COMPARE USP <71> TEST METHODS TO ALTERNATIVE METHODS

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Agenda

- Introduction to Sterility Testing
- Regulatory Information & USP <71>
- Methods
- Critical Elements & Bacteriostasis & Fungistasis (B&F) Testing
- Culture Media & Rinse Fluids
- Interpretation of Results
- Regulatory Guidance & Approvals
- ATP Bioluminescence for Rapid Sterility
Disclaimer

• The information in this seminar is given for the purposes of education and discussion.

• It is not intended to be, and it should not be used as a substitute for regulations or regulatory guidance.

• Decisions and actions should be based on the relevant regulations, guidance document and pharmacopeial chapters.

• Statements and opinions expressed are of the presenter and are not necessarily the views of MilliporeSigma.
INTRODUCTION TO STERILITY TESTING
Pharmaceutical Microbiology: Sterility Testing

- QC Microbiology Laboratory
- Environmental Monitoring
- Raw Materials
- Water
- In-process
- Support Data
- Sterility Testing
- Final Product
- Product Release
What is Sterility?

- Classical
  - The *complete* absence of life
  - Absolute term

- Practical
  - Absence of living material
  - *Demonstrated* by growth and reproduction
Why Perform a Sterility Test?

Because the GMP’s says so!

21CFR Part 211§167 (a)

“For each batch of drug product purporting be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed.”
02 REGULATORY INFORMATION
Regulations/Pharmacopeias/Guidance

- **Pharmacopeias**
  - USP Chapter <71>
  - Ph. Eur. section 2.6.1
  - JP section 54

- **Other Guidance Agencies**
  - PIC/S : Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme
  - FDA : Food and Drug Administration
  - TGA : Therapeutic Good Administration from Australia – September 2006
  - EMEA : European Agency for the Evaluation of Medicinal Products
  - PDA : Parenteral Drug Administration
  - AAMI : Association for the Advancement of Medical Instrumentation
  - ISO : International Organization for Standardization
  - WHO : World Health Organization
  - USDA: Department of Agriculture
Points to Consider from USP <71>

Provides details on Media, Microorganisms, Test Conditions, Sample Considerations, Suitability (Validation) and Methodologies

“The test may be carried out using the technique of Membrane Filtration or by Direct Inoculation of the Culture Medium with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits; that is, for filterable aqueous preparations, for alcoholic or oily preparations, and for preparations miscible with, or soluble in, aqueous or oily solvents, provided these solvents do not have an antimicrobial effect in the conditions of the test.”

“Use membrane filters having a nominal pore size not greater than 0.45 μm, in which the effectiveness to retain microorganisms has been established”  USP <71>
Points to Consider from USP <71>

- “These Pharmacopeial procedures are **NOT by themselves designed to ensure that a batch of product is sterile or has been sterilized.** This is accomplished primarily by validation of the sterilization process or of the aseptic processing procedures” – USP <71>

- Not intended as a sole product release test (See USP <1211>)
- Represents one set of data which contributes to the decision of whether or not the product lot meets the stated claims
- Pass/Fail Destructive test
- Not for viruses, mycoplasma or endotoxin
- Long incubation period: 14 calendar days (Harmonized in 1998)
- Probability-based
  - **Not statistically valid, not representative**
Products to be Examined & Sample Considerations

Number of products needed for testing is based upon several factors:

1. How the product is prepared
2. Type of product
3. Batch size
Membrane Filtration vs. Direct Inoculation

Membrane Filtration

Advantages
- Rinse away inhibitory or preservative agents
- Sample concentration:
  - High volume sample can be filtered
  - Statistically more valid
  - More sensitive
- Small volume samples can be diluted

Limitations
- Non-filterable samples
- Solids may plug membrane

Direct Inoculation

Advantages
- Fast, easy
- Wide selection of media
- Non-filterable samples

Limitations
- Limited sample size = lower sensitivity
- Turbidity
- Inhibition issues
Filtration  Retention  Recovery
Microbial Recovery Study

- Examine relationship of membrane pore size to growth characteristics and recovery on membranes used for enumeration tests
- Tested retention and recovery
- Numerous MCE membranes
  - 0.22µm, 0.45µm, 0.65µm, 0.7µm, 0.8µm, 1.2µm

