Poster #28

Evaluation of the Mango M6 System for Automated, Rapid Recovery of Microorganisms

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Abstract

The Mango system was evaluated by spiking Fluid A with seven different microorganisms: *Clostridium sporogenes* ATCC 11437, *Cutibacterium acnes* ATCC 6919, *Staphylococcus aureus* ATCC 6538, *Bacillus spizizenii* ATCC 6633, *Aspergillus brasiliensis* ATCC 16404, *Pseudomonas paraeruginosa* ATCC 9027, and *Candida albicans* ATCC 10231. For each microorganism, two replicates were prepared on spread plates, Oasis TSA cartridges, and Mango Cartridges. Each microorganism was tested three times by three different analysts. The inoculated control plates (Oasis and spread plates onto TSA medium) were incubated at 30-35°C for 24h to 7 days. The Mango test plates were incubated for 16 -160h. The study provides comparative data on the time to detection and recovery rates for the tested microorganisms. The results indicated that the Mango system consistently showed faster time to detection for aerobic organisms than traditional methods, however incubation time and conditions were microorganism-dependent, particularly for anaerobic bacteria such as *C. sporogenes* and *C. acnes*. In addition, this study showed that the rates of recovery between the Mango and traditional systems were equivalent and provides data on the filterability of different matrices. The findings indicate that the Mango system has potential to rapidly and consistently recover microorganisms from biopharmaceutical samples.

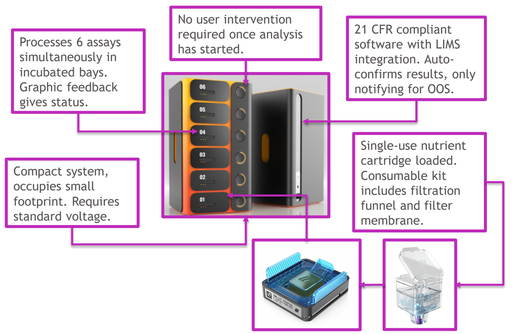
Background

Routine bioburden testing requires sample and method suitability testing. These assays require manual counting and reporting of colony forming units (CFU), which is labor intensive and typically takes 3-5 days to report results.

The M6 Mango system is an automated rapid method to detect and enumerate microorganisms. Assays can be completed in half the time using this system allowing for analysts to devote time to higher priority activities. In addition, the M6 Mango system removes the need for 2nd analyst verification through validation of the automated method.

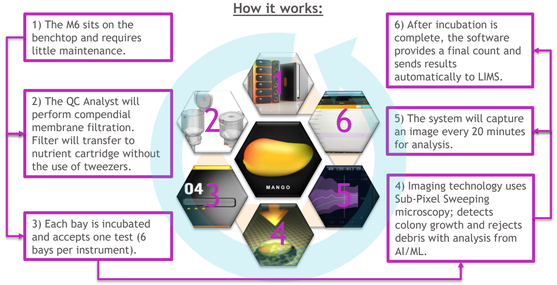
The purpose of this evaluation was to determine the feasibility of using the M6 Mango system as a solution for automated microbiology testing within the BMS network. Potential applications include automating bioburden method suitability enumeration, use with Car-T cell therapy for early microbial detection (business risk reduction), and use with biologics applications where rapid bioburden enumeration is required for business risk reduction.

The M6 Instrument & Features

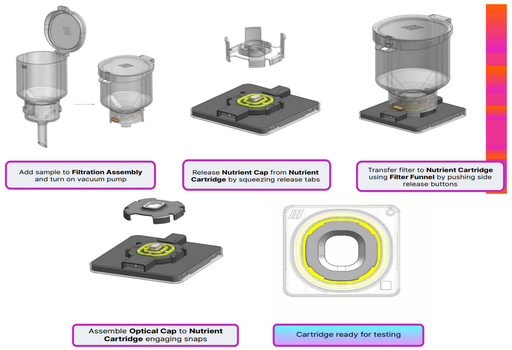


Mango System, Consumable, and Processing Images courtesy of Mango Inc.

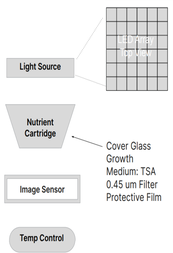
How it works:



Compendial Membrane Filtration Using Mango Consumables:



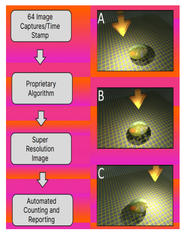
Automated Processing & Reporting of Results



Up to 64 images are captured in sequence at each timepoint (20min intervals).

A different illumination pattern is deployed with each image capture, and the resulting shifts are combined to create super resolution images with resolution down to ~560nm.

Collected high quality data lends itself well to AI/ML applications.



Experimental Design

A prototype Mango system was evaluated for accuracy, specificity, and robustness of colony counts as well as filterability of solutions. This was accomplished through three experiments as shown below. Challenge microorganisms and testing parameters are listed in Table 1.

