Rapid Sterility Testing as the Critical and Final Result for Product Release – Design Verification (DV) Data

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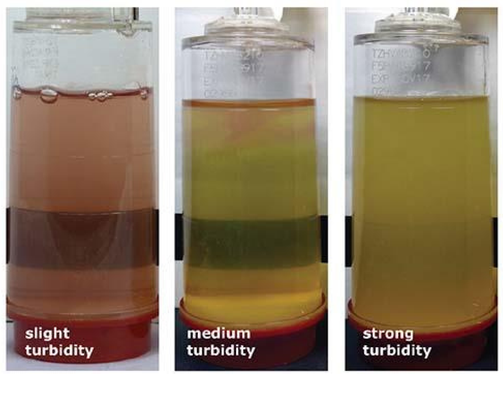
Introduction

Sterility testing is a mandatory release test for all manufactured sterile drug products. The testing is widely used to monitor and maintain sterility of the products and processes and ensure patient health and safety. The compendial sterility test is a 14-day test and in most cases, product cannot be released until the test is passed. It is a qualitative test that relies on the turbidity of the sterile sample. There are three different sampling techniques: Open funnel method, Direct inoculation and membrane filtration (the later being the major sampling technique). A defined portion of the finished product will be tested in Tryptic Soy Broth (TSB) and in Fluid Thioglycolic Media (FTM). Due to the importance of the sterility test to ensure product safety as a batch release critical sample, early confirmation on presence or absence of a contamination is patient and business critical. Over the past years, many different Rapid and Alternative methods have been invented and introduced into the Pharmaceutical industry. The Growth Direct® Rapid Sterility System is an automated, rapid microbial detection and enumeration system, based on the Growth Direct® System which has been evaluated in a DV Study to perform and utilize the Sterility test.

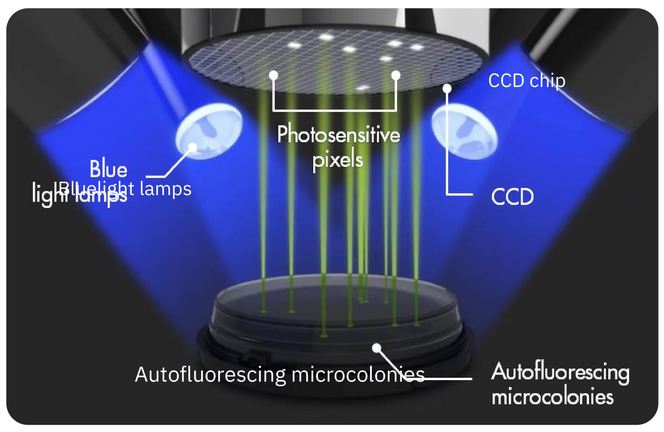


Technology

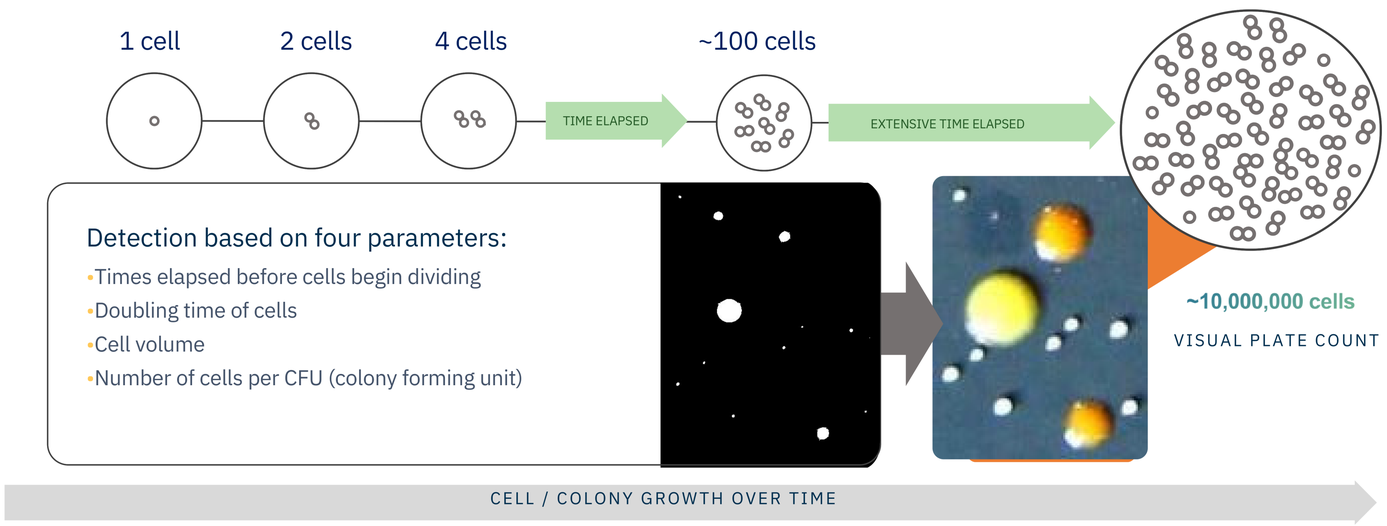
The Growth Direct® Rapid Sterility System is an automated, rapid microbial detection and enumeration platform suitable for sterility, in-process product testing, environmental, and water monitoring that integrates digital imaging, robotic cassette handling, incubation, and software control.