- Examined a variety of microorganisms
  - B. subtilis 13933
  - B. diminuta 19146
  - C. albicans 10231
  - C. sporogenes 11437
  - E. aerogenes 11437
  - E. coli 25922
  - M. luteus 9341
  - P. agglomerans
Recovery vs. Pore Size

Average %Recovery for 12 Runs

0.45 µm consistently showed >90% recovery; .2 µm did not.
Membrane Filtration with a Closed System

1) Pre-wet membranes/canisters
2) Filter test article through canisters
3) Rinse membranes
4) Add growth medium
5) Incubate
Membrane Filtration with a Closed System

**Advantages**
- USP accepted
- Pressure driven for faster filtration
- Reduced false positives/negatives
- Pre-sterilized canisters
- Pre-assembled canisters
- Product tested as used
- Pressure control/alerts

**Limitations**
- Test article vs membrane compatibility
- Product must be filterable or non-deformable
**Direct Inoculation**

Aseptically transfer sample to 8 to 15x volume of growth medium (depending on B&F testing)

Add solid samples to 100x volume of medium

Transfer equal sample sizes to each growth medium used

Incubate

Examine for turbidity
Direct Inoculation

**Advantages**
- Direct immersion of medical devices
- Non-filterable products may be tested
- Few manipulations depending on nature of the product

**Limitations**
- Antimicrobial product activity may be challenging; large volumes of media required
- Intrinsic product turbidity (Sub-culturing necessary)
- Aseptic technique training and validation required
- No volumes larger than 100 ml with individual test samples
- Unable to perform any rinses that may be required to remove inhibition
- Oily substances cannot be tested
CRITICAL ELEMENTS & B&F TESTING
Critical Elements for Sterility Testing

- Environment
- Training
- Method Development
- Method Suitability
Sterility Testing Environment

“The test for sterility is carried out under aseptic conditions. The test environment has to be adapted to the way in which the sterility test is performed.” USP <71>

Test Area
- Surrounding clean rooms & entry
- Immediate testing area – Horizontal or Laminar Flow hood
- Isolator/Barrier Environment and surrounding area

Gowning Effectiveness
- LFH: glove process and eye protection
- Isolator: Half suit and glove integrity testing

Surface disinfection & validation
- LFH: expiry and preparation of disinfectant
- Isolator: Load pattern validation, compatibility

Environmental Monitoring Program (methods, frequency, etc.)
Training

Operators
• QC Personnel
• Cleaning Personnel

External Training
• PDA TRI Courses
• Others

Internal Training Recommendation
• Initial Training Verification
• Verify Method Consistency
Method Development

The method for performing the Sterility Test must be confirmed before Method Suitability (a.k.a Validation)

- Filterability
- Chemical Compatibility
- Rinsing Fluids & Volumes
- Potential Inhibition issues
- Membrane Compatibility
- Quantity of Samples to be tested

Note: Data for removal of certain bacterial requirement will be figured here – This data can be used to petition the regulatory agencies for dismissal of this organism.
Why do products have B&F activity?

Products containing bacteriostatic or fungistatic agents need to be neutralized to overcome inhibition to allow the growth of viable microorganisms present in the product.

**The GOAL = NO RISK OF FALSE NEGATIVE RESULTS**

Need to PROVE that the Sterility Testing procedure will allow a perfect elimination of any growth inhibition before the incubation with the *BACTERIOSTASIS / FUNGISTASIS TEST* as described in Pharmacopoeias.
Why do products have B&F activity?

Antibiotics OR products containing preservatives:

- Microbial Resistance
  - Select for resistant strains
  - Microbes can live in most parenteral pharmaceuticals
    - 63% of small volume parenterals (SVP) w/o preservatives supported growth
    - 13% of SVP w/preservatives supported growth
How to test products with B&F activity?

USP <71>

- "Specifically adapted filters may be needed for certain products (e.g., for antibiotics)"
- Don’t use a membrane with high binding capacities (Nitrocellulose)
- Use a membrane with very low binding capacity like Polyvinylidene Fluoride (PVDF).
Choose the right membrane

Slowly pre-wet the membrane before filtration

Use the right fluid to prepare the membrane

Rinsing is more efficient when done slowly

Quickly filter the product and rinse immediately after filtration.

