Validation of the Mango System vs Compendial USP <61> Bioburden Testing

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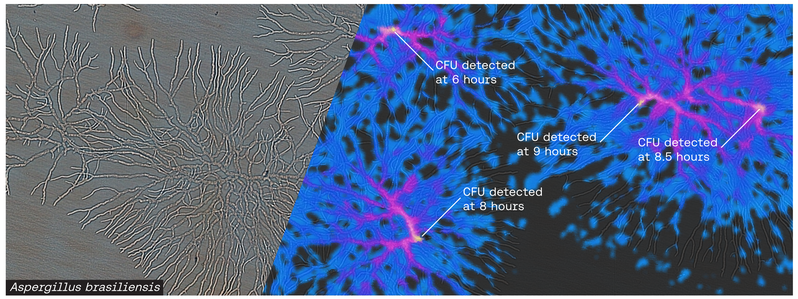
Abstract
In April 2024, a top-3 biopharmaceutical company conducted a Proof of Principle study comparing the Mango system with compendial USP <61> bioburden testing. The study found that the colony-forming units (CFUs) detected by the Mango system suggests equivalence to those manually counted on tryptic soy agar (TSA) plates. Additionally, the Mango system reported time-to-detection (TTD) of less than 5 hours for healthy yeasts and bacteria and less than 10 hours for mold compared to conventional counting methods which normally takes multiple days.

Introduction
The Mango System is an automated microbial incubation and enumeration device that aims to deliver compendial-equivalent CFU counts faster than any other growth-based bioburden test on the market. Compendial USP <61> bioburden testing takes as long as 3-7 days, which can lead to delays and at-risk release of materials. In comparison, the Mango System can generate time-to-detection of microbial growth in hours rather than days, thus enabling faster release, risk mitigation, and a faster response to contamination events.

Mango's core technology is Subpixel Perspective Sweeping Microscopy (SPSM) [0], which was originally developed by Prof. Changhuei Yang at Caltech. This technology enables micron-scale resolution across an entire filtration membrane. During incubation, the system captures an image every 20 minutes enabling the system to identify microscopic colonies long before they are visible to the human eye. Observations are purely optical, and no fluorescent markers, stains, PCR, or other reagents are employed. The test is non-destructive, allowing for downstream subculturing and microbial identification assays.



Left: capture taken using the Mango System. Right: CFUs identified by Mango ML.

Results
Mango System CFUs were comparable to compendial spread plate CFUs, with counts falling within the 50-200% acceptance range (Figure 1).

Detection times varied by organism (Figure 2). Yeast and bacterial colonies appeared as early as 1 hour, with 90% detected by 4.2 hours. *Aspergillus* mold spores showed initial detection at 5 hours, with 90% detected by 9.4 hours.

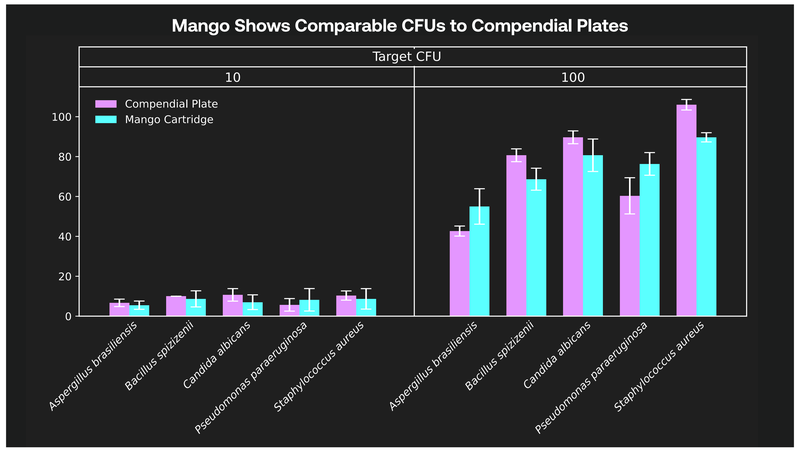


Figure 1. Mango CFUs compared to compendial CFUs for USP <61> microorganisms.

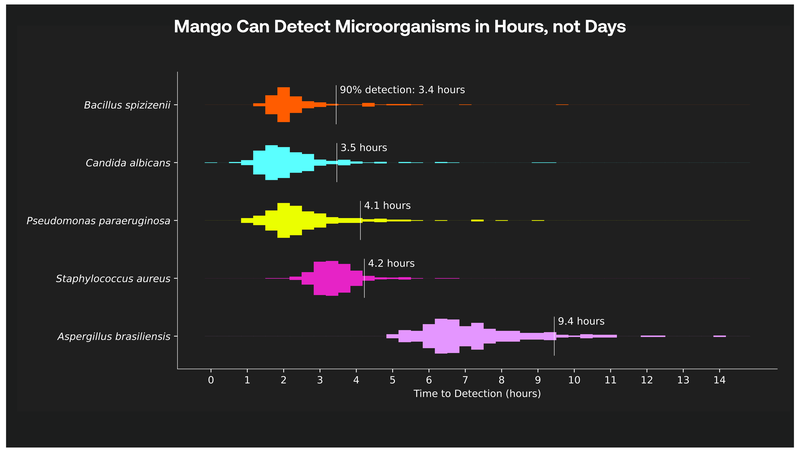
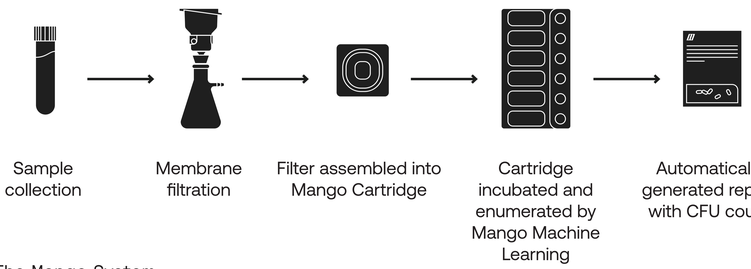


Figure 2. Mango Time-to-detection data for compendial USP <61> microorganisms.

Method
The test was a "spike and recover" of five microbial organisms: *Bacillus spizizenii* (ATCC 6633), *Candida albicans* (ATCC 10231), *Aspergillus brasiliensis* (ATCC 16404), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas paraeruginosa* (ATCC 9027). Organisms were prepared from bioMérieux BioBalls and are considered to be of healthy, non-stressed origin. Control TSA spread plates were spiked with 10 and 100 CFUs and incubated at 32.5°C ± 2.5°C for 5 days. Plates were inspected for growth every 24 hours.

For the Mango System comparison, 10 mL of PBS was spiked with 10 and 100 CFUs and vacuum-filtered through a Mango filtration funnel. The filter membrane was then assembled into a Mango cartridge and incubated within the Mango instrument at 32.5°C ± 2.5°C for no longer than 48 hours.



The Mango System

Conclusion
The Mango System's CFU enumeration is equivalent to USP <61> visual CFU counts, offering faster time-to-detection compared to the compendial method.

The top-3 biopharmaceutical company is validating the Mango System to expedite the release of in-process materials, respond more swiftly to contamination events, and prevent downstream material contamination.

Future studies are planned to characterize the accuracy, linearity, precision and limit of detection.

References
[0] Zheng G, Lee SA, Antebi YE, Elowitz MB, Yang C. The ePetri dish, an on-chip cell imaging platform based on subpixel perspective sweeping microscopy (SPSM). Proceedings of the National Academy of Sciences of the United States of America. 2011 Oct 3;108(41):16889–16894.