Application of a High Throughput Automated Colony Counting System Powered by AI to Environmental Monitoring

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Abstract

A system applied to clinical microbiology was adapted for the high throughput assessment of environmental monitoring plates collected from a sterile manufacturing site.

Proof of concept and industrialization of the instrument necessary to ensure a robust counting system where false negatives results could not be tolerated. Here we describe the side-by-side comparison of the system compared side by side to qualified microbiologists in routine use over multiple months

Introduction

Up to 30,000 Environmental Monitoring (EM) samples are analyzed each month at large AstraZeneca sites. Most of these samples are examined separately by two independent microbiologists to ensure data integrity of critical samples.

The majority (>98%) of these samples are negative for microbial growth (0 CFU), meaning that most of the microbiological work performed in our current environmental monitoring workflow is performed on samples without microbes.

Despite utilizing appropriately educated and trained microbiologists to examine samples, humans are not perfect, and many factors influence the quality of their work. Because of the lack of perfection, additional checks are employed to ensure the quality of the result. These checks add more work to samples which, as stated above, have very few microbes to begin.

It is desirable to replace the manual interpretation and counting of environmental monitoring samples with an automated solution to remove the variability of human performance. Additional benefits of automation would include reduced technician time that could be deployed elsewhere.

The challenge was finding an automation solution that would meet the needs of a diverse group of global microbiology labs.

Methods

The Automated Plate Assessment System (APAS) from Clever Culture Systems was selected for industrialization at AstraZeneca. The APAS is an automated agar plate reader that uses a camera system and algorithm trained by artificial intelligence to count and sort plates. A similar system from Clever Culture Systems is already approved for use in clinical microbiology in Australia, Europe and the USA. AstraZeneca partnered with Clever Culture Systems to develop the system for the pharmaceutical industry.

APAS processes approximately 200 plates per hour and sorts them into categories.

APAS Independence

Figure 1 Workflow with APAS

- Only samples with growth or processing errors are second checked. This greatly reduces the technician time.
- Data is automatically transferred to LIMS system (MODA). Elimination of the chance for transcription error.

Validation Approach

Primary Validation of the APAS was completed by Clever Culture Systems in March 2024.

Secondary Validation is currently in progress at AstraZeneca. A two-stage secondary validation approach was selected

1. Establish expected performance
   - Positive plates would be 'contrived' by exposing in general labs and interspersed with large enough number of negative plates to keep the humans 'reading' in representative manner. Target 710 positive plates (number of negatives not important) to give the desired sensitivity (non-inferiority to manual read (zero false negatives).
   - Present additional problem plates e.g., missing/cracked/dehydrated agar, cracked lids etc.
2. Establish real in-use performance
   - APAS instrument used as primary reader for real world EM plates.
   - All plates subsequently read and checked by humans as per normal process
   - APAS vs Human results compared for negatives and positives and count estimation

Results

Key Learnings from Industrialization

- Colony counting is impacted by areas of growth confluence. It is difficult to accurately count and can also be subjective between microbiologists
- For some species, larger and older colonies have significant morphological textural features that influence APAS counting algorithms.
- Our workflow doesn't rely on the APAS estimate of CFU for samples with growth and they are sorted as 'requiring human review'.

Figure 2 Example Image from APAS

Single colony not detected by human during industrialization

Figure 3 Example Image from APAS

Output of APAS shows visual of what instrument has counted

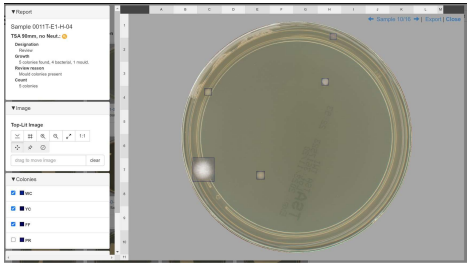

Table 1 Secondary Validation

Summary of results from the real-world use of APAS in environmental monitoring plates from sterile manufacturing site

Criteria	Number
Plates with growth recorded by human	51
Plates with growth recorded by APAS	51
Colony not counted by APAS on positive plate	7
Colony not counted by human and APAS	2
False Positive APAS	91
Total Plates Examined	969

Conclusions

- Accurate colony counting is impacted by areas of growth confluence. The "correct" colony count is also subjective between technicians
- APAS estimates CFU for plates with growth and sorts them as 'requiring human review'. Therefore, the false negative rate is more important
- APAS performance is showing as non-inferior to humans and is encouraging as a solution to colony counting for pharmaceutical QC laboratories

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