Dissolve and dilute the product properly.

Look at surface tension and different rinse buffer properties. Rinse fluids D & K are able to lower the capillary force.

Caution when using Fluid K: last rinse must follow with a fluid at lower detergent concentration (Fluid A)

How to test products with B&F activity?
“If the product has antimicrobial properties, wash the membrane not less than 3 times by filtering through it each time the volume of the chosen sterile diluent used in the validation test. Do not exceed a washing cycle of 5 times 100 ml, even if during validation it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity.” – USP <71>
Sterility Media & Rinse Solutions

Pharmacopeial Medium
- Soybean Casein Digest Broth (SCDB, TSB)
- Fluid Thioglycollate Medium (FTM)
- Clear Fluid Thioglycollate Media (CTM)

Pharmacopeial Rinse Buffers/Solutions
- Fluid A, D and K
# Sterile Rinse Buffers

<table>
<thead>
<tr>
<th>Type</th>
<th>Characteristics</th>
<th>Application</th>
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| Fluid A (USP) / neutral solution of meat or casein peptone (EP) | 0.1% Peptone: source of Carbon & Nitrogen pH 7.1 ± 0.2° maintained osmotic equilibrium (pH prior to sterilization) | • Suitable as a general rinse buffer  
• Works well with most samples  
• Excellent to dissolve or dilute samples  
• Excellent to reconstitute commercial microorganisms  
• Excellent transport medium for microorganisms |
| Fluid D (USP) | 1 l Fluid A + 1 ml polysorbate 80 (0,1%) Polysorbate 80: will neutralize some preservatives Peptone: source of Carbon & Nitrogen pH 7.1 ± 0.2° maintained osmotic equilibrium (° prior to sterilization) | • Suitable for testing specimens that contain lecithin or oil  
• Excellent for rinsing sterile pathways of devices  
• Works well with most antibiotics  
• Needed for rinse method testing of Medical Devices |
| Fluid K (USP) (neutral solution with emulsifying agent (EP)) | Beef extract and peptone: provide nutrients for recovery of injured and fastidious microorganisms **Polysorbate 80 at a concentration of 10 g/l (1 %)** Polysorbate 80 : will neutralize some preservatives pH 6.9 ± 0.2 maintained osmotic equilibrium (° prior to sterilization) | • Suitable for testing specimens that contain petrolatum  
• Suitable for oils and oily solutions  
• Excellent for rinsing pathways of Medical Devices  
• Good for “difficult” sample to filter or to dissolve samples |
Incubation Specifications

Current test conditions
- 14 calendar days

Harmonization
- 14 days incubation for all products
- Compensate for suboptimal growth
- Account for inherent slow growers
- Repair injured cells
- Questionable turbid reaction after 14 days
  - Subculture at least 4 additional days

Examination post 14 days not advisable
Verify slow growers from EM data
Interpretation of Results
Regulatory Requirements: USP/EP

Investigation

Invalid test?

a) The data of the microbial monitoring of the sterility testing facility show a fault
b) A review of the testing procedure used during the test in question reveals a fault
c) Microbial growth is found in the negative controls
d) After determination of the identity of the micro-organisms isolated from the test, the growth of these species may be ascribed unequivocally to faults with respect to the material and/or technique used in conducting the sterility test procedure.

If Yes & proved
Resample & Retest

If No or not sure
Reject Batch
Repeat Testing

If the test is declared to be invalid it is repeated with the same number of units as in the original test.

If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for sterility.

If microbial growth is found in the repeat test, the product examined does not comply with the test for sterility.
WHY
ALTERNATIVE/
RAPID
METHODS???
Why would an Alternative Method Benefit You?

