Roundtable Session 1 Table 8 – Use of New Rapid Sterility Testing Methods – Successes and Challenges

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Abstract:

Due to the nature of cell and gene therapy products, timely results for release testing are necessary for administration to patients. The final formulated drug product may be frozen prior to dosing or delivered fresh within hours of manufacture. In both cases, severity of patient prognosis also drives the need for speedy delivery to the patient. Current compendial sterility (USP<71>) and mycoplasma (USP<63>) testing provide results in 14 and 28 days, respectively. This means results may be received after patient treatment. While a preliminary gram stain test on the drug product is recommended prior to release, the patient is still at risk. To combat this, rapid sterility tests are being developed to accurately detect bacterial and fungal contamination. For sterility testing, blood culture systems such as Bactec and BacT/Alert, which are automated, allow for continual monitoring with objective detection under aerobic and anaerobic condition. These methods allow for final results reported after 7 days of culture with readouts at various intervals to allow detection of a rapidly growing organism prior to the full 7 days of culture. With proper qualification and proof of equivalence to USP<71>, health authorities (HA) have recognized these tests as acceptable alternatives to the compendial methods. Sequencing-based assays have been developed to detect microbial contamination, specifically for mycoplasma. While commercially available, minimal assay validation is still needed to support limit of detection across laboratories and programs. In this roundtable, we will discuss the improvements to rapid sterility testing, future hurdles, and cooperation between Sponsors and HAs to further development.

Recommended reading: Sterility Testing for Cellular Therapies: What Is the Role of the Clinical Microbiology Laboratory? https://pmc.ncbi.nlm.nih.gov/articles/PMC7315024/

Discussion Questions:

1. In what ways have the Bactec and BacT/Alert systems improved release testing for sterility and what challenges do you foresee in implementing further improvements on the system, especially for slower growing organisms?

2. Current compendial and alternative sterility testing are limited in the number of organisms tested for, basing on manufacturing risk and exposure. As trials continue to expand manufacturing globally and evolving microorganisms, how have Sponsors and health authorities considered the need to expand the testing array?

3. What benefits and challenges are associated with the development of sequencing-based assays to detect microbial contamination?

Notes:

Several cell therapies have been approved using **BacT/ALERT-based sterility assays**. In these cases, sponsors were required to validate the non-compendial assay and demonstrate comparability to compendial methods. There is growing interest in implementing **rapid technologies**, such as **qPCR** and **ddPCR**, for sterility release testing. However, there remains uncertainty regarding what constitutes an acceptable **qualification/validation strategy** from a regulatory standpoint.

This uncertainty may stem from difference in the extend of feedback provided during regulatory reviews, particularly between early-stage input from **OPT reviewers at the FDA** and later-stage evaluations conducted by **DBSQC experts** in analytical procedures related to safety. While the Agency typically offers additional comments on validation during the **BLA review**, this internal misalignment can create challenges for sponsors.

It may be worthwhile submitting a formal inquiry to the FDA specifically requesting clarification on validation requirements for alternative methods to compendial sterility tests. In doing so, sponsors should explicitly ask for feedback from **DBSQC reviewers**. Although the Agency may not provide formal feedback due to time constraints, **informal guidance** could still help support BLA submissions.

Mycoplasma testing using qPCR is already in practice using commercial kits (e.g., PMC10192841). The validation process for qPCR-based sterility testing is not expected to differ significantly. **NGS-based sterility methods** are also in development, though their **sensitivity** and **false-positive rates** remain key concerns. **Sample enrichment** may be necessary to achieve optimal performance for these methods.

Currently, the FDA accepts **Gram stain plus BacT/ALERT** as a sterility release strategy for **short shelf-life products**, provided that justification is scientifically sound. Some sponsors have released product batches based on Gram stain results first, supported by a protocol included in their **IND/BLA** outlining follow-up steps in the event of a positive BacT/ALERT result post-release. This strategy has precedent in at least one **commercially approved short shelf-life cell therapy**.

Additional experience shared by roundtable participants included:

- Releasing product based on **7-day BacT/ALERT results**, with mitigation protocols in the IND in case the **14-day result** is later positive.
- Using **in-process data** from **intermediates** and **wash buffers** to expedite release timelines while still meeting sterility requirements in a clinical context.

USP <1071>, *Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach*, supports the use of rapid sterility methods, including **nucleic acid amplification technologies**. The approach of using rapid sterility methods are encouraged in the corresponding **Ph. Eur. chapter**.

Contract testing organizations developing novel methodologies may engage the FDA through the **CBER Advanced Technologies Team (CATT)** to seek designation for advanced methods. One challenge with this pathway is that while a method may not be new per se, its application in the cell therapy context may be novel. Even with CATT designation, **use of a Master File** can be limited, as the FDA typically requires **product-specific data** to be included directly in the BLA to enable comprehensive review, rather than relying on external master files.

FDA can also be influenced through organizations such as NIST and NIIMBL (https://www.niimbl.org/) rapid analytical procedures for adventitious agent testing including sterility and to provide clarity on requirements to be met to register such assays.