

Guidance for Industry

Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products

DRAFT GUIDANCE

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I. INTRODUCTION

We, the Food and Drug Administration (FDA), are providing you, manufacturers of cellular therapy and gene therapy products, with recommendations on the validation of growth-based Rapid Microbiological Methods (RMMs) for sterility testing of your products. This guidance addresses considerations for method validation and determining equivalence of an RMM to sterility assays described in Title 21 Code of Federal Regulations, 610.12 (21 CFR 610.12)¹. This guidance, when finalized, will address relevant issues and facilitate the implementation of an RMM for sterility testing.

This guidance applies to somatic cellular therapy and gene therapy products. This guidance does not apply directly to human cells, tissues, and cellular and tissue products (HCT/Ps) which are regulated solely under Section 361 of the Public Health Service Act as described under 21 CFR 1271.10, or HCT/Ps which are regulated as medical devices under 21 CFR Part 820. Such products are not subject to the sterility testing provision in 21 CFR 610.12, or to the requirement in 21 CFR 610.9 to demonstrate that an alternative RMM is equivalent to that sterility method. However, HCT/P and device establishments seeking to validate an RMM may find these recommendations useful.

Some of the principles of RMM validation discussed in this guidance might also be applicable to products other than cellular and gene therapy products that are subject to sterility testing under 21 CFR 610.12. RMM testing for such other products may involve additional considerations for validation of RMMs. Therefore, you should discuss RMM validation for your product with the appropriate review office in the Center for Biologics Evaluation and Research (CBER).

¹ 21 CFR 610.12 sterility method is similar but not identical to USP <71> "Sterility Tests" (Ref. 1).

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II. BACKGROUND

Human somatic cell therapy and gene therapy products are an emerging class of products that present multiple challenges to safety, purity and potency. Gene therapy products (see definition in section II.D.2), include vectors (i.e., nucleic acid, virus, or genetically modified microorganisms) that are administered directly to patients, and cells that are transduced with a vector ex-vivo prior to administration to the patient. Certain genetically modified microorganisms (e.g., bacteria and yeast), cellular therapy products, and cells transduced with a gene therapy vector present similar challenges to sterility assurance. For the purposes of this document these categories of cellular and gene therapy products are called cell-based products.² Many cell-based products cannot be cryopreserved or otherwise stored without affecting viability and potency. Most cell-based products are manufactured using aseptic manipulations because they cannot undergo sterile filtration or terminal sterilization (Ref. 2). Rapid and effective testing is needed because many cell-based products have a potentially short dating period, which often necessitates administration of the final product to a patient before sterility test results are available. Because of the challenges associated with cell-based products, there is a significant need to develop, validate, and implement sterility test methods that are more rapid than the sterility test methods described in 21 CFR 610.12.

A. What are Rapid Microbiological Methods (RMMs)?

Rapid microbiological methods are methods designed to provide performance equivalent to the sterility testing methods described in 21 CFR 610.12, while providing results in significantly less time. To show that an alternative method is equivalent to a test method specified in 21 CFR Part 610, such as the sterility testing described in 21 CFR 610.12, an applicant must demonstrate in a Biologics License Application (BLA) or supplement to a BLA that the alternative method will provide assurances of the safety, purity, potency and effectiveness of the biological product equal to or greater than the assurances provided by the specified method (21 CFR 610.9). Under this provision, an applicant would need to show that an RMM provides assurances of sterility that are equal to or greater than the assurances provided by the method described in 21 CFR 610.12.

² We recognize that not all gene therapy products contain cells modified ex vivo. The recommendations in this guidance are most directly applicable to gene therapy products that contain cells transduced with a gene therapy vector ex vivo. This guidance also applies to gene therapy products that do not contain cells, and we are willing to discuss with applicants recommendations for validation of RMMs for such gene therapy products.

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In general, RMMs are based on technologies which can be growth-based, viability-based, or surrogate-based cellular markers for a microorganism (i.e., nucleic acid-based, fatty acid-based). RMMs are frequently automated, and many have been utilized in clinical laboratories to detect viable microorganisms in patient specimens. These methods reportedly possess increased sensitivity in detecting changes in the sample matrix (e.g., by-products of microbial metabolism), under conditions that favor the growth of microorganisms.

