PDA Parenteral Drug Association

Midwest Chapter

Microbial Contamination and Control Conference

May 13th & 14th

# Rapid Sterility Testing

*Implementing Alternative Sterility Methods for Modern Pharmaceutical Manufacturing*

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A Sotera Health company

# Agenda

- Selecting a Rapid Method
- Rapid ATP-Based testing for product sterility (USP ⟨73⟩ and USP ⟨1223⟩ examples)

# Poll Question #1

Which sterility testing method is primarily used at your organization?

- Traditional compendial sterility testing
- Alternative or rapid sterility testing
- A combination of both
- Not sure

# Poll Question #2

What would be the most compelling reason for your organization to implement an alternative or rapid sterility test method?

- Faster time to product release
- Improved operational efficiency
- Enhanced data objectivity and automation
- Regulatory alignment and modernization
- Cost reduction
- To ask the lab for results (more quickly)

# Poll Question #3

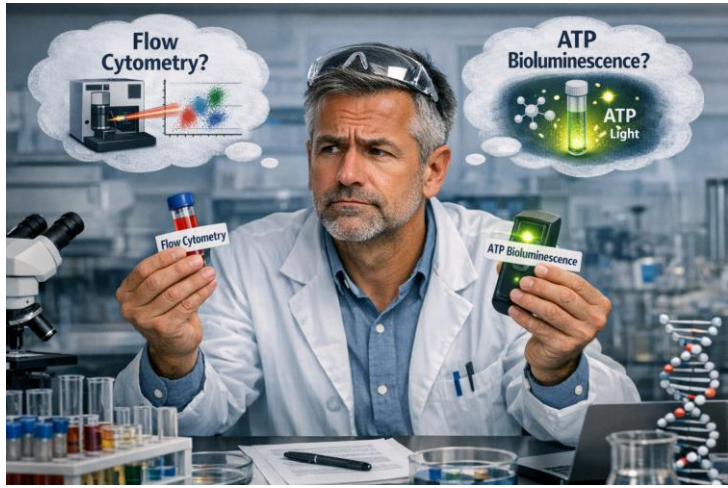
Where is your organization in implementing alternative or rapid sterility testing?

- Not currently considering it
- Exploring options
- In validation phase
- Fully implemented
- Previously evaluated but not adopted

# A Brief History

# Which Method is Right for My Use Case?

- Growth based
  - Electrochemical Measurement
  - $CO_2$ Detection
  - Biochemical/Carbohydrate Substrates
  - Digital Imaging / Auto-fluorescence
  - Laser Excitation
  - Selective Media
  - Head Space Pressure
  - Microcalorimetry
- Viability Based
  - Flow Cytometry
  - Laser Scanning Solid Phase Cytometry
  - Direct Epifluorescence Filter Microscopy
- Nucleic Acid Amplification
  - PCR
  - RT PCR
  - Ribotyping
  - Gene Sequencing
- Cellular Component Based
  - ATP Bioluminescence
  - Fatty Acid Profiling
  - MALDI-TOF
  - SELDI-TOF
  - FT-IR
  - Endotoxin Detection
- Optical Spectroscopy
  - Light Scattering/Intrinsic Fluorescence
  - Raman Spectroscopy
- Micro-Electrical-Mechanical Systems (MEMS)
  - Microfluidics
  - Microarrays



# Which Method is Right for My Use Case?

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

Chart from USP ⟨1071⟩

# Consult with your Regulators!

- Work with regulatory agencies/notified bodies on proposed AMM validation plan
- Define product requirements based on critical factors (filing info, 510(k) vs PMA, MDR status, etc.)
- Understand regional requirements, as these may vary

# User Requirement Specifications

- A rapid result time
- Microorganism Detection:
  - Low quantity
  - Wide rage
  - Specific organisms of concern
  - Investigations

- Samples:
  - Quantity
  - Batches
  - Handling

- Instrument:
  - Aseptic test material handling
  - Availability of instruments and reagents
  - Reference material and Controls
  - Ease of use/simplicity of test and data interpretation
  - Low rates of false positive and false negative results
  - Manual vs automatic preparation methods
  - Automation and automated continuous/periodic monitoring

