Title: Rapid Sterility Testing Applied to Tissue Products

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Background: Rapid sterility testing has been a topic for many years, but is often not applied to tissue products using a platform that has been approved by regulatory agencies, including FDA. The validation of these systems, as well as determination of appropriate steps to verify their use on new tissue product types, can be complex and often not well understood. This presentation will describe the validation efforts that go into general validation of a rapid sterility test system, followed by details on how the validation can be applied to distinct product types, including tissues, using a verification process rather than requiring full validation.

Hypothesis: This presentation is intended to provide the science behind rapid sterility test systems, particularly the Charles River Celsis® system, and provide general details on validation and verification practices as well as the potential benefits to a tissue bank. Discussions with tissue banks are ongoing at this time in an effort to gain specific experience with tissue products.

Methods: As part of the presentation the test methods associated with use of the Charles River Celsis® system will be provided. This system is specifically designed to be utilized as a means of detecting growth in a sterility test in a shorter period of time than the traditional 14-day incubation. It does allow for continued incubation and identification of any microorganisms that grow in the sterility test media.

Results: In the presentation results will be provided that have been obtained to date, and examples will be shared of the process of applying the Celsis system to new products, including challenges that occurred during validation/verification, as well as the resulting incubation time to detection of growth.

Conclusions: The presentation will describe successful ways of incorporating rapid sterility test approaches to tissue products and provide points to consider when assessing the use of such systems.

Ethical Considerations: The only potential ethical consideration to address is that any data shared from a customer will be general in nature and approval of the content will be obtained prior to presentation.

RAPID STERILITY TESTING OF TISSUE

GENERAL INFORMATION

Charles River Celsis® system detects microbial growth in a sterility test before it can be seen with the unaided eye.
Growth has been validated for detection at 6 days compared to the 14-day sterility test.
Historically, significant work with pharma companies to help in faster product release.
Can benefit tissue primarily in two ways:

1. Faster release for short-shelflife products
2. Eliminate need for subculturing sterility tests where the product creates turbidity

Sterility tests continue to incubate, if desired, for identification of positive test samples.
Can test using both TSB and FTM, or only one if desired.

APPLIED TO TISSUE

Same process for pharma can be applied to tissue.
Verify that neutralizers added to media are suitable for Celsis® process through sample effects testing. Antibiotics and other residuals on tissue assessed in the same way.
Sample effects testing:

- Background ATP and contamination
- ATP bioluminescence interference

Does the product or solution contain non-microbial ATP or other components to inhibit ATP and enzymatic reactions?

VALIDATION OUTCOMES

Equivalency testing:

- LOD = not statistically different
- Detection of 0.1, 1, and 10 CFU at 6 days compared to 14 days = non-inferior to USP
- Ruggedness = met %CV < 30%
- Robustness = passed all acceptance criteria

MICROORGANISMS USED IN VALIDATION

Aspergillus brasiliensis
Staphylococcus aureus
Candida albicans
Pseudomonas aeruginosa
Bacillus spizizenii (aka *B. subtilis*)
Clostridium sporogenes
Micrococcus luteus (stressed)
Staphylococcus epidermidis (stressed)
Cutibacterium acnes (stressed)
Burkholderia cepacian
Methylobacterium extorquens
Penicillium citrinum

Celsis® Advance II™
~80 samples in one hour

criver.com/products-services/qc-microbial-solutions/microbial-detection

CELSIS® PROCESS

1. Perform sterility test following SOP/standard.
2. At 6 days gently agitate bottle and aseptically remove ~2 mL of media from bottle and place 50µl aliquots into duplicate cuvettes.
3. Place cuvettes into Celsis® system and initiate program.
4. Results are provided as positive or negative for growth.
    a. Sample results are compared to baseline media.
    b. Microbial enzyme-enhanced ATP causes bioluminescence, demonstrating the presence or absence of viable microorganisms.
5. Continue incubation for samples scored as positive for growth by Celsis® to obtain microbial identifications.

METHOD SUITABILITY (B/F)

Due to being a rapid method, recommend an enhanced method suitability test. Perform with same 12 microorganisms from validation, but without stressed process. If only using TSB can remove *Clostridium*.
Testing process is identical to USP, but after 5 days of incubation, remove aliquot and test in Celsis®.

CHALLENGES DURING VALIDATION

Background inference can cause high relative light unit (RLU) readings in media. However, it can be greatly reduced by specific reagents included in the process and verified via an LOD equivalency qualification.

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