The principles of RMM validation described in this guidance apply only to growth-based RMMs. Growth-based RMMs, like traditional methods of detecting viable microorganisms as described in 21 CFR 610.12, rely on the ability to recover and detect organisms from the product and demonstrate their viability by multiplication in liquid media. The specific recommendations in this document may not be applicable for non-growth-based RMMs which detect microbiological surrogates. This guidance focuses on RMMs with qualitative results (i.e., detection of microorganisms). If your RMM does not have the capability to speciate microorganisms, an additional method for speciation will be needed for investigation of detected contaminants. Early discussions with product review staff at CBER are encouraged for individuals intending to use or develop an RMM at any time in the product lifecycle using growth-based, viability-based, or surrogate-based RMMs, or RMMs that provide quantitative results.

B. What are Possible Benefits of RMMs for Cell-Based Products?

RMMs potentially offer significant improvements in timeliness compared to the test methods in 21 CFR 610.12. Although traditional methods are effectively used for the isolation of microorganisms in the manufacturing setting, these methods present limitations which are becoming more apparent with the emergence of new classes of biological products, such as cell-based products with low production volumes, limited manufacturing time, and short dating periods. Requirements for large sample volumes, manual examination of cultures, and growth detection based on visual observation are some of the limitations intrinsic to traditional methods. These limitations have motivated sponsors and manufacturers to develop RMMs based on techniques which reportedly yield accurate and reliable test results in less time and often with less operator intervention. Therefore, the use of RMMs for cell-based products might provide rapid results that could be used for product release.

C. Why is Validation of RMMs Necessary?

Reliance on validated sterility testing methods is a critical element in assuring the safety of a product. Validation of most methods for final product testing, including those assessing sterility, is required under 21 CFR 211.165(e) and 21 CFR 211.194(a)(2). Proper validation of critical methods, including RMMs, demonstrates that the methods are suitable for their intended purpose and provides assurance that the results obtained are accurate and reproducible.

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Although RMMs are now routinely used in limited settings, such as clinical testing, they have not been comprehensively validated for use in a variety of manufacturing settings. Cell-based products present especially challenging manufacturing issues, as these products often introduce additional product or process variables that would likely affect the test outcome. These variables should be considered in a validation study to demonstrate that the method is suitable for its intended product and application.

D. What Products are Included in This Guidance?

This guidance applies to the following products.

1. *Somatic cell therapy products*: Autologous (i.e., self), or allogeneic (i.e., intraspecies) cells that have been propagated, expanded, selected, pharmacologically treated, or otherwise altered in biological characteristics *ex vivo*, to be administered to humans and applicable to the prevention, treatment, cure, diagnosis, or mitigation of disease or injuries (Ref. 3).
2. *Gene therapy products*: All products that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms. The products may be used to modify cells *in vivo* or transferred to cells *ex vivo* prior to administration to the recipient (Ref. 4).

E. Does This Guidance Document Replace Any Existing Document?

No. This guidance document is not intended to replace any existing document. Rather, it is intended to address issues relating to validation of RMMs for cellular and gene therapy product testing that are not addressed in other guidance documents. This guidance also supplements other guidance documents that address similar subject areas.

III. GENERAL CONSIDERATIONS FOR VALIDATION OF AN RMM FOR CELL-BASED PRODUCTS

A. Where Might RMMs be Used in the Production of Cell-Based Products?

RMMs have the potential to replace the traditional methods for microbiological testing in the manufacturing process, including the following:

1. component (e.g., raw material, excipient) testing
2. in-process testing

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3. drug substance testing
4. drug product in its final container

B. How Can Risk Assessment Help in Designing Validation of RMMs?

During the manufacturing of cell-based products, microorganisms may be introduced from multiple sources, including biological and non-biological source material components, poorly controlled process streams, or failures in aseptic processing techniques (e.g., environment, personnel). We recommend that you perform an assessment of the production process to identify potential routes of microbial contamination. You should use the results of this assessment to determine the most appropriate samples and time points, and to identify the types of microorganisms associated with your process. You should incorporate these findings in the design of your RMM validation protocol.³ This type of risk-assessment could include the following:

- source materials (e.g., human cells or tissue that cannot be sterilized)
- level of manipulation (e.g., multiple or few manual processes, automated processes, no manipulation)
- processing path (e.g., open, partially closed, closed, sealed)
- cell culture or incubation duration
- cell culture vessel ((e.g., open system (flask, tube, dish); partially closed (sterile collection bag); closed (bioreactor))
- other relevant manufacturing components or materials

C. What Elements Should Be Considered to Validate an RMM?

The International Conference on Harmonization (ICH) Q2 (R1) “Validation of Analytical Procedures: Text and Methodology” (Ref. 6), states that “the objective of validation of a procedure is to demonstrate that it is suitable for its intended purpose.” We recommend that validation include comparison studies between the RMM and the method described in 21 CFR 610.12 to demonstrate equivalence as required under 21 CFR 610.9. For this purpose, equivalence may be demonstrated by evidence that the performance of the RMM assay provides assurances equal to or greater than assurances provided by the method in 21 CFR 610.12. Because validation studies of microbiological methods encompass a large degree of variability, the concept of the RMM being “equal to” or “greater than” the traditional method is demonstrated in parallel comparisons to the traditional method using as many identical parameters as possible to claim equivalence. Based upon the ICH Q2 (R1) terminology, validation characteristics that should be considered for a qualitative RMM are listed in Table 1.

³ This assessment is also useful in designing and implementing appropriate current good manufacturing practice (CGMP) controls of the manufacturing process (Ref. 7).

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Table 1: Validation Characteristics for an RMM Validation

Validation Characteristic	Definition
Limit of Detection	The lowest number of microorganisms detectable in the sample matrix. A limit of detection is essential to define what is considered to be contaminated. The limit of detection is determined by inoculating samples with serial dilutions of viable contaminants, with Colony Forming Units (CFUs) confirmed at inoculation by plate counts. Challenge testing is performed with each challenge microorganism using an amount less than 100 CFU but greater than 10 CFU. Any growth is recovered and identified to confirm identity.
Specificity	The ability of the test method to detect a panel of organisms within the sample matrix established in the validation protocol. Specificity is demonstrated by challenging the RMM to detect variations in microbial growth characteristics and concentrations. Challenge testing is performed with organisms including those recovered in the manufacturing environment and from sterility test positives, as well as common technical and operator contaminants. Known quantities of organisms inoculated into product samples need to be detected within the detection limit (see limit of detection), recovered, and identity confirmed.
Ruggedness	The degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions, such as different analysts, different instruments, and different reagent lots. Ruggedness is the lack of influence on the test results of operational and environmental variables of the microbiological method and should be assessed by having different analysts prepare multiple samples as specified in procedures. Positive and negative controls should be included and should test true to their characteristics. Ruggedness is evaluated by tabulating the mean time to detection for each inoculate and determining the difference between the means for each analyst.
Robustness	The capacity of a method to remain unaffected by small, but deliberate, variations in method parameters (from ICH Q2A) (e.g., changes in reagent concentration or incubation temperatures).

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D. How Do I Select a Panel of Appropriate Challenge Microorganisms for Validating an RMM?

You should develop a panel of microorganisms relevant to the product and process to challenge the performance of your RMM. We recommend that you include in your panel microorganisms which represent the following categories:

- Gram-negative bacteria
- Gram-positive bacteria
- aerobic bacteria
- anaerobic bacteria
- yeast
- fungi
- isolates detected in starting materials
- isolates detected by in-process testing or during preliminary product testing
- isolates detected by environmental monitoring of your manufacturing facility
- isolates from your production areas which represent low nutrient and high stress environments
- microorganisms from commercial sources that have continually been exposed to high nutrient growth media
- slow growing bacteria (such as *Propionibacter acnes*)
- fastidious microorganisms for the RMM
- microorganisms reported in the scientific literature to be common isolates from a particular product type

Microorganism source differences can translate directly into differences in growth rate kinetics (i.e., lag and log phases) that significantly affect incubation times and temperatures necessary to detect growth. Growth of isolates under aerobic and anaerobic conditions, particularly with respect to products manufactured in closed systems, should be an element of the challenge.