Bioburden Test Method Assessment
- Comparison of Spread Plates, Oasis Filtration, and Mango Filtration challenged with <100 CFU microorganism spike
- Assessed accuracy of CFU counts, % recovery of microorganisms, time to detection, and time to results

Microorganism Recovery Assessment
- Comparison of Spread Plates, Oasis Filtration, Mango Filtration challenged with <100 CFU microorganism spike
- Assessed recovery of aerobes vs anaerobes and pure vs mixed cultures
- Assessed recovery of aerobes from ICR Swabs

Assay Variability Assessment
- Assessed results generated on different Mango readers by multiple analysts, with multiple microorganism preparations, and on multiple testing days

Table 1: Challenge Microorganisms and Testing Parameters

Experiment(s)	Microorganism[1]	Sample Matrix (Volume)	Rinse Fluid[2]	Incubation Temp / Time
Bioburden Test Method & Microorganism Recovery Assessment (aerobes)	*Bacillus spizizenii* ATCC 6633 (BS) *Staphylococcus aureus* ATCC 6538 (SA) *Pseudomonas paraeruginosa* ATCC 9027 (PA) *Candida albicans* ATCC 10231 (CA) *Aspergillus brasiliensis* ATCC 16404 (AB)	Fluid A (100mL)	Fluid A (5mL)	30-35°C Oasis/Spread: 24h: BS, PA, SA 48h:AB 72h: CA Mango: 16h all
Microorganism Recovery Assessment (anaerobes)	*Clostridium sporogenes* ATCC 11437 (CS) *Cutibacterium acnes* ATCC 6919 (CAC)	Fluid A (100mL)	Fluid A (5mL)	30-35°C Oasis/Spread: 7 days (aerobic & anaerobic) Mango: 48h: CS, 160h: CAC
Microorganism Recovery Assessment (mixed cultures)	*Pseudomonas paraeruginosa* ATCC 9027 (PA) *Staphylococcus aureus* ATCC 6538 (SA) *Bacillus spizizenii* ATCC 6633 (BS)	Fluid A (10mL)	Fluid A (5mL)	30-35°C Oasis/Spread: 24h: BS, PA, SA Mango: 16h all
ICR Swab Study (aerobes)	*Bacillus spizizenii* ATCC 6633 (BS) *Staphylococcus aureus* ATCC 6538 (SA) *Pseudomonas paraeruginosa* ATCC 9027 (PA) *Candida albicans* ATCC 10231 (CA) *Aspergillus brasiliensis* ATCC 16404 (AB)	TSB / Fluid A (10mL)	Fluid A (5mL)	30-35°C Oasis/Spread: 24h: BS, PA, SA 48h:AB 72h: CA Mango: 16h all

[1]All microorganisms were prepared from commercially available Bioball® Multishot (550CFU/mL) vials and rehydrated per manufacturer's instructions. 100uL of each preparation was used to inoculate each sample, resulting in a final concentration of <100 CFU per sample. For mixed culture assessments, 33uL of each preparation was used to inoculate the sample, resulting in a final concentration of <100 CFU per sample.
[2]Rinse was only performed for the Mango filtration units.

- To assess pure culture recovery, duplicate samples were inoculated, filtered, plated on Oasis TSA and Mango TSA cartridges, and incubated per Table 1.
- To assess mixed culture recovery, quadruplicate samples were inoculated, filtered, plated on Oasis TSA and Mango TSA cartridges and incubated per Table 1.
- To assess ICR swab recovery, the reservoir was snapped, and ~2mL TSB was vortexed vigorously with the swab for 10 seconds. The swab was removed from the tube, and the TSB was inoculated. 1mL aliquots of inoculated TSB was then transferred into 9mL of Fluid A in duplicate, processed using the Oasis and Mango filtration systems, and incubated per Table 1.
- For all experiments described above, spread plates were performed concurrently during testing as an inoculum verification control and incubated per Table 1. Anaerobic control plates were incubated both with and without oxygen.
- Assay variability was assessed by repeat runs with multiple instruments, analysts, and test days.

Results

Average results from the performed experiments were analyzed and compared across filtration types for CFU counts, percent recovery, time to detection, and time to results. Figure 1 and 2 illustrate examples of microcolony growth on the Mango cartridges and CFU growth on the Oasis cassettes and control spread plates.

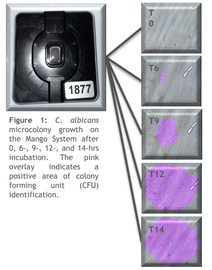


Figure 1: *C. albicans* microcolony growth on the Mango System after 0, 6-, 9-, 12-, and 14-hrs incubation. The pink overlay indicates a positive area of colony forming unit (CFU) identification.

