Alternatives to the Pharmacopoeial Sterility Test

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Presentation Overview

• Critical quality attributes for sterile injectable products
• USP <71> as a release test
• Alternative tests to USP <71>
• Method validation as described in USP <1223>
• Candidate rapid sterility tests and their technology
• USP evolving position on method equivalency
• Ph. Eur. 2.6.27 Microbiological Examination of Cell-based Preparations
• Development of a compendial rapid sterility test
Critical Quality Attributes

The critical quality attributes of a sterile injectable product include:

- Identity
- Strength
- pH
- Osmolality
- Essentially free of visible particulate matter
- Absence of viable microorganisms
- Control of bacterial endotoxins
- Container-closure integrity
- Antimicrobial effectiveness (multiple-use products only)
GMP Requirements

• 21 CFR 211.165 *Testing and release for distribution*
  (a) For each batch of drug product, there shall be appropriate laboratory determination of satisfactory conformance to final specification for the drug product, including the identity and strength of each active ingredient, prior to release.

• Where sterility and/or pyrogen testing are conducted on short-lived radiopharmaceuticals, such batches may be released prior to the completion of the test, provided such testing is completed as soon as possible.
Absence of Viable Microorganisms

- USP <71> Sterility Tests describes the procedures to determine whether a sterile pharmacopeial article complies with the monograph requirements with respect to the test for sterility.
- However, due to the limitations of the sterility test, the sterility assurance of a injectable product is determined by the microbiological quality of the ingredients, bioburden control, facility design, efficacy of the sterilization and aseptic processes not the sterility testing.
Harmonization

The compendial sterility test in the three major pharmacopeia are harmonized. They are the following:

• USP <71> Sterility Tests
• JP 4.06 Sterility Test
• Ph. Eur. 2.6.1 Sterility Testing

Note: A sterility test developed and qualified using the harmonized test will be suitable for global regulatory submission.
USP <71> Sterility Tests

• Products are tested preferably using the membrane filtration method, where the nature of the products allows, or if the membrane filtration technique is unsuitable by the direct inoculation method.

• Two media are employed, Fluid Thioglycollate medium incubated at 30-35°C and Soybean-casein digest medium incubated at 20-25°C for at least 14 days.

• The quantity of product tested per media and the total number of units tested as related to lot size is given in Tables 2 and 3 in USP <71>.

• If no evidence of microbial growth is found the product tested complies with the test for sterility.
USP <71> Sterility Tests

• Suitability testing includes confirmation of the sterility of each batch of media, growth promotion testing, and method suitability testing for each specific product.

• The ability of less than 100 cfu of the specified bacteria and fungi to grow in the media is demonstrated by adding the inoculum to the final rinse for the membrane filtration method or to the media in the direct inoculation method. Incubate the media at the prescribed temperature and clearly visible growth of the microorganisms must be obtained compared to the control without the product.
Limitations of the <71> Sterility Tests

What are the limitations of the USP <71> sterility test?

• The selection of the media, i.e., Fluid Thioglycollate Medium and Soybean-Casein Digest Media, incubation temperature and incubation time were a compromise, not all microorganisms will grow under these conditions, and over 30% of the sterility failures occur between 7 and 14 days of incubation. Scoring growth in the media in form of turbidity, pellicle formation, precipitation and floccular growth is subjective.

• The test with a 14-day incubation is not suitable for short life products like compounded sterile preparation, PET preparations and cell therapy products.
Method Qualification

Compendial microbial tests methods are considered validated and are subject to method qualification.

- For example, USP <71> Sterility Tests describes the method suitability testing that is conducted for each product tested to demonstrate that the product does not interfere with the recovery of microorganisms.

Alternative methods to the compendial tests are subject to method validation.

- Demonstration of performance equivalence using the validation parameters, e.g., accuracy, precision, specificity, limit of detection, limit of quantification, robustness and ruggedness, appropriate for the specific test as described in USP <1223>.
Validation Strategy

• According to USP <1223> *Validation of Alternative Microbiological Testing Methods* the validation parameter for an alternative sterility test method are Limit of Detection, Specificity, Robustness, and Ruggedness as required for a qualitative test.