After processing samples and loading onto the Growth Direct® Rapid Sterility System, cassettes are automatically moved every four hours by a robotic system and illuminated by the blue-light technology which generates auto-fluorescence. The auto-fluorescence from the microorganism is captured by a camera and a proprietary software does the rest, the incubation continues with data monitoring and traceability throughout the incubation period.

- All microbial cells fluoresce in the yellow-green spectrum when illuminated with blue light.
- Charged Coupled Device (CCD) captures images with illuminated pixels where fluorescence from microbial cells is detected.
- Image analysis software counts the clusters of illuminated pixels that represent underlying microcolonies.



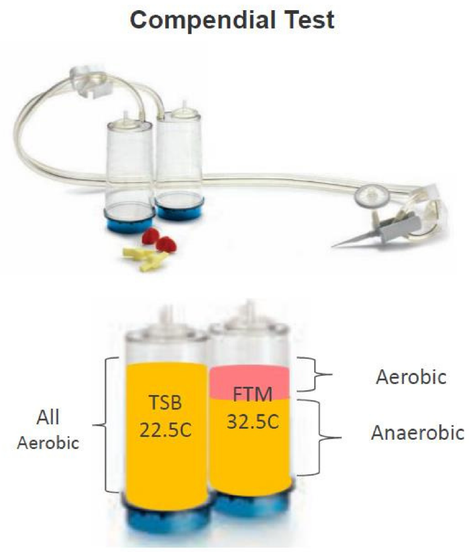
Growth Direct® technology finds colonies much earlier than operators using visual plate inspection



Methods

The Design Verification study involved comparison of "real-world" application; comparison of the compendial test with the Rapid Sterility Kit. In these study, different bacteria, molds, and yeast have been spiked with a predefined number of microorganisms to challenge the software in signaling at the first sign of microorganism detection.

Samples were processed using the Growth Direct Rapid Sterility Kit, composing of three different cassettes that will ensure the detection of the contamination. Sample as well as media is evenly distributed onto the different cassettes which will be loaded into the Growth Direct® Rapid Sterility System after sampling completion.



