

The primary advantages of these ATP bioluminescence-based methodologies for detecting viable microorganisms are 1) the broad equivalency to compendial methods, 2) early detection of microbial growth, and 3) isolation of the organism for identification. Further benefits may include the detection of cultures without interference from product-related turbidity, the automation of data analysis, reporting, and archiving. Disadvantages of these methods include the inability to detect microorganisms that do not grow under the culture conditions utilized and products with significant ATP background that cannot be reduced.

#### CULTURE MEDIA AND INCUBATION TEMPERATURES

For recovery of aerobic microorganisms, culture media is to be incubated at 20°–25° and 30°–35° and for the recovery of anaerobic microorganisms, culture media is to be incubated at 30°–35°. Other or additional incubation temperatures ranging from 20°–37° can be applied, where relevant, to appropriately recover potential microorganisms associated with the product/manufacturing process based on risk and a suitable justification.

The choice of culture media and incubation conditions should be risk-based in consideration of manufacturing process parameters (e.g., temperature, oxygenation level). For example, for fully aerobic processes the evaluation of anaerobic microorganisms may not be required.

Culture media with relatively consistent low ATP levels should be sourced.

#### GROWTH PROMOTION TEST OF AEROBES, ANAEROBES, AND FUNGI

Perform growth promotion of culture media using the strains of microorganisms listed in [Table 1](#). Incubated at not more than 100 colony-forming units (CFU). Incubate for not more than 3 days for bacteria or not more than 5 days for fungi. Use seed lots so that the viable microorganisms used for inoculation are not more than 5 passages removed from the original master seed lot. The culture media are suitable if all microorganisms are detected.

The liquid culture media are suitable if ATP bioluminescence is detected at the predefined positive cutoff level compared to the background of the culture media without microbial inoculation. For solid culture media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum.

#### METHOD SUITABILITY TEST

As a prerequisite, a primary validation of the method must be available as described in [USP <1071> 13301463\\_2.en.pdf](#). The ATP bioluminescence-based method must demonstrate growth of the test microorganisms inoculated at not more than 10 CFU into the culture media containing the product. In case a sample pretreatment is necessary, test microorganisms are added prior to the treatment.

The microorganisms used for the method suitability should include the test strains listed in [Table 1](#) and a selection of test strains

USP <1071>

## INTRODUCTION

- This chapter is intended to be used as a **risk-based** test for the detection of microbial contamination in **short-life products** and encompasses short shelf-life products and/or **short manufacturing times** where the product must be administered as soon as possible.  
*(See [Rapid Microbiological Methods for the Detection of Contamination in Short-Life Products — A Risk-Based Approach for more details \(1071\)](#))*
- It may also be used as an ***in-process control*** for the testing of product intermediates, cell culture media, or process solutions.

### \*(72) RESPIRATION-BASED MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

#### INTRODUCTION

This chapter is intended to be used as a risk-based test for the detection of microbial contamination in short-life products and encompasses short shelf-life products and/or short manufacturing times where the product must be administered as soon as possible. (See [Rapid Microbiological Methods for the Detection of Contamination in Short-Life Products — A Risk-Based Approach \(1071\)](#) for more details.) It may also be used as an in-process control for the testing of product intermediates, cell culture media, or process solutions. Growth-based methods that use detector signals other than visible signs of microbial growth or precipitation within the culture media (i.e., turbidity, particle formation, or floccular growth) have been used to test a variety of products. Instrumentation for respiration-based methods is based on blood culture systems using the clinical setting that detect colorimetric or fluorescent signals to detect an exponential increase in carbon dioxide as an indicator of microbial growth. The application of respiration-based methods for the detection of contamination in a variety of short-life products, such as cell therapy products, has been published in peer-reviewed literature.

