USP Microbiology Expert Committee

Current Activities and Standards Development Update

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Dublin, Ireland
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Topics

• USP revision process
• Chapter revisions now official
• Changes to Validation of Microbial Recovery  <1227>
• New General information for Rapid Microbial Contamination Test  <1071>
• New *B. cepacia* method  <60>
• New General information for Bacterial Endotoxins Testing  <1085>
• New Revision Activities of Microbiology Expert Committee

Don Singer
Development & Revision of Official USP Standards

1. Proposal is published in *Pharmacopeial Forum* (PF) for public review and comment

2. Comments on PF proposal reviewed by USP Scientific Staff and Expert Committee

3a. If major revisions to the proposal are needed, the comments, responses, and the revised proposal are published in *PF* for additional public review.

3b. If no further revisions or minor revisions are needed, Expert Committee recommends for official adoption and the comments and responses are published in the *Commentary Section*

4. Board of Trustees approves for official adoption
USP Ongoing Review

• Public review and comment is welcome at any time.
USP GC- Microbiology Expert Committee 2015-2020

- David Hussong, Ph.D., Chair
- Edward Tidswell, Ph.D.
- James Akers, Ph.D.
- James Agalloco, M.S., M.B.A.
- Anthony Cundell, Ph.D.
- Karen McCullough, M.S.
- Russell Madsen, M.S.
- Dennis Guilfoyle, Ph.D.
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- David Roesti, Ph.D.
- Donald Singer, M.S.
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- Randa Melhem, Ph.D., (FDA / CBER Liaison)
- Marla Stevens-Riley, Ph.D. (FDA/CDER Liaison)
- Richard Friedman, (FDA/CDER Liaison)
- David Lau (FDA/ora Liaison)
- Laura Huffman (FDA/CVM Liaison)
- Radhakrishna Tirumalai, Ph.D., USP Staff Liaison
2018-2019 Revisions

• <1227> Validation of Microbial Recovery From Pharmacopeial Articles
• <1071> Rapid Microbial Tests For Release of Sterile Short-Life Products: A Risk-Based Approach
• <60> Microbiological Examination of Nonsterile Products- Tests for Burkholderia Cepacia Complex
• <1085> Guidelines on the Endotoxins Test
• <1229.16> Prion Inactivation
Introduction

“This chapter provides guidelines for...recovery methods...”.

“The test procedures...<51>,...<71>,...<61>, and <62> are considered validated. However, use of compendial methods require establishment of suitability of the method...in presence of product.”
Validation of Microbial Recovery from Pharmacopeial Articles

“Neutralization may be achieved by...washing and dilution, filtration, and rinsing,...any combination of these methods.”

“When the product displays intrinsic antimicrobial activity...and....the risk of microbial contamination is low, the method could be considered fit for purpose providing a strong rational.”
Validation of Microbial Recovery from Pharmacopeial Articles

Recovery on Agar Medium

“...a mean count of any of the test organisms not differing by a factor greater than 2, i.e., 50%-200%, from the value of the control in the absence of product.”

Recovery in Liquid Medium

“The method....validated...clearly visible growth, visually comparable to that in the control vessel without product within the indicated time...”
Rapid Methods Expert Panel

STIMULI TO THE REVISION PROCESS
USP PF 43(5) Sep-Oct 2018
“The Development of Compendial Rapid Sterility Tests”