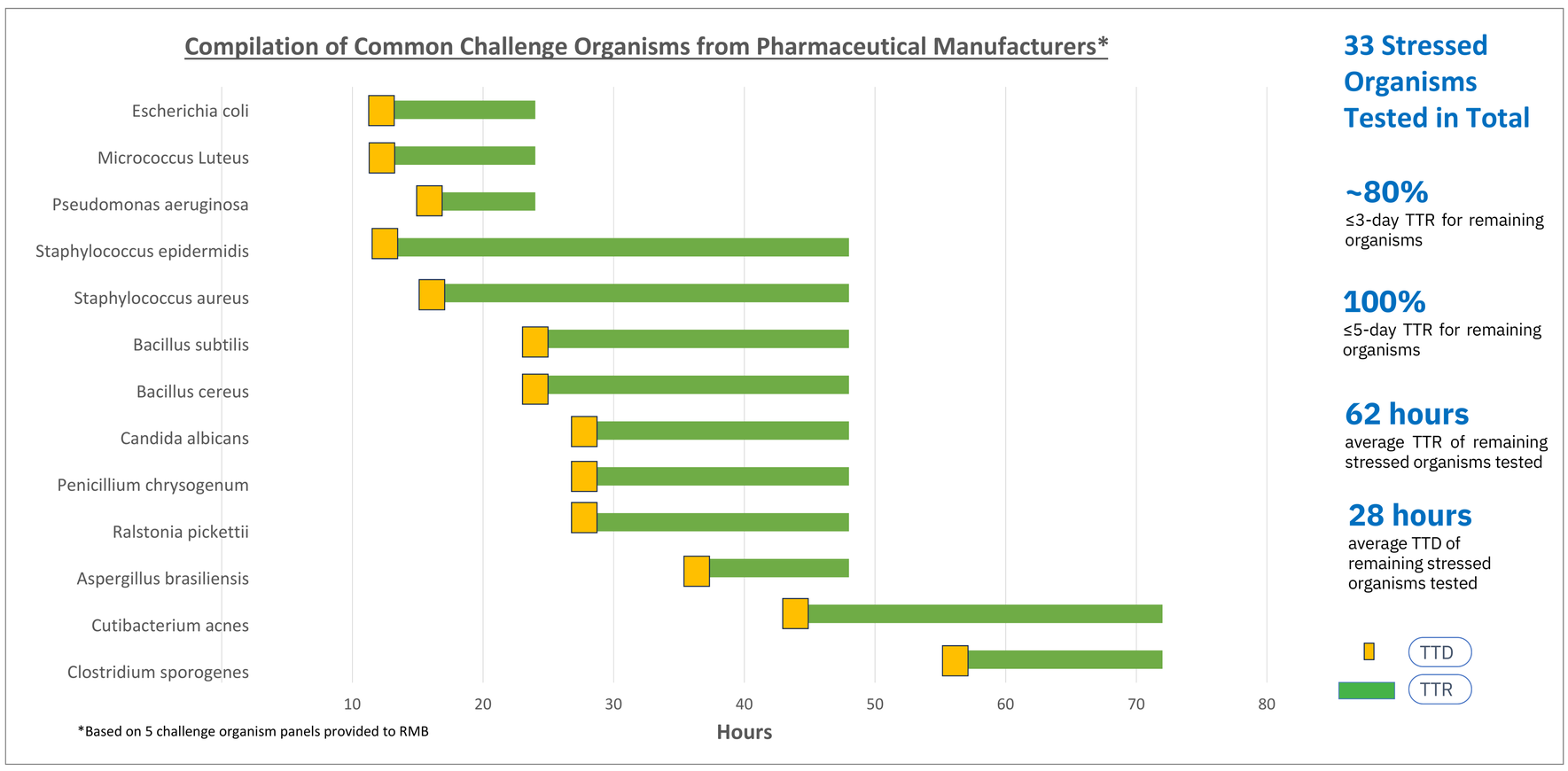
Relevant Species

The selection of relevant species for the Design Verification study was based on Compendial requirements, literature research and supporting data from Pharmaceutical companies. And because microorganisms vary by location and climate conditions, the following Microorganisms were identified at sites globally, internal isolates, compendial requirement and commonly referenced isolates in scientific literature (1,2).

TYPE	MICROORGANISM	TYPE	MICROORGANISM
Aerobic Bacteria	Acientobactor lwoffii Bacillius cereus Bacillius licheniformis Bacillus subtilis Corynebacterium tuberculostericum Escherichia coli Klebsiella oxytoca Kocuria rhizophila Microbacterium liquefaciens Micrococcus luteus Paenibacillus lautus Pseudomonas aeruginosa Ralstonia pickettii Salmonella enterica Staphylococcus aureus Staphylococcus epidermidis	Anaerobic Bacteria	Bacteroides vulgatus Cutibacterium acnes Clostridium sporogenes
		Yeast	Candida albicans Cryptococcus sp. Candida parasilosis Komagataella phaffii Sporidiobolus salmonicolor Wickerhamomyces anomalus
		Mold	Aspergillus brasiliensis Aspergillus fumigatus Cladosporium cladosporidies clade1 Epicoccum sp. Penicilium chrysogenum Pithomyces chartarum Fusarium oxysporium Trichophyton rubrum

Time to Detection (TTD) and Time to Result (TTR) for stressed Microorganisms

The detection of a contamination is time critical, for patients and Pharmaceutical company alike. Therefore, having the lowest possible Time to Detection (TTD) and Time to Result (TTR) is crucial. Therefore, during Design Verification testing to determine the TTD and TTR of the Growth Direct Rapid Sterility Kit, we tested the relevant species in a stressed state as this will mimic the worst condition that microbiologist will find the microorganisms in.
Stress was performed using Heat stress in a water bath, achieving at least a 50% kill rate of the initial Inoculum.



Impact

The technology has shown to reduce the time-to-detection (TTD), enabling a faster release and remediation process:

- Deliver a TTD in as little as 12 hours
- Deliver a TTR in as little as 1-3 days
- Enables rapid responses to contaminations with real-time detection notification
- Reduce risk by automating traditionally human processes rife for error like sample handling and data processing
- Maintain test integrity by reducing the risks of secondary contamination with a closed-loop design and non-destructive sample processing

Conclusion

Rapid, automated sterility testing with the Growth Direct® Rapid Sterility System in combination with the Rapid Sterility Kit has proven within the Design Verification a TTD of as little as 12 hours and a TTR of as little as 1-3 days within a wide range of different microorganisms and species. These technology enables patients and manufacturer earlier access to critical drugs and enables faster and safer market supply.

References

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3. Matthew R. England, Frida Stock, James E. T. Gebo, Karen M. Frank, Anna F. Lau: Comprehensive Evaluation of Compendial USP<71>, BacT/Alert Dual-T, and BacTec FX for Detection of Product Sterility Testing Contaminants. Journal of Clinical Microbiology 2019 Volume 57, Isue 2 e01548-18

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