- Improved patient safety
- Robustness
- Reliability

# ATP Bioluminescence: Pros and Cons

## Advantages

- Broad equivalency to compendial methods
- Early detection of microbial growth
- Isolation of organism for identification
- Easily fits into current workflow
- Objective Results
- Vendors and reagents widely available

## Disadvantages

- Inability to detect organisms that do not grow under conditions utilized
- Products with significant ATP background that cannot be reduced
- Increased labor time and cost of testing

# Rapid Product Sterility: AMM Support in Standards

- USP ⟨1223⟩: Validation of Alternative Microbiological Methods
- Ph. Eur 5.1.6: Alternative Methods for Control of Microbiological Quality
- PDA Technical Report 33: Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods (New revision effective soon)

Revised or newly official 01 Aug 2025

- USP ⟨1071⟩: Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach
- USP ⟨72⟩: Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products
- USP ⟨73⟩: ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products

Others:

- USP ⟨74⟩: Solid Phase Cytometry-Based Microbiological Method for the Detection of Contamination in Clear Aqueous Solutions (published in PF51(3) in May 2025)
- USP ⟨75⟩: Nucleic Acid Amplification Method for the Detection of Contamination in Short-Life Products (under development)

# Requirements for USP ⟨73⟩

- **Short-life products:** Products with a short-shelf life or with a short time between manufacturing and administration and for which a sterility test requiring a 14-day incubation is not suitable.
- Primary validation of the method per USP ⟨1071⟩
  - Does **not** require equivalence/comparability to USP ⟨71⟩
- Should include standard method suitability organisms AND a **selection of organisms relevant** to product/manufacturing process.
    - If relevant, slow-growing organisms and local isolates may be included
- Inoculum level for method suitability is ≤10 CFU
- Perform sample interference study
- Incubation time determined via calculation plus **safety factor**

# Generation Time Calculation for USP ⟨73⟩

**GENERATION TIME**

- Generation time = time required for microbial cell number to double

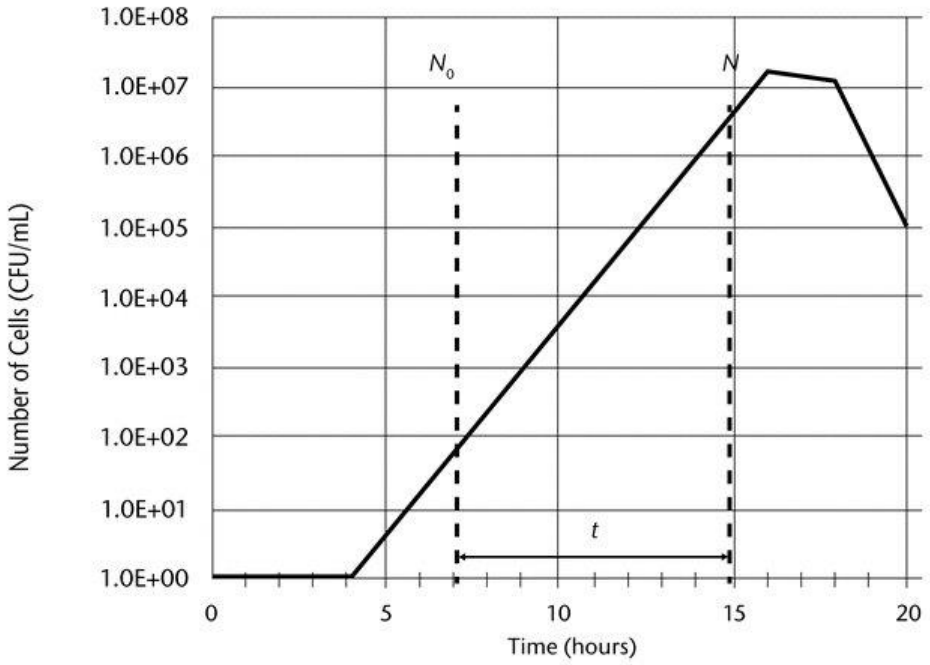
$$G = \frac{t}{3.3 \times \log_{10}(\frac{N}{N_O})}$$

$t$ = time interval (h)