### \*(73) ATP BIOLUMINESCENCE-BASED MICROBIOLOGICAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS

#### INTRODUCTION

This chapter is intended to be used as a risk-based test for the detection of microbial contamination in short-life products and encompasses short shelf-life products and/or short manufacturing times where the product must be administered as soon as possible. (See [Rapid Microbiological Methods for the Detection of Contamination in Short-Life Products — A Risk-Based Approach \(1071\)](#) for more details.) It may also be used as an in-process control for the testing of product intermediates, cell culture media, or process solutions. Growth-based methods using detector signals other than visible signs of microbial growth or precipitation within the culture media (i.e., turbidity, particle formation, or floccular growth) include adenosine triphosphate (ATP) bioluminescence assays used for the detection of growth.



# Specificity

ATCC compendial microbial panel for sterility:  
Ph. Eur. 2.6.1.; JP 4.06; USP <71> **USP <72> & <73>**

## **Aerobic bacteria:**

- *Staphylococcus aureus*
- *Bacillus spizizenii* (formerly *subtilis*)
- *Pseudomonas paraeruginosa* (formerly *eruginosa*)

## **Anaerobic bacteria:**

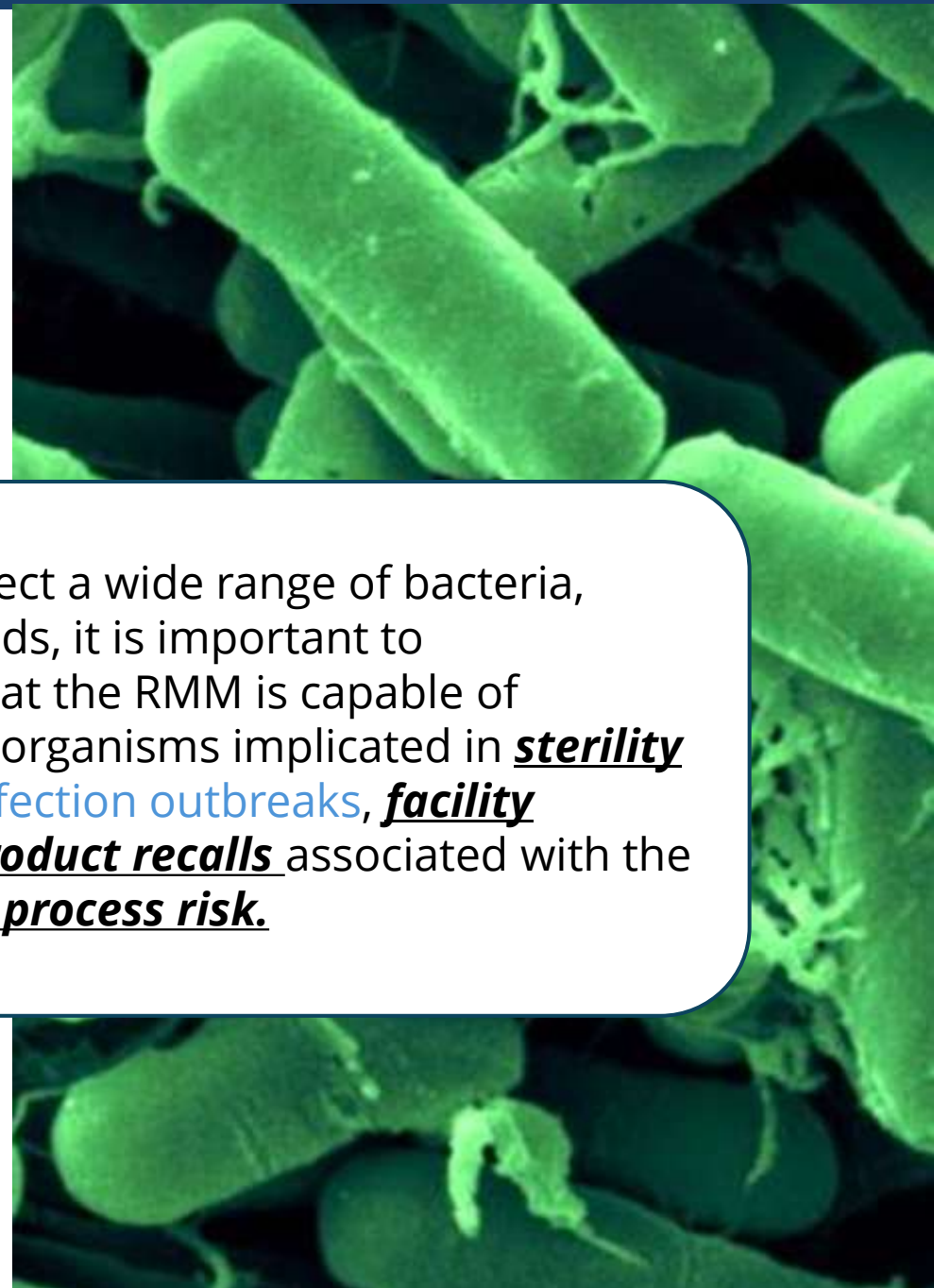
- *Clostridium sporogenes*

## **Fungi:**

- *Candida albicans*
- *Aspergillus brasiliensis*

- ... ability to detect a wide range of bacteria, yeasts, and molds, it is important to demonstrate that the RMM is capable of detecting microorganisms implicated in **sterility test failures**, **infection outbreaks**, **facility isolates**, and **product recalls** associated with the **manufacturing process risk**.

**requisito minimo !**





## Microbial Examination of cell-based Preparations

Table 2.6.27.-2. – Micro-organisms used for method suitability