Members of the USP Modern Microbiological Methods Expert Panel

USP Modern Microbiological Methods Expert Panel Members (Listed alphabetically with affiliation):
Thierry Bonnevay, Sanofi Pasteur; Randolph Breton, Infuserve; Claudio Denoya, Particle Measuring Systems Technology; Anthony M. Cundell, USP Microbiology Expert Committee (Co-chair); John Duguid, Vericel; Matthew Jenkins, UVA Medical Center; Felix Montero Julian, bioMerieux; James Kenney, FDA/CBER; Amy McDaniel, Pfizer; Michael Miller, Microbiology Consultants, LLC; Gary du Moulin, Massachusetts College of Pharmacy and Health Sciences; David Newton, USP Compounding Expert Committee; David Hussong, Chair, USP Microbiology Expert Committee; Kuldip Patel, Duke University Hospital; Steven Richter, Microtest Laboratories; David Roesti, USP Microbiology Expert Committee; Edward Tidswell, USP Microbiology Expert Committee (Co-chair); Yongqiang Zhang, BD; Steven Zigler, USP Chemical Medicines Expert Committee.
Rapid Microbial Tests for Release of Sterile Short Life Products – A Risk-Based Approach

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• CRITICAL OPERATING PARAMETERS TO BE USED IN DETERMINING A RISK-BASED RAPID MICROBIAL TEST FOR THE RELEASE OF STERILE SHORT-LIFE PRODUCTS
• SITUATIONS WHEN <71> IS UNSUITABLE FOR PRODUCT RELEASE TESTING
  Sample Size Consideration
  Limit of Detection
  Ability to Detect a Wide Range of Microorganisms
• RAPID MICROBIAL TEST METHODS FOR THE RELEASE OF STERILE SHORT-LIFE PRODUCTS
  Brief Descriptions of the Technologies
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• GLOSSARY
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Rapid Microbial Tests for Release of Sterile Short Life Products – A Risk-Based Approach

THE CONCEPT OF RISK-BASED MICROBIOLOGICAL MONITORING AND RELEASE TESTING

“...some risk-based decisions would need to be made... in terms of time to results, LOD, sample size, and range of microbes detected to allow the use of such tests... The stakeholders should carry out a risk assessment for choosing an RMT in cases where the current compendial sterility test is unsuitable.”

Monitoring

“...rapid microbiological methods (RMMs) may be used as in-process controls prior to the final product release sterility test to provide faster information on the effectiveness of microbial controls and the early detection of gross contamination (enabling to investigate and restart production sooner) or probability that a product may fail sterility.”
Rapid Microbial Tests for Release of Sterile Short Life Products – A Risk-Based Approach

CRITICAL OPERATING PARAMETERS TO BE USED IN DETERMINING A RISK-BASED RAPID MICROBIAL TEST

<table>
<thead>
<tr>
<th>Candidate Technology</th>
<th>LOD (cfu)</th>
<th>Time to Result</th>
<th>Sample Size Range (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain (for comparative purposes only)</td>
<td>$10^4$–$10^5$</td>
<td>30 min</td>
<td>0.1</td>
</tr>
<tr>
<td>Theoretical LOD of 1–3 cfu based on a Poisson distribution</td>
<td></td>
<td>14 days</td>
<td>40–500</td>
</tr>
<tr>
<td>Adenosine triphosphate (ATP) bioluminescence</td>
<td>1–10</td>
<td>2–7 days (including pre-enrichment)</td>
<td>1–1000</td>
</tr>
<tr>
<td>ATP bioluminescence</td>
<td>$10^1$</td>
<td>30 min</td>
<td>1–1000</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>10–100</td>
<td>6–8 h (pre-enrichment)</td>
<td>0.1–2</td>
</tr>
<tr>
<td>Isothermal micro-calorimetry</td>
<td>$10^4$</td>
<td>2–7 days</td>
<td>1</td>
</tr>
<tr>
<td>Nucleic acid-methods$^a$</td>
<td>10–100</td>
<td>2–4 h</td>
<td>0.2–2</td>
</tr>
<tr>
<td>Respiration</td>
<td>1–10</td>
<td>Overnight to 7 days</td>
<td>Up to 10</td>
</tr>
<tr>
<td>Solid phase cytometry</td>
<td>1–10</td>
<td>2–3 h</td>
<td>1–1000</td>
</tr>
</tbody>
</table>

$^a$ For these methods, the signal would be in genomic units.
SITUATIONS WHEN <71> IS UNSUITABLE FOR PRODUCT RELEASE TESTING

SAMPLE SIZE- “The sample size tested may need to be reduced based on either the sample processing capability of the technology or the need to conserve the much-needed product.”