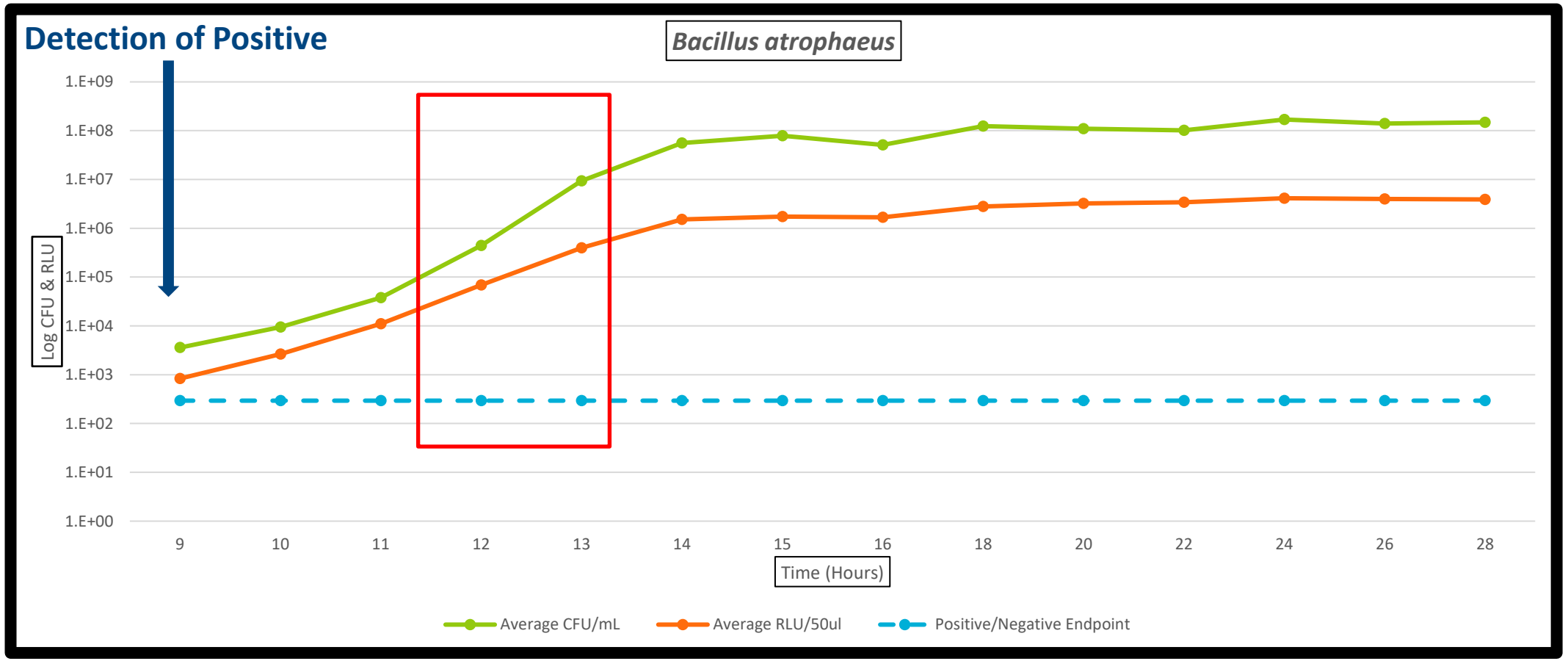
$N$ = number of cells/CFU at the end of the time interval

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

**EXAMPLE**



# Generation Time Example



# Incubation Time Calculation for USP ⟨73⟩

## USP ⟨73⟩ CALCULATION EXAMPLE

- Slowest organism time to detection of 48 hours
- Generation time of 1 hour

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

$$T = 48h + 3.32h \times 1$$

$$T = 51.3h \; or \; 2.1 \; days$$

$T$ = incubation time for microbial detection in the product to be examined (h)

$t_{ttd}$ = longest time to detection in the method suitability test (h)

G = generation time slowest growing organism (h)

## *BACILLUS ATROPHAEUS* CALCULATION EXAMPLE

- Time to Detection (+): 9 hours
- Generation (*G*) Time: 0.29 hours
- T = 9h + $log_2$(10) x *G*
- T = 9h + (3.32h x 0.29)
- T= 9.96h (approximately a 10h minimum incubation time)