<b>Aerobic medium</b>	
<i>Aspergillus brasiliensis</i>	for example, ATCC 16404, IP 1431.83, IMI 149007
<i>Bacillus subtilis</i>	for example, ATCC 6633, CIP 52.62, NCIMB 8054
<i>Candida albicans</i>	for example, ATCC 10231, IP 48.72, NCPF 3179
<i>Pseudomonas aeruginosa</i>	for example, ATCC 9027, NCIMB 8626, CIP 82.118
<i>Staphylococcus aureus</i>	for example, ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518
<i>Streptococcus pyogenes</i>	for example, ATCC 19615, CIP 1042.26, NCIMB 13285
<i>Micrococcus sp.</i>	for example, ATCC 700405, NCTC 7567
<b>Anaerobic medium</b>	
<i>Clostridium sporogenes</i>	for example, ATCC 11437, CIP 79.3, NCTC 532 or ATCC 19404
<i>Propionibacterium acnes</i>	for example, ATCC 11827

- Growth-based method
- Instrumentation for **respiration-based methods** is based on blood culture systems used in the clinical setting that utilize **colorimetric or fluorometric sensors** to detect an **exponential increase in carbon dioxide** as an indicator of microbial growth.
- The application of respiration-based methods for the detection of contamination in a variety of short-life products, such as cell therapy products, has been published in peer-reviewed literature.
- **Primary advantages:**
  - 1. the **broad equivalency** to compendial methods
  - 2. the **automated** and **periodic monitoring** for earlier detection of microbial growth
  - direct inoculation of cultures without interference from product-related turbidity,
  - automation of data analysis, acquisition, reporting, and archiving,
  - isolation of the organism for **identification** and **investigation**.
- **Like all culture-based methods, disadvantages** include the inability to detect microorganisms that do not grow under the culture conditions utilized.

