



Identifying Faster Sterility Tests for Biological Products

Regulatory Research Seeks to Reduce the Time Needed to Ensure the Safety of Critical Products

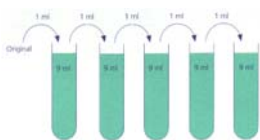
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The Need for Rapid Sterility Test

The Food and Drug Administration requires that all parenteral biological products undergo sterility testing using the compendial sterility method to ensure that products such as vaccines are safe when they reach the market. This method is based on the observation of turbidity in liquid culture media due to the growth of bacteria, yeast, and fungi and can detect extremely small numbers of microorganisms. However, this method takes 14 days to provide results. This two-week period can be a significant limiting factor in the timely release of biologicals, particular for pandemic vaccines and products with short shelf lives. Researchers in the Division of Product Quality (DPQ) in the Office of Compliance and Biologics Quality (OCBQ) are working to resolve this problem by evaluating rapid microbial methods that significantly reduce the time needed for sterility testing of biologicals. As part of that effort a team of researchers in OCBQ compared the ability of several alternative rapid tests to detect microorganisms in either solid or liquid media in the presence and absence of thimerosal, the organomercury compound used as an antibacterial and antifungal preservative in certain biological products.

Comparing Rapid Sterility Methods to the Compendial Sterility Method



Microorganisms were spiked into 10 ml of Fluid A with & without 100 ppm thimerosal, and then evaluated for detection by Compendial Sterility Methods (Filtration and Direct inoculation method) and Rapid Sterility Methods (Milliflex Detection System, BacT/Alert, BACTEC). Three rapid microbial methods and the compendial sterility method were comparatively evaluated for the sensitivity and speed of detection of spiked microorganisms.

Direct inoculation method in liquid medium



Compendial Sterility Method



Filtration method using liquid medium

*Media Used in Compendial Sterility Method:
Fluid Thioglycollate Medium and Tryptic Soy Broth*

The DPQ team developed criteria for an alternate rapid sterility method to be equivalent in sensitivity and accuracy to the Compendial Method, but provides results within seven days rather than two weeks. The researchers evaluated the ability of three alternative methods to meet the criteria and compared the results to the standard compendial sterility method. To compare the various methods they spiked growth media with various organisms and used the methods to detect, isolate and identify the spiked organisms.

Alternative Methods

BACTEC™



- Direct inoculation into standard aerobic and anaerobic liquid media
- Early detection of bacterial growth based on CO₂ fluorimetric detection

BacT/ALERT®

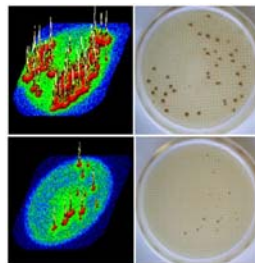


- Direct inoculation into iAST and iNST liquid media
- Detection of bacterial growth based on CO₂ colorimetric detection

Rapid Milliflex®



- Growth on solid media: Tryptic soy agar (TSA), Sabouraud dextrose agar (SDA), and Schaedler blood agar (SBA)
- Inoculum filtered by membrane; membrane sprayed with ATP-releasing and bioluminescence reagents
- Photons generated ATP bioluminescence are captured and detected by photon counting imaging tube; picture displayed on computer monitor. Some colonies identified even before visual growth on medium.



*Images generated by photons captured by Milliflex
ATP bioluminescence compared to actual growth
on solid media.*

Major Findings

- Compendial method, Rapid Milliflex, BacT/Alert, and BACTEC showed equivalent sensitivity at detection of lowest spiked microorganism level.
- Milliflex Detection System detected the lowest spiked of *P. acnes* and *B. vulgatus* within 5 days versus 9-10 days for compendial sterility method, BacT/Alert, and BACTEC.
- Milliflex consistently detected spiked organisms at 1 CFU/10 ml within 5 days (usually 1-3 days). Similar results were obtained at 10 and 100 CFU/10 ml.
- Solid media (TSA, SDA, and SBA) showed faster growth of spiked organisms (within 5 days) compared to liquid medium.
- BACTEC and BacT/ALERT did not show growth in experiments using matrix containing thimerosal for most of the microorganisms.
- Unlike TSA and SDA media, SBA supported growth of spiked organism in the presence of thimerosal and after treatment of organisms with Rapid Milliflex ATP-Bioluminescence reagents.

Milliflex Detection System appears to be a promising alternative to the compendial sterility test for filterable biological products.

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