You should perform studies to demonstrate the RMM's growth promoting qualities and detection system capabilities. We recommend that such studies include the initial challenge test panel of organisms indicated in Table 2, cultured with the indicated media, or a recognized equivalent media, at the prescribed temperatures. You should address the other categories of microorganisms listed above as appropriate for your product in subsequent validation studies for your RMM. You should perform a minimum of three replicates for each challenge organism for each sample and method.

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Table 2: Recommended Microbial Test Panel and Conditions

Culture Medium	Incubation Temperature.	Microorganism	Microorganism Characteristics
Fluid Thioglycollate	30-35°C	<i>Bacillus subtilis</i> <i>Clostridium sporogenes</i> Environmental contaminants isolated from manufacturing	Organisms that produce spores under anaerobic conditions
Fluid Thioglycollate	30-35°C	<i>Candida albicans</i> <i>Kocuria rhizophila</i> (<i>Micrococcus luteus</i>) <i>Bacteroides vulgaris</i> Environmental contaminants isolated from manufacturing	Organisms that grow well under anaerobic conditions
Soybean Casein Digest or Tryptic Soy Broth	20-25°C	<i>Bacillus subtilis</i> <i>Aspergillus niger</i> Environmental contaminants isolated from manufacturing	Organisms that produce spores under aerobic conditions
Soybean Casein Digest or Tryptic Soy Broth	20-25°C	<i>Candida albicans</i> <i>Kocuria rhizophila</i> (<i>Micrococcus luteus</i>) <i>Propionibacter acnes</i> <i>Staphylococcus capitis</i> <i>Staphylococcus epidermidis</i> Environmental contaminants isolated from manufacturing	Organisms that grow well under aerobic conditions

E. How Do I Design The Method Comparison Studies?

You should carefully consider the composition of the test sample so that it is representative of all types of product samples that you intend to test with the RMM. Among the items you should consider are cell concentration, media and additives, and preservation agents or antimicrobial agents that may have a bacteriostatic or fungistatic effect. It might be possible to choose a “worst case scenario” sample matrix that could be used to validate the method for multiple sample types of the same cellular product. For example, you may wish to validate the RMM for both in-process and final product samples. The in-process sample may contain antibiotics in

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the cell culture medium, and the final sample may contain a preservation agent such as dimethyl sulfoxide (DMSO) and a higher cell concentration than the in-process sample. It might be appropriate to perform the validation study using the worst-case scenario sample matrix that would be the higher cell concentration (same concentration as final product) suspended in the cell culture medium containing the antibiotic (same concentration as the in-process sample) and DMSO (same concentration as in the final product). However, the use of multiple sample matrices might still be necessary for successful validation. You should note that validation data from one type of cell-based product may not be applicable to other products.

To validate the performance of a commercially available testing system, you should perform studies using characteristic organisms and isolates under prescribed assay conditions and compare the results with vendor specifications. For parallel testing of the RMM and traditional method, systems are compared using a microbial panel such as described in Table 2. Spike known quantities (measured in CFU/volume) of potential microbial contaminants into specific product configurations that demonstrate microbial recovery and identification, detection limits, and assay performance under actual testing conditions. Prepare dilutions of challenge organisms such that final concentrations of solutions contain between 10-99 CFU/sample for currently approved sterility methods. Ensure that CFU determination accurately reflects the actual number of CFUs mixed with the sample(s), such as by plating respective dilutions of the inoculation.

F. What Types of Positive and Negative Controls are Necessary in the Validation Study?

In the validation study design, the potential for the materials being tested to generate false positive or false negative results should be evaluated using the appropriate controls. This will depend greatly on the product matrix, additives and preservatives, and unique characteristics of the product. For example, if a freshly isolated cellular product is tested for sterility using a detection method based on CO₂ production, then the same freshly isolated cellular product should serve as a control to determine whether uncontaminated cells generate levels of CO₂ that would produce a false positive result. Use of additional positive and negative controls containing product components, but not cells, is also recommended.

G. How are Data from Validation Studies Evaluated and Submitted to FDA?

To aid in interpretation of your RMM validation study data, you should submit to your regulatory file (e.g., IND, BLA, Master File, as applicable) a complete description of the RMM, including the media used, incubation temperatures, detection technology and accompanying software, etc. If any of this information is available in a regulatory file for which you are not the sponsor (e.g. a drug master file), you should provide a letter from the holder of such file granting permission to cross-reference the information. You should provide copies of any published literature you wish to cite to describe the RMM or support your validation studies.