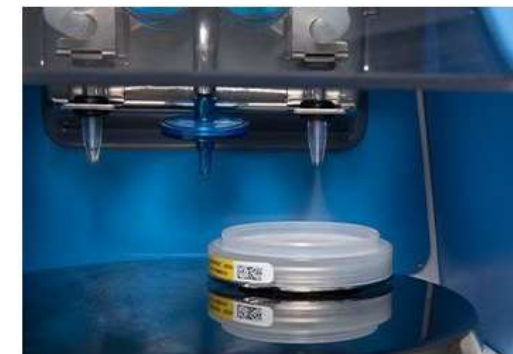


- CULTURE **MEDIA** AND **INCUBATION** CONDITIONS (20°–25° and 30°–35°)
- Growth Promotion Test (**GPT**) of Aerobes, Anaerobes, and Fungi (not more than **100 CFU**; incubate for not more than 3 to 5 days)
- METHOD **SUITABILITY** TEST (see USP <**1071**> and <**1227**>; inoculating NOT more than **10 CFU** per test culture media)
- DETERMINATION OF THE **INCUBATION TIME** IN THE PRODUCT TO BE EXAMINED
- TEST FOR MICROBIAL DETECTION IN THE PRODUCT TO BE EXAMINED (see USP <**1071**> and <**71**> and <**1113**>).

USP <1113> Microbial Characterization, Identification, and Strain Typing

USP <1227> Validation of Microbial Recovery from Pharmacopeial Articles

- Growth-based method.
- Adenosine triphosphate (ATP) bioluminescence luciferin-luciferase cascade emitting light measurement for the detection of growth in liquid culture media (nutrient broth) or on membranes placed on solid culture media (
- Measured light (optical luminometer) is interpreted in a readout signal such as relative light units (**RLU**) or counts per charge coupled device (**CCD**) pixel.
- ATP bioluminescence-based methods for detecting viable microorganisms have a long history of use in the pharmaceutical,... The application of ATP-based methods for the detection of contamination in a variety of products has been published in peer reviewed literature
- **Primary advantages:**
  - 1) the broad equivalency to compendial methods,
  - 2) early detection of microbial growth,
  - 3) isolation of the organism for identification.
  - detection of cultures without interference from product-related turbidity,
  - automation of data analysis, reporting, and archiving.
- **Like all culture-based methods, disadvantages** include the inability to detect microorganisms that do not grow under the culture conditions utilized + products with significant ATP background that cannot be reduced.







- CULTURE **MEDIA** AND **INCUBATION** CONDITIONS (20°–25° and 30°–35°)
- Growth Promotion Test (**GPT**) of Aerobes, Anaerobes, and Fungi (not more than **100 CFU**; incubate for not more than 3 to 5 days)
- METHOD **SUITABILITY** TEST (see USP <**1071**> and <**1227**>; inoculating NOT more than **10 CFU** per test culture media
  - Volume of Articles to be Tested
- MONITORING AND INTERPRETATION OF RESULT
  - Direct Inoculation Method - Contamination is considered to be detected if the RLU level of the contaminated sample has an RLU value that exceeds the predefined positive cutoff level as compared to the non inoculated nutrient medium controls.
  - Membrane Filtration Method - Contamination is considered to be detected if at least one count of microorganism is measured. For both methods, contaminating microorganisms should be

- DETERMINATION OF THE **INCUBATION TIME** IN THE PRODUCT TO BE EXAMINED
- The **longest time** to detection determined in the method suitability test **plus a safety margin** (10-fold increase in amount of the **slowest growing organism**) in hours:

$$T = t_{\text{ttd}} + (\log_2(10) \times G)$$

- $T$  = incubation time for microbial detection in the product to be examined
- $t_{\text{ttd}}$  = longest time to detection in the **method suitability test**
- $G$  = generation time slowest growing microorganism

$$T = t_{\text{ttd}} + (\log_2(10) \times G)$$

- $G$  = generation time slowest growing microorganism

$$G = \frac{t}{3.3 \times \log_{10}(N/N_0)}$$

$t$  = time interval (h)

