Environmental Monitoring: Developing tests to challenge artificial intelligence models for colony counting using the APAS® Independence

Steven Giglio Ph.D, Scientific Director, Clever Culture Systems

ABSTRACT

- Healthy skepticism surrounds the use of automated systems in microbiology to reliably detect and count colonies on an agar plate
- Compendial guidelines covering the assessment of alternative microbiology methods are well established (e.g. USP<1223>) which provide critical measurements of method suitability
- The appetite for technology interrogation, however, is high and driven by a desire to understand any limitations as part of risk profiling for an application
- The ability to detect colonies on the perimeter and affixed labels on the plate has been a concern for automated platforms, for which there are no established methods or acceptance criteria, or existing detection rates when compared to the manual process
- When performing primary validation for the APAS® Independence, this challenge test was developed. Bespoke AI application tools were used for microbiologists to annotate >14,000 colonies on the APAS® Independence images
- These images were then objectively and computationally compared to the APAS® Independence result, on a colony-by-colony basis and stratified by location (edge, bottom label, other)
- This is a unique approach to a unique challenge test developed for this type of colony counting technology, based on CCS experience (Figure 1) tests in a statistically and regulatory defensible manner

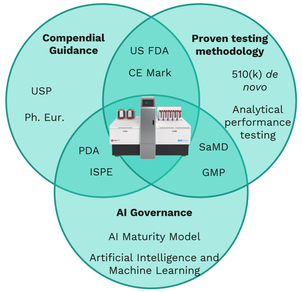

Figure 1. Scientific provenance of developing proven and robust AI tools for analysis

METHOD

- No compendial methods exist for this combination of test and technology
- CCS has leveraged 10+ years of regulatory experience with the US FDA for Artificial Intelligence (AI) and microbiology (Figure 1), resulting in the development of a bespoke AI tool (APAS Labeller) which is fit-for purpose for microbial challenge testing
- Microbiologists use this tool to digitally annotate colonies on raw images as either edge, bottom label, or other (Figure 2)
- From an APAS ® raw image capture (Figure 3A), the instrument will produce 2 results – the first being a boxed colony count (Figure 3B) and a digital result of all colonies identified is extracted into imaging software (Figure 3C, red boxes)
- The microbiologist–labelled colonies are digitally transformed using imaging software (Figure 3D, green boxes)
- A composite image is produced (Figure 3E), demonstrating overlap (yellow boxes) of the two digital images
- A successful result is when the APAS® label has >50% coverage of the microbiologist label
- Tests were performed on 3-day cultures of *S. epidermidis, S. aureus, M. osloensis,* and *M. luteus,* on both TSA and TSA + neutralizer

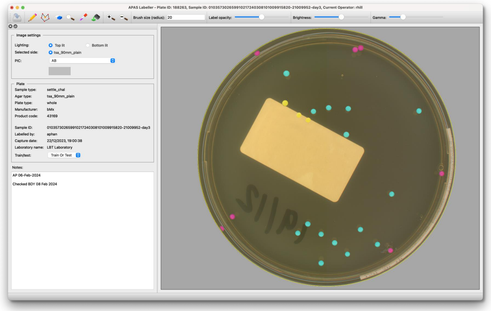

Figure 2. Microbiologist-driven annotation of colonies for challenge testing

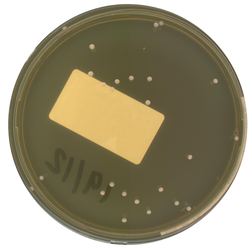

Figure 3A – APAS raw image (M. osloensis)

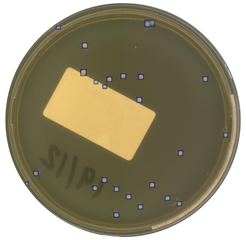

Figure 3B – APAS raw image with AI-bounding boxes identifying colonies

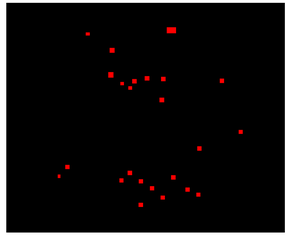

Figure 3C – APAS digital colony image

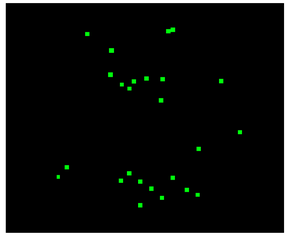

Figure 3D – Microbiologist-labelled digital colony image

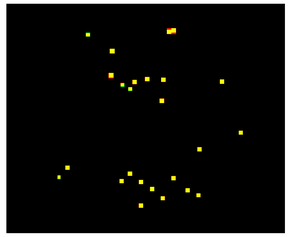

Figure 3E – Composite image of 3C and 3D

RESULTS

- A total of 14,102 unique colony events, across 3 major media manufacturers, were included in this performance testing set
- Colonies on agar were detected at almost 100%, with ≥99.86% detection rate on bottom labels

Percent of colonies detected			
	Media 1	**Media 2**	**Media 3**
Colony location - All	99.26	99.05	99.26
Colony location - Edge	96.11	96.95	96.98
Colony location - Bottom Label	99.90	99.86	100
Colony location - Other	99.97	100	100

- Detection on the edge of the plate, defined as where petri-dish molding is present and towards the edge (typically the outer 5 mm) was lower than other areas, with a range of 96.11-96.98%. A large proportion of these were likely due to experimental artefact, where semi-circular colonies growing on the meniscus presented challenges (Figure 4)

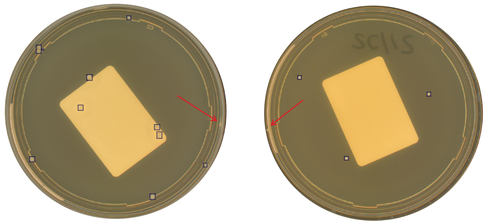

Figure 4 – M. luteus with semi-circular colonies on the meniscus

CONCLUSIONS

- A unique approach to challenge testing has been developed and APAS® Independence has a very high overall colony detection rate across all areas of the plate
- Testing is designed to challenge limitations of the technology which should be considered when developing a risk-based strategy for setting acceptance criteria for instrument performance qualification and evaluating equivalency with current methods

CLEVER CULTURE SYSTEMS

Contact information steven.giglio@cleverculturesystems.com