LOD – “Setting an LOD of a single viable cell with all technologies is an unrealistic barrier of entry for any sterility test, especially when the signal is not the colony-forming unit that is amplified by cultural enrichment.”

ABILITY TO DETECT – “Although all...platforms should have...ability to detect...range of bacteria, yeasts, and molds, it is of practical importance...that technology chosen for an RMT is capable of detecting microorganisms implicated in sterility test failures, infection outbreaks, and product recalls associated with either CSPs, radiopharmaceuticals, cell therapies, or manufactured pharmaceuticals.”
Rapid Microbial Tests for Release of Sterile Short Life Products – A Risk-Based Approach

RAPID MICROBIAL TEST METHODS FOR THE RELEASE OF STERILE SHORT-LIFE PRODUCTS

“Technologies recommended based on their match to the URS discussed...suitable for an RMT are listed alphabetically as follows:

- Adenosine triphosphate (ATP) bioluminescence
- Flow cytometry
- Isothermal microcalorimetry
- Nucleic acid amplification
- Respiration
- Solid phase cytometry”
Burkholderia cepacia – Why?

Gram negative, opportunistic bacteria
- *B. cepacia* complex comprised of 20 closely related species

Can overcome antimicrobial preservative systems and antiseptics

Low nutrient environment is common for Bcc to survive
➢ Can grow in preserved aqueous oral and nasal liquids and topical products

Has caused serious infections in patients with cystic fibrosis, and those who are mechanically ventilated or immunosuppressed or with serious underlying disease
Timely addition of a test to recover *B. cepacia*

Selection of a common media for recovery of *B. cepacia* complex-BCSA

A starting point for compendial development- No monographs state absence of *B. cepacia*
Microbiological Examination of Nonsterile Products – Tests for Burkholderia Cepacia Complex

Introduction

“The tests...to determine...preparation complies with an established specification...for inhalation use or aqueous...oral, oromucosal, cutaneous, or nasal use- contain members of the Bcc.”
Microbiological Examination of Nonsterile Products – Tests for Burkholderia Cepacia Complex

Growth-Promoting and Inhibitory Properties of the Media and
Suitability of Test Method

“Microorganisms for Growth-Promoting Properties: B. cepacia, B. cenocepacia, B. multivorans...”

These are 3 clinically significant strains of Bcc.
Microbiological Examination of Nonsterile Products – Tests for Burkholderia Cepacia Complex

**Recommended Culture Media**

Burkholderia cepacia Selective Agar

Agar contains Gentamycin, Vancomycin, Crystal Violet and Polymyxin B

- Substances that are inhibitory to microorganisms (other than B. *cepacia* complex) common in respiratory secretions

‘Alternative’ media can be used but must meet similar suitability and indicative properties; and may require best practice ID methods.
Testing of Products
Sample Preparation and Pre-incubation

“Soybean Casein Digest Broth...mix and incubate at 30-35°C for 48-72h.”
“Mix and subculture by streaking...BCSA, and incubate at 30-35°C for 48-72h.”

Incubation time allows for ‘slow-growing’ strains.
Method suitability testing should challenge the shortest time.
Guidelines on the Endotoxins Test

Why?

FDA LAL Guideline (1987) was retired.