$N$  = number of cells/CFU at the end of the time interval

$N_0$  = number of cells/CFU at the beginning of the time interval

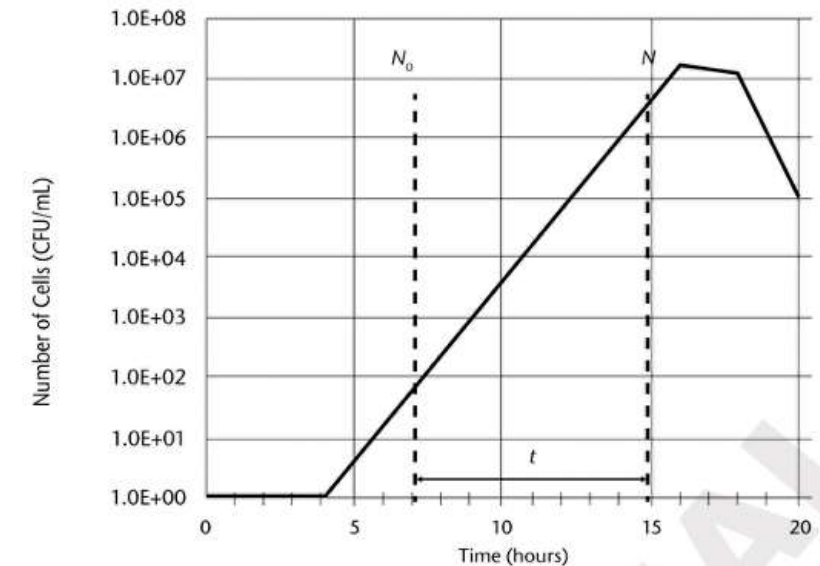


Figure 1. Growth phase curve.



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# Thank you for your attention

## Websites:

<https://www.pda.org>

[www.pda-it.org](http://www.pda-it.org)



*Dr. Lucia Ceresa*  
*Freelance Pharmaceutical Consultant*  
*PDA Italy Chapter Board Committee*  
*[Lucia Ceresa](#) / [LinkedIn](#)*

Phone: +39-327-288-0132  
Email: [lceres2@gmail.com](mailto:lceres2@gmail.com)





# Additional Regulatory RMM References

- The cfu has been in use for > **125 years** and continues to be specified as the unit of microbial enumeration in all current USP monographs. However, it is important to understand that the **cfu** has always been an estimation of microorganisms present, rather than an actual count.
- Rapid or modern microbiological methods typically produce signals in units other than **cfu** for microbial estimation
- Observations of cell counts that **differ from cfu** results are not a concern if the different methods and their **different signals** of cell presence are equivalent to or are non-inferior to referee methods in terms of assessing the microbiological safety of an article.



(1) USP Chapter < 1223 > Validation of Alternative Microbiological Methods

- Annex 1: § 4.24 and 4.25 Qualification and Validation refer to Annex 15



## Annex 15

**Effective March 2015**  
Qualification and  
Validation

### Annex 15 - § 1.8.

Appropriate checks should be incorporated into **qualification** and **validation** work to ensure the **integrity of all data obtained**.

- THE reason to introduce Rapid Microbial Methods:
  - Remove subjective interpretation
  - Remove potential transcription errors
  - Introduce automatic results reporting and exporting

[https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2015-10\\_annex15.pdf](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2015-10_annex15.pdf)

## Why considering Rapid Microbial Methods ?

General Notices, 6.30 Alternative and Harmonized Methods and Procedures :

*“Alternative methods and/or procedures may be used if they provide advantages in terms of **accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, or in other special circumstances.**”*



## Annex 1, 2022

The adoption of **suitable alternative monitoring systems** such as **rapid methods** should be considered by manufacturers in order to expedite the detection of microbiological contamination issues and **to reduce the risk to product (9.28)**.

## Ph. Eur. 5.1.6

Alternative methods for the control of microbiological quality have shown potential for real-time or near to real time results with the possibility of **earlier corrective action**. These new methods, if validated and adapted for routine use, can also offer **significant improvement in the quality of testing**.