• In addition, the method suitability, i.e., a demonstration that a sterile compounded preparation does not affect the ability of the test to detect microorganisms must be conducted for each specific preparation.
Pharmacopeial Guidance

- USP General Notices 6.30 *Alternative and Harmonized Methods and Procedures*
- USP <1223> *Validation of Alternative Microbiological Test Methods*
- Ph. Eur. 5.1.6 *Alternative Methods for the Control of Quality.*
Other Guidance

• ICH Guidance Q2 (R1) Validation of Analytical Procedures: Text and Methodology, May 1997

• ASTM Standard E2935 Standard Practice for Conducting Equivalence Testing in Laboratory Application, 2013e

• PDA TR#33 (Revised) The Evaluation, Validation and Implementation of New Microbiological Methods 2014
Alternative Methods

• USP General Notices 6.30: “Alternate methods 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.

• Such alternate methods shall be validated as described in the General Informational Chapter Validation of Compendial Procedures <1225> and must be shown to give equivalent or better results.”
Alternative Methods

ICH Q6A, 2.7 *Alternative Procedures*: 
• “Alternative procedures are those which may be used to measure an attribute when such procedure control the quality of a drug substance or drug product to an extent that is comparable or superior to the official method.”
Revisions to USP <1223>

• The revisions included a discussion of the limitations of the CFU, the acceptable procedure concept, the performance, results and equivalence options for alternative method validation, and application of non-inferiority test as a statistical tool.

• The revised chapter will be published online in the July-August Pharmacopeial Forum with an official date of December 1, 2015.
Alternative Sterility Tests

• USP <797> *Pharmaceutical Compounding – Sterile Preparations* states that high-risk compounded sterile preparations shall meet the sterility test (see Sterility Tests <71>) before they are dispensed or administered.

• The chapter states that a method not described in the USP may be used if verified results demonstrate that the alternative is as least as effective and reliable as the <71> test.
FDA Support for Alternative Methods

• The 2004 FDA Aseptic Processing Guidance for Industry states that 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 methods).
FDA Support for Alternative Methods

• The current FDA strategic plan acknowledges that analytical technologies are rapidly changing and leading to dramatic improvements in sensitivity, resolution, and precision in the detection of contaminants.

• In order to better reduce the risk of microbial contamination of products, the following needs will be addressed:

  *Develop sensitive, rapid, high-throughput methods to detect, identify, and enumerate microbial contaminants and validate their utility in assessing product sterility.*
Alternative Sterility Tests

Candidate rapid sterility tests and their technology include:

- Growth Direct (Advanced Imaging)
- Scan RDI Microbial Detection System (Solid Phase LASER Scanning Cytometry)
- BacT/ALERT Microbial Detection System (CO$_2$ sensor)
- BACTEC System (pH sensor)
- Milliflex Rapid System (ATP Bioluminescence)
- MagNA Pure 96 & ABI 7500 System (RT-PCR)
Growth Direct System
Growth Direct System

• Advantages of the system: Aerobic incubation at 20-25°C and 30-35°C and anaerobic incubation at 30-35°C for equivalency to USP <71>, 7-day incubation, automation of the incubation and reading, suitable for conducting testing under a laminar flow hood or isolator, and recovery of contaminating microorganisms.

• Disadvantages of the system: Growth-based system with a 7-day incubation, only suitable for filterable products, and high capital costs.
Scan RDI Microbial Detection
Scan RDI Microbial Detection

• Advantages of the system: Non-growth-based system with time to result 2-3 hours and a robust viability measurement.

• Disadvantages of the system: Product must be filterable and not contain autofluorescent particles and results must be verified using a fluorescence microscope.
BacT/ALERT Microbial Detection System
BacT/ALERT Microbial Detection System

• Advantages of the system: CO$_2$ detection system independent of the filterability of the product and turbidity of the media, 7-day incubation time, dual temperature incubation and aerobic and anaerobic media to give equivalency to USP <71> sterility test, and continuous monitoring to detect growth.

• Disadvantages of the system: Sample size limited to 10 mL per media vial, and growth-based system with a 7-day incubation.
Milliflex Rapid System
Milliflex Rapid System

• Advantages of the system: ATP bioluminescence a widely accepted technology, 5-7 day incubation, and convenience of reagent handling.

• Disadvantages of the system: Growth-based method, and product must be filterable.
USP Evolving Position

• As Alternative Microbiological Methods (AMM) move further away from classical methods based on the colony-forming unit, the USP needs to respond to this RMM validation challenge.

• The current compendial methods are unsuitable for the emerging short-lived cell-derived drug products, compounded sterile preparation and radiopharmaceuticals as they are growth based.

• We need a new paradigm.
USP Evolving Position

• The USP believes that the widespread implementation of AMMs for both in-process and finished product testing will improve good manufacturing practices, maintain product quality and promote patient safety.