- Faster product release
- Quicker Corrective/Preventative Action implementation
- More sensitive detection
- Keeping up with Industry and Regulatory Standards
- Data integrity
Issues to Consider When Selecting a Method

- Risk of False Positives (sample manipulation, amount of steps, background)
- Risk of False Negatives (ability to rinse inhibition)
- Quantitative vs. Qualitative
- Limit of Detection
- Sample prep and volume
- Complexity (Validation, Ease of Use)
- Time to result
- Ability to ID
- Cost
- Regulatory
Developing A User Requirement Specification

- Understand your current method
  - What is the **volume/weight** of product that you sample?
  - Does your product have **inhibitory** properties?
  - **Sample prep**: method, time and throughput?
  - **Validation requirements**
Developing A User Requirement Specification

- Level of sensitivity
- Range of organisms detected
- Time to detection
- Sample prep
- Data management (involve IT)
- Functions and characteristics (space, utility, software, etc.)
- Instrument qualification (IQ/OQ/PQ), SOPS, & training
  - Design qualification: Does the supplier have documentation to show design of equipment is validated for specific application?
- Method suitability and validation (consider equivalency)

Because every requirement may not be met by one product, categorize requirements as “Mandatory” or “Desirable”.
Examples:

Main functionality requirements of the system:

- “must be able to obtain fast and reliable enumeration of organisms”
- “must be able to store the obtained results as data...access to altering parameters, properties and results must be restricted to the appropriate personnel. Software must be 21CFR Part 11 compliant”
- “must be able to reduce incubation time by X amount of time”
- “must be able to meet a throughput demand of X”
- “sample preparation must be based upon Membrane Filtration”
- “Health and Safety of operators must not be changed with the addition of this new method”
- “the vendor will continue to support the product for a min. period of X years after purchase”
## Regulatory Guidance

### Pharmacopeia Specific Chapter

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<thead>
<tr>
<th>Pharmacopeia</th>
<th>Specific Chapter</th>
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<tbody>
<tr>
<td><strong>EP 7th edition (7.2)</strong></td>
<td>5.1.6: Alternative methods for control of microbiological quality (aligned with TR33)</td>
</tr>
<tr>
<td><strong>USP 34-NF 29 through second supplement</strong></td>
<td>(&lt;71&gt;, &lt;1231&gt;, &lt;1111&gt;, \text{etc. compendial chapters}) (&lt;1223&gt;) Validation of alternative microbiological methods (2015)</td>
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<tr>
<td><strong>PDA TR No. 33</strong></td>
<td>Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods (2013)</td>
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<tr>
<td><strong>FDA</strong></td>
<td>21 CFR610.12 Sterility Amendment to sterility test requirements for Biological products (2012)</td>
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PDA Technical Report Number 33 (revised 2013)

- Updated Technology Review
- Risk Assessment
- User Requirement Specifications
- Comparison of compendial/classic methods to rapid/alternative methods
- Method Suitability
- Equivalence
- Validation Process
- IQ/OQ/PQ
- Use of Statistics
- Implementation Strategies
- Regulatory submission and expectations
USP <1223>: CFU Versus Alternate Signal

- CFU is an estimate of cell count
- Other signals or measurement units can be used
- Direct cell counts from staining or from autofluorescence
- Differences in CFU and signal should not be a concern as long as methods are equivalent to or non-inferior to referee method
USP <1223>: Qualification of an Alternative Method

**Qualitative Tests:**
- Specificity
- Limit of Detection
- Ruggedness
- Robustness
- Repeatability
- Equivalency

**Quantitative Tests:**
- Accuracy
- Precision
- Specificity
- Limit of Quantification
- Linearity
- Operational Range
- Ruggedness
- Robustness
- Repeatability
- Equivalency
Qualitative Tests

Specificity

“The specificity of an alternative qualitative microbiological method is its ability to detect a range of microorganisms that may be present in the test article.” 100 CFUs is recommended for growth based methods. TR33 & EP discuss using stressed organisms and mixed cultures.

Limit of Detection

“The limit of detection is the lowest number of microorganisms in a sample that can be detected under the stated experimental conditions. A microbiological limit test determines the presence or absence of microorganisms.” Not necessarily quantified.

Ruggedness

“Ruggedness can be defined as the intrinsic resistance to the influences exerted by operational and environmental variables on the results of the microbiological method.” For example, different analysts, instruments, and reagent lots. Performed by supplier.
Qualitative Tests

Robustness

“We refer to the robustness of a qualitative microbiological method as its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability during normal usage.” For example, incubation time. Performed by supplier.