## **Annex 1, 2022 - Quality Control (QC)**

- **10.7** For some products it may ***not be possible to obtain a sterility test result prior to release*** because the shelf life of the product is too short to allow completion of a sterility test. In these cases, the **additional considerations of design of the process** and **additional monitoring** and/or **alternative test methods required to mitigate the identified risks should be assessed and documented.**
- **10.10** ... For **products with short shelf life**, the environmental data for the time of manufacture may not be available; in these cases, the compliance should include a review of the most recent available data. Manufacturers of these products should consider the **use of rapid/alternative methods.**
- **10.11** Where **rapid and automated microbial methods** are used for **general manufacturing purposes**, these methods should ***be validated for the product(s) or processes concerned.***

## **Guidelines on GMP specific to Advanced Therapy Medicinal Products -**

### **2.3. Examples of the application of the risk-based approach in connection with the testing strategy**

**2.39** The application of the **sterility test** to the finished product in accordance with the **European Pharmacopoeia (Ph. Eur. 2.6.1)** **may not always be possible** due to the **scarcity of materials available**, or it may not be **possible to wait for the final result** of the test before the product is released due to **short shelf-life or medical need.**

In these cases, the strategy regarding **sterility assurance has to be adapted.** For example, **the use of alternative methods** for preliminary results, **combined with sterility testing of media or intermediate product** at subsequent (relevant) time points could be considered."



# FDA US cGMP: Rapid Methods



GUIDANCE DOCUMENT

## Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice

*Guidance for Industry*

OCTOBER 2004

### D. Alternate Microbiological Test Methods

- Other **suitable microbiological test methods** (e.g., **rapid test methods**) can be considered for **environmental monitoring, in-process control testing, and finished product release testing** after it is demonstrated that the methods are equivalent or better than traditional methods (e.g., USP).



# PDA Point-To-Consider for Microbial Control in ATMP Manufacturing



## Points to Consider for Microbial Control in ATMP Manufacturing

- Control through Facility Design
- Control through Equipment and Instrumentation Design and Maintenance
- Control through Analyst and Operator Gowning and Qualification
- Control Confirmed by Microbiological Process Monitoring

<https://www.pda.org/bookstore/product-detail/6754-ptc-for-microbial-control-in-atmp-manufacturing>







# PIC/S Annex 2A



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April 2021

### Revision of PIC/S GMP Guide (PE 009-15)

Geneva, 23 April 2021: The PIC/S GMP Guide to Good Manufacturing Practice (GMP) for Medicinal Products has been revised to include a new Annex 2A and 2B:

- Annex 2A: Manufacture of Advanced Therapy Medicinal Products for Human Use (ATMP); and
- Annex 2B: Manufacture of Biological Medicinal Substances and Products for Human Use

Annex 2A provides PIC/S GMP requirements for ATMP - it is not a standalone document but it enables reasonable harmonisation with the standalone ATMP Guidelines published by the European Commission. Annex 2B had very minor revisions and continues to harmonise with the EU Annex 2 for human use biological medicinal substances and products.

<https://picscheme.org/en/news/revision-of-pics-gmp-guide-pe-009-15>



# PIC/S Annex 2A

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April 2021

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Annex 2A provides PIC/S GMP requirements for ATMP - it is not a standalone document but it enables reasonable harmonisation with the standalone ATMP Guidelines published by the European Commission. Annex 2B had very minor revisions and continues to harmonise with the EU Annex 2 for human use biological medicinal substances and products.

<https://picscheme.org/en/news/revision-of-pics-gmp-guide-pe-009-15>

# BioPhorum

Table 2: ATMP production process category examples

Category	Manufacturing process				Characteristics			Example product type
	Aseptic end to end	Low-bioburden steps	Aseptic cell culture	Aseptic fill finish	Batch size	Fill finish	Scaled out/ scaled up	
1	x				Ultra-small or small	Manual or semi-automated	Scaled out	Ex vivo cell editing
2		x	x	x	Small or standard	Manual or automated	Scaled out or scaled up	Viral vector based in-vivo gene editing
3		x		x	Ultra-small, small or standard	Manual or automated	Scaled out	Individualized cell-free mRNA products or E. coli-based DNA products