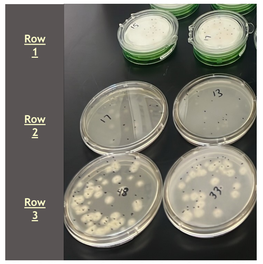


Figure 2: Microorganism Growth on Oasis System Cassettes and TSA Spread Plates. Row 1: Oasis membrane filtration cassettes. Row 2: *P. paraeruginosa* spread plates. Row 3: *A. brasiliensis* spread plates.

Figures 3,4, and 5 illustrate the CFU counts, recovery rates, time to detection, and time to results for pure, mixed, and ICR Swab microorganism cultures, respectively. Results indicate that acceptable recovery (75.1%-122.7%) was obtained for all tests using the Mango M6 system. The only exception was for *C. acnes* and *C. sporogenes* as these organisms were unable to grow under the aerobic Mango incubation conditions. In addition, time to results was reduced by up to 92.6% (65.8% - 92.6%) and the Mango system was able to detect microorganism growth within 4 hours (2.7 - 4.0hrs). This data was consistent across all experiments and was performed across multiple days using 6 instruments, 4 analysis, and multiple lots of Bioball® Multishot 550 preparations. This shows that the assay and automated reading using the Mango is robust and the data meets the criteria defined in USP <61>/EP 2.6.12JP 4.05 for method suitability.

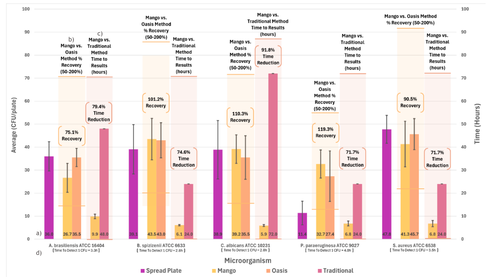


Figure 3: Pure Microorganism Culture Results a) Overall CFU Count Averages b) Percent Recovery of Mango Cartridges vs. Oasis Cassettes c) Mango Cartridge vs. Traditional method time to results d) Mango Cartridge time to detection. Similar counts and acceptable recovery of all tested microorganisms were observed when comparing the Oasis and Mango filtration systems. Inoculum control plates indicated that the samples were inoculated with < 100 CFUs.
NOTE: Results for anerobic microorganisms are not shown since there was no growth detected by the Mango system during incubation (30-35°C, aerobic).

Results (continued)

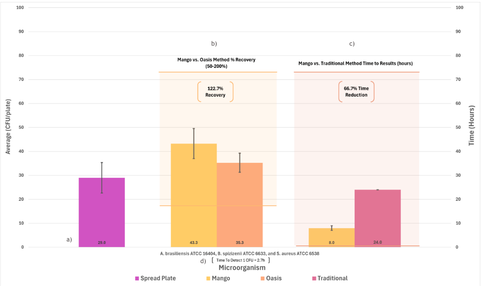


Figure 4: Mixed Microorganism Culture Results a) Overall CFU Count Averages b) Percent Recovery of Mango Cartridges vs. Oasis Cassettes c) Mango Cartridge vs. Traditional method time to results d) Mango Cartridge time to detection. Similar counts and acceptable recovery was observed when comparing the Oasis and Mango filtration systems. Inoculum control plates indicated that the samples were inoculated with a total of < 100 CFUs.

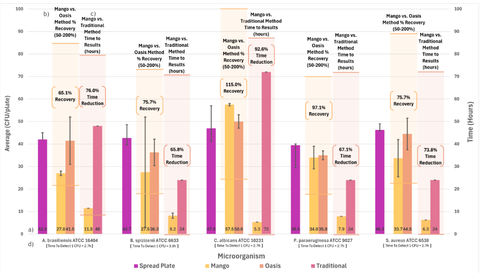


Figure 5: ICR Swab Microorganism Culture Results a) Overall CFU Count Averages b) Percent Recovery of Mango Cartridges vs. Oasis Cassettes c) Mango Cartridge vs. Traditional method time to results d) Mango Cartridge time to detection. Similar counts and acceptable recovery of all tested microorganisms were observed when comparing the Oasis and Mango filtration systems. Inoculum control plates indicated that the samples were inoculated with < 100 CFUs.

Conclusion

A summary of the evaluation data and pros/cons of using the system is presented below. Overall, the evaluation proved the system performed equivalently to the traditional method and was accurate, robust and specific in detecting aerobic microorganisms in at least 65% less time and can be used to process ICR swabs. However, the Mango system was unable to detect anaerobic or aerotolerant microorganisms. More testing will occur to determine how the system performs using buffer, raw material, cell culture, and DS/DP matrices in the future.

Pros
- Accurate enumeration
- At least 65% reduction in time to results
- Classified as an automated reading method
- Easy to use
- Ability to see growth over time and multiple cell morphologies
- Does not require additional reagents

Cons
- Requires consumables specific to Mango (single source)
- Data size of video capture for retention
- Currently limited to aerobic organisms only

Points to Consider
- Evaluation of product matrix filterability with final filter format not yet tested
- Integration with LIMS not yet evaluated
- Other media types/incubation conditions to be developed in the future

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