Current activities of the USP Microbiology Expert Committee

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Topics

- Bioburden Characterization
- Rapid Microbial Tests
- Recombinant Reagents for Bacterial Endotoxins Test

- **Bioburden Characterization**

How Do You Gain Assurance of Sterility?

Sterility assurance for a lot/batch of product does not come from passing the tests in *USP* <71> *Sterility Tests.*

How Do You Gain Assurance of Sterility?

- Sterility assurance can be established only through the use of adequate sterilization cycles and subsequent aseptic processing, if any, and adherence to appropriate current good manufacturing practice.

- *USP* Chapters <1211> *Sterility Assurance* and *USP* <1229.x> series of *Sterilization chapters* address this.

Bioburden

- ISO 11737-1:2006 describes bioburden as

"… the sum of the microbial contributions from a number of sources, including raw materials, manufacturing of components, assembly processes, manufacturing environment, assembly/manufacturing aids (e.g., compressed gases, water, lubricants), cleaning processes and packaging of finished product …"

Why Bioburden?

- Articles intended to be sterile must attain a ≤10−6 PNSU i.e., less than or equal to one chance in one million that viable bioburden microorganisms are present.
- Bioburden is the ‘true’ target of sterilization processes and thus knowledge of its resistance becomes essential.
- The selection of a particular sterilizing treatment and the details of its execution often represent a compromise between the conditions required to destroy or remove the bioburden to the desired level and the impact of the sterilization process on the materials being processed.

Bioburden Control

- Control over pre-sterilization bioburden is necessary to assure the final product conforms to the requisite finished product quality sterility criterion.
- To this end, bioburden control is mandated through 21 CFR Part 211.110, Production and Process Controls which states *"…written procedures shall be established and followed that describe the in-process controls, and tests, or examinations to be conducted on appropriate samples of in-process materials of each batch."*

Bioburden Control

- In the manufacture of terminally sterilized products validated sterilization processes are performed which impart the microbial quality attribute of sterility upon the finished product.
- These lethal sterilization processes may be designed, developed, qualified and routinely controlled based upon a consideration of the bioburden on or in the material.
- Given the diversity of bioburden (i.e., quantity, physiological state, physical state, resistance) coupled with the technical constraints of sampling, and analysis, bioburden control is one of the greatest challenges in the manufacture of terminally sterilized products.
- Moreover, the determination of bioburden resistance is especially deserving of careful consideration; permitting the optimum monitoring and control strategy for effective, efficient and compliant manufacture.

What is the Sterilization Objective?

- A minimum PNSU of 10^{-6} is desired for all items.
- That means that in routine operation of the sterilizer, the possibility for a surviving **bioburden** microorganism must be less than 1 in 1,000,000.
- That has very little to do with the biological indicator resistance, and even less to do with BI population.

BI vs Bioburden Resistance

- Killing the bioindicator is certainly safe, but this approach arbitrarily increases the adverse process impact on the product. The real target in sterilization is always the bioburden, which is generally far easier to kill. Therefore, the sterilization process should be developed with that as the objective.
- Adoption of sterilization processes qualified using the bioburden/BI or bioburden methods therefore requires a robust routine means of effectively monitoring both the population and resistance of bioburden.

<56> Sterilization Process Resistance Determination for Microorganisms

- Published in PF late last year…

- With the possible exception of overkill processes, information regarding the resistance of the bioburden to the sterilization process is essential to confirm the efficacy of the sterilization process and is a required component of a parametric release program. Spores exhibit inherent and varying levels of resistance to all modalities of sterilization.

- Boil Test-The boil test relies upon the fundamental observation that spores generated from differentiating microorganisms will retain their viability, while vegetative cells swiftly lose viability at 100 degrees C.

<56> Sterilization Process Resistance Determination for Microorganisms

- The test requires that a sample or item under evaluation is uniformly subjected to a temperature of 100 degrees C for a specified duration of time; ensuring a constant thermal challenge over time.