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In general, an acceptable RMM would demonstrate equivalent sensitivities or have more sensitive endpoint detection systems than the 21 CFR 610.12 sterility test methods. Demonstration that at least equivalent results are obtained from both methods should be based on statistical evaluation (e.g., probability of detecting an organism in sample size). If the RMM is based on the same principal as current methods, such as multiplication of organisms in a growth-based assay, any modifications in sample size, challenge inoculation, and media quantity should be described and justified with respect to currently approved sterility test methods. Other adjustments to test parameters, including actions taken in consideration of elements that contribute to false negative and false positive results, should be described and discussed.

H. Is Validation of an RMM Required to Initiate its Use in Testing Investigational Products?

To support the use of your proposed RMM for sterility testing of your investigational product, submit data prior to substitution of the RMM for the 21 CFR 610.12 test method. The validation studies should follow elements listed in Table 2. The completeness of your RMM validation study during the product development should reflect assessment of risk associated with the application of the method (e.g. stage of the manufacturing process). We recommend early discussion of your RMM validation plans with CBER.

I. When is Re-Validation of a Method Required?

Any changes in product manufacturing, including formulation, or changes in the RMM, which can potentially inhibit or enhance detection of viable microorganisms may trigger a need to re-validate the RMM. Changes in cell types, harvesting procedures, culture media, additives, critical processing steps or post processing handling can potentially affect the detection of viable microorganisms. Initial validation can be designed to minimize the effect of some changes by designing assay conditions that encompass known or proposed changes (e.g., worst case – matrix approach). Re-validation of your RMM should be performed whenever there are changes in the process that could potentially inhibit (e.g, the addition of antimicrobials) or enhance detection of viable microorganisms. Verification of critical parameters of the test method post process, or product changes using the microorganisms most difficult to detect, can serve as an indication of the need to re-validate the method. Validation of your sterility test should be performed on all new products.

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IV. GLOSSARY

Challenge Organism – An organism supplied by a certified vendor or isolated from laboratory processes and well characterized by colony morphology, gram staining, growth attributes and biochemical profile that is used to evaluate the performance of a sterility test methodology.

Challenge testing – Involves inoculating the product or component with a known quantity of organisms and then evaluating the product to determine if growth of organisms can be detected using the test method.

Closed System – An enclosure designed to maintain a sterile environment for production or testing operations. It is used to isolate the product and process intermediates from personnel and the surrounding environment.

Colony Forming Unit (CFU) – A microbiological term that describes the formation of a single macroscopic colony after the introduction of one or more microorganisms to microbiological growth media. One colony forming unit is expressed as 1 CFU.

Equivalence – A demonstration that results obtained from the RMM are equal to (same as) or better than results obtained from the sterility method described in 21 CFR 610.12, and support the conclusion that the RMM will provide assurances of the safety, purity, potency, and effectiveness of the biological product equal to or greater than the sterility method in 21 CFR 610.12.

False Negative – A negative result from a positive sample. An example of a false negative would be a sample inoculated with 100 CFU that is reported by the system as a negative.

False Positive – A positive result from a negative sample. An example of a false positive would be a positive result in a sample containing no contamination.

Growth-Based Method – A method that relies on the ability to recover organisms from the product and demonstrates their viability by multiplication in either solid or liquid media. In most cases, this is achieved by adding a sample suspected of containing a contaminant to a general media such as Tryptic Soy Broth or Fluid Thioglycollate.

In-Process Testing – Checks performed during production in order to monitor and, if appropriate, to adjust the process and/or to ensure that the intermediate or API conforms to its specifications.

Qualitative Microbial Method – A method that detects the presence of viable microorganisms in the test sample but provides no indication of their number.

Viable – Viability in the microbiological context refers to the ability of an organism to grow and reproduce under appropriate conditions.

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V. REFERENCES

1. United States Pharmacopeia 28 and National Formulary 23 (USP 28 NF 23), Sterility Tests, General Chapter 71, pp. 1-13 (2005).
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