• Existing method validation approaches are limiting AMM development, validation and implementation.
USP Evolving Position

- The colony-forming unit (CFU) is an estimate of viable bacterial or fungal numbers in a water, air, soil, food or drug sample.
- Unlike direct microscopic counts where all cells, dead and living, are counted, CFU estimates viable cells that are capable of dividing in the solid plate count medium under the incubation conditions employed.
USP Evolving Position

Signals other than colony-forming units and growth liquid media that may be used for microbial enumeration and detection include:

• Autofluorescent cells detected by flow cytometry
• Vital stained cells detected by solid-phase and fluid fluorescent microscopy
• PCR amplified nucleic acid targeted sequences
• Number or weight of genomic units
• ATP levels measured by bioluminescence
USP Evolving Position

• Typically these signals may be numerically higher than CFU and may not be directly statistically related.
• However, they should move directionally with CFU so as to detect adverse bioburden trends.
• Materials with an established fitness for use using CFU will retain these qualities despite the measurement of an alternate signal.
• To take advantages of emerging technologies we may need cut the ties to the CFU as a so-called gold standard.
USP Evolving Position

Since the USP <1223> revision, four options are available to establish the equivalence of an alternative method are:

• 1) acceptable procedures, i.e. merely meeting a minimum performance requirement without demonstrating equivalence to the compendial method,

• 2) performance equivalence to the compendial method,

• 3) results equivalent to the compendial method,

• 4) decision equivalence to the compendial method.
Equivalency Options

• Equivalency was fully discussed in the stimuli article Hauck et al, 2009 *Acceptable, equivalent or better approaches for alternatives to official compendial procedures*. Pharm. Forum. 35(3):772-778.

• The concepts of acceptable, performance, results and decision equivalency were introduced to the USP stakeholders in this article.
Option 1: Acceptable Procedure

- This is not strictly an equivalence option that requires direct comparison to an official compendial method but with the scientific literature.
- For example, with a nucleic acid-based technology a highly purified bacterial genome could be used expressed as copies/mL.
- The Limit of Detection would be the lowest concentration where 95% of the diluted samples would be positive, i.e. that could be 50-80 copies per mL.
- The dynamic range of a qPCR method may be 10 to 10,000 copies/mL
Option 1: Acceptable Procedure

With a nucleic acid-based technology (cont.):

• The linearity of the PCR reaction will have a regression coefficient greater than 0.95

• At the limit of quantification, i.e. 100 to 200 copies/mL the Relative Standard Deviation (RSD) will be less than 10%
Option 2: Performance Equivalence

• In general, performance equivalence requires the demonstration of equivalence or better with respect to classical validation criteria, e.g. accuracy, precision, specificity, limit of detection, limit of quantification, robustness and ruggedness, appropriate for the type of test.

• This is current paradigm.

• However, it is possible that the alternative method may be worse for one or more of the validation criteria and still be acceptable in terms of the USP General Notices.
Option 3: Results Equivalence

• For results equivalence, the alternative and compendial test methods should give equivalent numerical results.

• Because the same sample cannot be tested in microbiology, typically a tolerance interval is established when comparing the two methods, with the alternative method determined to numerically superior or non-inferior.
Option 4: Decision Equivalence

- A decision equivalence is similar to a results equivalence but differs in that a numerical result is not generated; instead a pass/fail result is obtained.
- With this approach, the frequency of positive results generated should be no worse than with the compendial method.
- Examples where this option would be applied are sterility testing and absence of a specified microorganism.
Ph. Eur. Approach to Cell-Based Preparations

• Ph. Eur. developed 2.6.27 *Microbiological Examination of Cell-based Preparations* for cell therapies where the 2.6.1 sterility test cannot be performed due to the nature of the preparation, when microbial contamination can occur, the short shelf-life of cell therapy products, as well as the amounts available for testing and sampling-related issues.

• Ph. Eur. positioned this test, not strictly as a sterility test, but as a test to screen for microbial contamination. We can learn from this approach.
Product Shelf-Life:

- The shelf-life will depend on the cell characteristics and preservation conditions.
- For non-cryopreserved cell-based preparation, the shelf-life usually does not exceed 3-4 day and occasionally not more than a few hours.
- The preparation would typically be administered to the patient before the 2.6.1 sterility test is completed.
Sample Composition:

• Unlike most pharmaceutical products, microbial contaminants could reside on the surface or even inside the cellular components and not detected only in the supernatant, such as culture or transport media.

• The requirements is the test sample must representative of all the components of the cell-based preparation.
Sample size:

• With the use of a single donor or manufacturing-related capacity restraints, the sample volume available for testing may be limited.

• Due to sampling error, which may lead to microbial contamination not being detected, the sample size must be sufficient to ensure suitable sensitivity and specificity of the chosen test method.
Sample size, for cell-based preparations, where the total volume (V) is between 1 mL and 1 L in a single container.

<table>
<thead>
<tr>
<th>Cell-based Preparation Volume (mL)</th>
<th>Total Inoculum Volume</th>
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<tbody>
<tr>
<td>10 ≤ V &lt; 1000</td>
<td>1% of the total volume</td>
</tr>
<tr>
<td>1 ≤ V &lt; 10</td>
<td>100 μL</td>
</tr>
<tr>
<td>V &lt; 1</td>
<td>NA</td>
</tr>
</tbody>
</table>
Ph. Eur. 2.26.7 Microbiological Examination Options

Three options are given in the Ph. Eur. chapter:

• Automated Growth-Based Testing
• Combination of Preculturing and Alternative Method Detection
• Direct Detection by Alternative Methods
Sterility Testing Requirements for CSPs

• USP <797> Pharmaceutical Compounding – Sterile Preparations requires all high-risk level Compounded Sterile Preparations prepared in groups of more than 25 identical individual single-dose packages, i.e. ampules, IV bags, syringes or vials, or in multiple-dose vials for administration to multiple patients or are stored for longer than 12 hours at refrigeration temperature and longer than 6 hours at room temperature shall meet the USP <71> Sterility Tests requirements before they are administered.
Sterility Testing Requirements for CSPs

• In the proposed revision to USP <797>, if a Category 2 CSP is assigned a beyond-use dating that requires a sterility test, the testing must be performance as specified in USP <71> Sterility Tests.

• An exception to Table 3: Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch is that the sterility test may be performed with 10% of the batch, rounded up to the next whole number, when the batch size is less than 40 units.
USP Expert Panel

• As a follow-up to the March, 2014 USP Workshop on Alternative Microbiological Methods held at the USP Headquarters in Rockville, MD an expert panel was established to recommend to the USP Microbiology Expert Committee on the direction to take in writing a general test chapter on rapid sterility testing.
USP Expert Panel

• A two-phase approach was decided upon with the user requirement specifications for the different stakeholders established first.

• Base on these user requirements, the most appropriate technologies for a compendial rapid sterility test(s) would be recommended to the Microbiology Expert Committee.
USP Expert Panel

• At the February 2016 Face-to-Face Expert Panel Meeting at the USP Headquarters the panel assembled high, medium and low priority user requirements for a compendial rapid sterility test method.

• At two subsequent teleconferences these user requirements were refined.

• The expert panel was divided into Red, Blue and Green Teams respectively and each team was assigned 5 high priority user requirements for definition.
Ranked User Requirements Specifications

High Priority (N = 15)

• Ability to detect a wide range of microorganisms, i.e., specificity
• Availability of instruments and reagents from multiple vendors
• Availability of Reference Standards
• Data integrity
• Ease of use/simplicity of test and data interpretation
• Low false positive and negative rates
• Limit of detection
Ranked User Requirement Specifications

High Priority (continued)

- Method suitability
- Improved patient safety
- Regulatory acceptance
- Robustness and reliability of equipment
- Sample preparation
- Sample quantity i.e., minimum number of articles tested and quantity per container tested
- Time to result
- Aseptic test material handling, i.e., open vs. closed systems
## User Requirement Specifications Matrix

### User Requirement Matrix

<table>
<thead>
<tr>
<th>User Requirement</th>
<th>Definition</th>
<th>Specific Stakeholder Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>Ability to detect a wide range of microorganisms</td>
<td>Sterile Compounding</td>
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<tr>
<td></td>
<td></td>
<td>PET</td>
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<td></td>
<td></td>
<td>Cell Therapy</td>
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<td>Manufactured Pharmaceuticals</td>
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</table>
Expert Panel Status

• The teams have conducted weekly teleconferences and on schedule to complete their definition of the user requirements by the middle of April, 2016.

• After the Users Requirement Specifications are finalized, the Expert Panel will select candidate technologies for a Compendial Rapid Sterility Test(s).

• The final report to the USP Microbiology Expert Committee is due June 30, 2016.
Development of Compendial Rapid Sterility Tests

• The USP Microbiology Expert Committee will draft the test chapters, conduct proof of concept studies, validate the methods as per USP <1223> and publish the test chapter for stakeholder comment.

• This ambitious undertaking will be completed in the 2015-2020 cycle.

• Thanks for your attention.