- Any surviving viable microorganisms are then recovered by culture-based methods permitting their enumeration and isolation for further analysis depending upon the purposed application of the test.

- This chapter outlines methods that can be used to determine the presence of spores and establish the moist heat sterilization resistance of vegetative cells, spores and biological indicators (BI).

Rapid Microbial Tests

The Sterility Testing Dilemma

- The currently required 14-day incubation period imposes a significant burden on the manufacturer, who must quarantine product until successful completion of the test.

- The long incubation time (14 days) is unsuitable for numerous small lot size products including cytotherapy, radiopharmaceuticals, pharmacy compounded sterile products, and some clinical trial materials.

Rapid Microbial Tests

- Establish user requirement specifications for Rapid Microbial Tests for release of short shelf life sterile products

- Recommend suitable technologies for the rapid microbial testing of short-life sterile products.

Limit of Detection

- This user requirement is perhaps the most challenging.
- Within the limitations of preparing inocula with one or more colony-forming units, growth-based sterility tests can be shown to have at least a theoretical Limit of Detection (LOD) of 1 CFU or 1 to 3 CFU based on a Poisson distribution.
- Setting a LOD of a single viable cell of all technologies is a huge barrier of entry for a rapid sterility test especially when the signal is not the colony-forming unit that is amplified by cultural enrichment.

Time to result

- The incubation time for growth-based *USP* <71> *Sterility Tests* is at least 14 days, which makes it unsuitable for positron emission tomography and cell therapy as these short-life products would be administered prior to the completion of the test, marginal for sterile compounding, but generally suitable for pharmaceutical manufacturing.
- PET radiopharmaceuticals are usually administered within several hours of preparation due to the short half-life, so candidates for a rapid sterility test need to be real time.
- For compounded sterile preparations and cell therapies, rapid sterility tests need to be completed at best within 24 hours or within 48 hours at the most.

Time to result

- Signals employed by different technologies may be amplified by enrichment culture with a 24-48 hour incubation or by concentration e.g. filtration, selective adsorption and elution or centrifugation, to reduce the time to result and lower the limit of detection.

Patient Safety

- It is widely accepted that a rapid microbial test for release of compounded sterile preparations, radiopharmaceuticals and cell therapies will improve patient safety, especially if contaminated materials can be detected prior to administration to patients.
- Furthermore, rapid microbial test methods that continuously monitor for the presence of viable microorganisms and report when a failure is detected would enable the laboratory to report to the clinician who could intervene with patient will provide an additional advantage.

Sample Quantity

- The minimum number of articles tested and quantity per container tested per media is defined in Tables 2 and 3 of *USP* <71> *Sterility Tests*.
- Whereas this sampling plan is suitable for manufactured pharmaceuticals, it is unsuitable for products generated by sterile compounding pharmacies, PET facilities, and cell therapy manufacturing facilities due to the small batch size of their products and the volume of the products to the individual patients. A further consideration is the sample size limitation of the advanced technology.

Sample Quantity

- Alternative sampling plans have been proposed in other compendial chapters.
- For example, the recommended approaches to sterility testing of compounded sterile preparation (CSPs) and cell therapy products as found in *USP* <797> and *Ph. Eur*. 2.6.27.

Sample Quantity

- Example from USP<797> for Compounded Sterile Preparations (CSP)
- If between 1 and 39 CSPs are compounded in a single batch, the sterility testing must be performed on a number of units equal to 10% of the number of CSPs prepared, rounded up to the next whole number. For example: If 1 CSP is compounded, 10% of 1 rounded up to the next whole number would indicate that 1 additional CSP must be prepared for sterility testing.
- If 39 CSPs are compounded, 10% of 39 rounded up to the next whole number would indicate that 4 additional CSPs must be prepared for sterility testing

Example from EP 2.6.27 Microbiological Examination of Cell- based preparation

- The contamination test sample size for a cell preparation with a volume between 10 and 1000 mL would be 1% of the total volume, for a cell preparation with a volume between 1 and 10 mL it would be a sample size of 0.1 mL and for a cell preparation less than 1 mL,surrogate testing, in-process testing or other appropriate testing should be used and justified.