Repeatability

“The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of the same suspension of microorganisms and uses different suspensions across the range of the test.” Subset of precision
USP <1223>: Equivalency Options

- **Acceptable Procedure**
  - No direct comparison
  - Using standard inoculum or pure nucleic acid as a reference material
  - No example of a technology was provided

- **Performance Equivalence**
  - Multiple validation criteria to show equivalence or better results
  - May not meet certain validation criteria but could still be acceptable
  - Similar approach to TR33 and EP

- **Results Equivalence**
  - Both methods give an equivalent numerical result with a tolerance interval
  - If a technology is non-growth based, calibration curve could be used to show correlation.

- **Decision Equivalence**
  - Qualitative result
  - Positive and negative results should be no worse than compendial method
  - Based upon historical performance
FDA Approval

- Submit Comparability Protocol (CP) as prior approval supplement
  - Validation Studies
  - Acceptance Criteria
- Complete validation as outlined in CP
- Notify FDA
  - Submission of a Special Report (21 CFR 314.81 (b)(3)(ii))
  - Changes Being Effectected (CBE-30 or CBE-0) to allow for routine use

*If in-process assays are not included in product submission (NDA or ANDA), implementation is internal thru standard change control. However, still discuss validation plan with FDA.
EU Approval

- Commission Regulation (EC) 1234/2008 as of 2010
  - Can group variations under same Marketing Authorization

- EMA Scientific Advice Procedure
- Post approval change management protocol (PACMP)
ATP
BIOLUMINESCENCE
FOR RAPID
STERILITY
ATP Bioluminescence Technology

ATP + D-luciferin + Oxygen (O₂)

Firefly Luciferase
Magnesium

AMP + PPi + Oxyluciferin + CO₂

LIGHT
ATP Bioluminescence with Membrane Filtration

Membrane Filtration & Growth Based Enrichment on Agar
- Similar to “Compendial method” for easier validation
- Large sample volumes can be tested
- Allows removal of B&F effects and free ATP

ATP Bioluminescence
- Automated reagent application
- Allows micro-colonies to be detected
- 75% time savings in incubation times

Advanced Detection & Image Analysis
- The detection limit is 1 CFU
- Easy, automated counting
- Continuity in the data interpretation
Method Review

1- Filtration
2- Reduced incubation
3- Reagent dispensing
4- Signal detection
5- Data acquisition
Detecting micro-colonies

“Micro” Colonies = Results Up to 75% faster than conventional methods

Typical Growth curve

Number of Organisms

Visible Colonies

Micro colonies

Time (days)

T1

T5

10^6

10^2
Automated Application of Reagents & Image Analysis

Application of:
1) ATP Releasing Agent
2) Bioluminescence reagent

Total time to process a sample ~13 minutes after completion of the incubation step

Imaging & Counting <60s

22 Colony forming units
Data Acquisition

Parameters (intensity and size of signal vs background) set in the system (tower and software) allow discrimination between true signals and background.

The validated detection limit of the system is

1 cfu
Automated Analysis & Reporting
Sample results of a mixed culture

Total Viable Count: Mix of *P. aeruginosa* + *E. coli* + *B. cepacia*
Compendial USP <71> vs. ATP Bioluminescence for Rapid Sterility

- Compendial method EP/USP
  - Membrane filtration
  - Test in liquid medium (2 media)
  - Incubation: 14 days
  - Reading: visual turbidity observation
  - Result: qualitative (turbidity)

- Alternative method
  - Membrane filtration
  - Test on solid agar medium
  - Incubation: target = 5 days
  - Result: quantitative (CFU)
Novartis & FDA Publications

**Introduction of a Rapid Microbiological Method as an Alternative to the Pharmacopoeial Method for the Sterility Test**

**Identifying Faster Sterility Tests for Biological Products**
Regulatory Research Seeks to Reduce the Time Needed to Ensure the Safety of Critical Products

**The Need for Rapid Sterility Test**

**Identification of Micro-Organisms after Milliflex Rapid Detection — A Possibility To Identify Nonsterile Findings in the Milliflex Rapid Sterility Test**

**Growth-promoting Properties of Different Solid Nutrient Media Evaluated with Stressed and Unstressed Micro-organisms: Prestudy for the Validation of a Rapid Sterility Test**
THANK YOU!!!