New FDA Guidance: Pyrogens and endotoxins testing, Questions and Answers (2012)

Analysts continue to have questions about basic understanding of bacterial endotoxin testing.
INTRODUCTION

BACKGROUND: PYROGENS AND ENDOTOXINS

ENDOTOXINS

PRELIMINARY TESTING

RSE: CSE Calibration
CSE Calibration/Potency Determination Using the Gel-Clot Method
CSE Calibration/Potency Determination Using Quantitative Kinetic and Endpoint Assays
Activity Determination for a Liquid CSE
Screening and Qualification of Consumables: Compendial Requirement
Analyst Qualification
Equipment and Instrumentation Calibration and Qualification
Laboratory Environmental Conditions

METHOD SUITABILITY

Calculating Endotoxin Limits for Drug and Biological Products
Relevance of Limits for Compounded Sterile Preparations
Calculating Endotoxin Limits for Active Substances and Excipients
Calculating Endotoxin Limits for Combination Products
Calculating Endotoxin Limits for Medical Devices
Maximum Valid Dilution
Method Suitability Testing
Qualifying Test Preparation Methods Other than Dilution

ROUTINE TESTING

Sampling
Pooling
Calculation of Endotoxin Content
Out-of-Specification Results and Retesting Considerations
Standard Curve Control

ALTERNATE TEST METHODS

GLOSSARY

REFERENCES
Guidelines on the Endotoxins Test

Endotoxins

• “...current USP Endotoxin Reference Standard (RSE) and control standard endotoxins (CSE) are extracted from the GNB cell membrane...purified to remove any surrounding cell membrane components...Additionally, these ...are often formulated with stabilizing agents. As such, LPS prepared using extraction methods, cannot contaminate pharmaceutical products because they do not exist in this form in nature. Endotoxin standards, attributed to differences in source and manner of preparation, could “react differently from native sources of endotoxins”. It is possible that standards extracted by...denaturing methods...may not always be representative surrogates for modeling the behavior of natural endotoxins in some pharmaceutical, biopharma, or medical device extraction experiments.”
Guidelines on the Endotoxins Test

Analyst Qualification

“Classroom training delivered by a subject matter expert (SME) develops an understanding of the principles and limitations of the test methods as well as the effects of the analyst’s technique on the test result.”

“Training effectiveness should be confirmed by the demonstration of analyst competency in performing the test.”
Calculating Endotoxin Limits for Combination Products

“If the combination product...a prefilled syringe...may be tested as a filled unit and the endotoxin limit for the drug product prevails. Any endotoxins contributed by the container (device) are assumed to be eluted with...product during sample preparation...are accounted for in the product’s assayable endotoxins.”

“...two drugs...administered simultaneously [IV or intramuscular (IM)], then... endotoxin content of the combined dose may not exceed the endotoxin limit for drugs of 5 EU/kg/h for IV or IM...or 0.2 EU/kg/h for IT...”

“...a kit containing multiple components...administered as a single entity (e.g., a lyophilized product/diluent/syringe) the endotoxin content of the combined dose may not exceed the endotoxin limit for drugs of 5 EU/kg/h for IV or IM...or 0.2 EU/kg/h for IT administration.”
New Activities of USP Microbiology Expert Committee

New Chapter for <1229> series
Alternative Endotoxin Test Plan
Revision of <1117> Microbiological Best Laboratory Practices
Prion Inactivation

Prions are related to Transmissible spongiform encephalopathies (TSE)
Also related to BSE

Proteinaceous substances that are potential contaminants of materials and cells from mammalian origin

Chemical and thermal methods are discussed as ‘inactivation’ methods
Alternate Bacterial Endotoxin Test – rFC

Recombinant Factor C test is commercially available

It is not referenced in USP <85> or the Ph Eur or JP harmonized monographs

As an alternative test, it is acceptable for use with product specific validation

USP is
1) developing a proposal for publication in Pharmacopeial Forum and
2) also interested in simultaneously collecting and reviewing of independent data for evaluation of rFC test
USP <1117> is being revised to add more relevant topics based on stakeholder input

Current topics in <1117> will be updated with improved clarity

Please review the new chapter when it is published in Pharmacopeial Forum (mid to late summer issue) and send us your comments!
Thank You!