A Risk-based Approach

- There are obvious trade-offs between limit of detection, sample size and time to results. Detecting a contaminated unit prior to administration will improve patient safety.
- It is therefore pragmatic to develop risk-based rapid microbial tests that provide a certain level of LOD suitable for different stakeholders. For example, for a short lived product with a shelf life of less than a day, the time to result is a critical user requirement specification from a patient safety perspective.
- So a rapid microbial test with a time to test of less than a day would promote patient safety even if it had a limit of detection greater than one viable microbial cell.

Rapid Microbial Tests that match URS

Candidate rapid microbial tests for release of short life products include:

- Respiration
- ATP Bioluminescence
- Solid Phase Cytometry
- Flow Cytometry
- RT-PCR
- Isothermal Microcalorimetry

Candidate Technologies

- It is acknowledged that no single method will work for all types of products/ product matrices and one or more of these analytical platforms may be found to have insurmountable technical limitations, which may prevent them from becoming compendial test methods.

Where are now?

- <1071> is effective December 1, 2019 in 2S to USP42-NF37
- This is being followed by development of test methods chapters (chapters numbered less than 1000, based on technologies that can be advanced as compendial methods.
- Proof of concept testing may be needed for certain technologies prior to chapter development.

<72> and <73> Proposals

- <72> Respiration based Rapid Microbial Methods for release of Short shelf Life Products
- <73> ATP Bioluminescence based for release of Short shelf Life Products
- Generic Methods that takes into accounts various formats of the respective technologies available from multiple vendors
- Proposals Targeted to appear in the November 2020 issue of PF.

Recombinant Reagents for Bacterial Endotoxins Test

Bacterial Endotoxins Test

- USP <85> Bacterial Endotoxins Test (BET) describes different methods for the detection and quantitation of endotoxins activity.
    - Reagents described in <85> are naturally-derived lysates of amebocytes (blood cells) sourced from horseshoe crabs (HSC) either *Limulus polyphemus* or *Tachypleus tridentatus*
- Limulus Amebocyte Lysate (LAL) and Tachypleus Amebocyte Lysate (TAL) contain a cascade of zymogen proteases

Recombinant Reagents for BET

- New variants of the traditional naturally-sourced lysate reagents, using one or more *recombinant* zymogen proteases cloned from the cascade, represent possible alternatives to the natural lysate reagents.
- Currently three different recombinant variants are available
  - A fourth variant to be available soon.

Where are we now?

- A revision to <*85*> that allows the use of Recombinant Reagents as an alternative to LAL in the end point chromogenic/fluorometric method was published for public comments in PF 45 (5), 2019.
    - Based on stakeholder feedback the revision proposal was canceled

Stakeholder Recommendations

- Stakeholders recommended:
  - ✓Development of an independent chapter <xxxx> rather than inclusion in a harmonized chapter
  - ✓Include instructions on how to demonstrate comparability between recombinant reagents and LAL reagent in the BET
  - ✓Provide guidance on QA oversight of recombinant reagents for BET which is currently not regulated by the FDA

Where are we now?

- A new chapter proposal <1085.1> that allows the use of recombinant reagents in BET was developed.
- Published in the latest issue (September 2020) issue of PF. Comment Period Closes Nov 30,2020.
- Inclusion of instructions on how to demonstrate comparability between recombinant reagents and LAL reagent in BET.
- This proposed chapter also provides suggestions for the evaluation of a recombinant reagent supplier
- Use of methods in this proposed chapter in place of the ones indicated in <85> to meet the requirements of BET will require demonstration of non-inferiority/equivalence and approval by the regulatory agency