# ⟨1223⟩ VALIDATION OF ALTERNATIVE MICROBIOLOGICAL METHODS

Qualitative tests must meet certain validation parameters

Examples of qualitative tests: 1) sterility test and 2) test for absence of specified microorganisms

Validation parameters that are required include the following

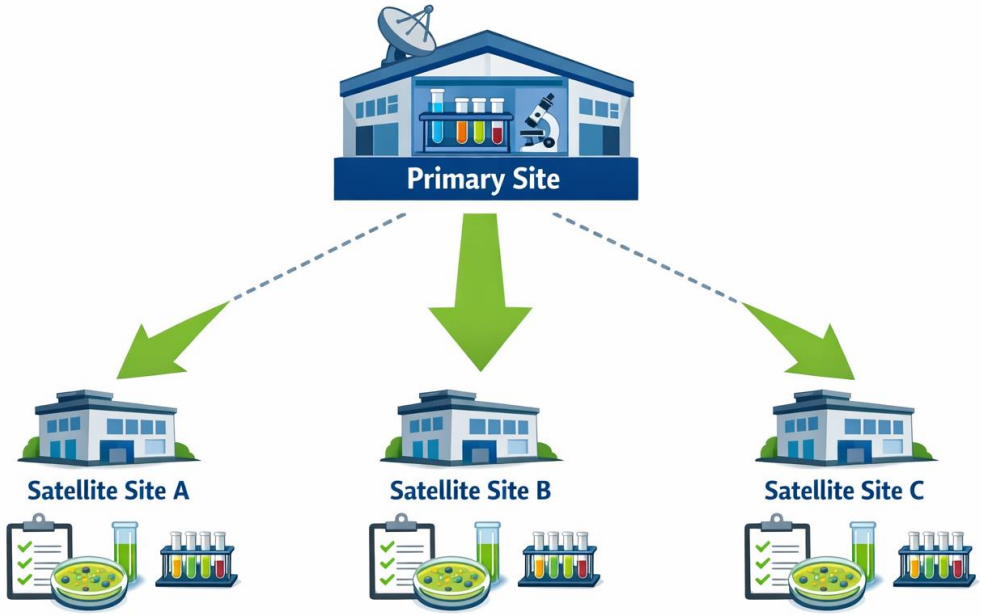
| | | | | | |
|---|---|---|---|---|---|
| Specificity | Limit of Detection | Robustness | Repeatability | Ruggedness | Equivalency |

# Suitability

| Requirements | ⟨73⟩ | ⟨1223⟩ |
|---|---|---|
| **Method Suitability:** | | |
| USP ⟨71⟩ Organisms | ✓ | ✓ |
| Environmental Isolates (3) | ✓ | ✓ |
| ≤ 10 CFU | ✓ | |
| ≤ 100 CFU | | ✓ |
| **False Positive/Negative:** | | |
| Sample Interference | ✓ | ✓ |

# Multi-Site Implementation

- Are there any differences in the sites?
  - Temp, rH, altitude
  - Microflora
  - Data recording
- Keep IQ/OQ consistent
- Reduced PQ
  - Ensure all site-specific materials are assessed




ARE YOU BETTER THAN A
RAPID STERILITY
METHOD?

# The Rules

- There are 5 samples, note which are positive and which are negative
- The samples were processed through the ATP Bioluminescence reader to determine a Relative Light Unit (RLU) value which correlates to ATP activity in the sample
- If the RLU value is 3 times greater than the negative control, it is considered positive

# The Results

| Sample # | Visual Result | RLU Result | Sample Type |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |

# An Even Simpler Approach

| Requirements | Do it Yourself | Contract Lab |
|---|---|---|
| IQ | ✓ | Already established |
| OQ | ✓ | Already established |
| PQ | ✓ | Already established |
| ⟨71⟩ Equivalence Testing | ✓ | Already established |
| Suitability | ✓ | ✓ |

# Conclusions

Consult with your regulators early and have a plan

Know your user requirements

Rapid sterility is an impactful way to shorten release times and improve patient safety

# Questions?

# Useful Links

## Sotera Health Academy:

## White Paper:

## LinkedIn: