CLEVER CULTURE SYSTEMS

Data Integrity Challenges in the Pharmaceutical Microbiology Laboratory, Considerations for Current State and the Application of Technology to Mitigate Risk

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ABSTRACT

A key foundation to Good Manufacturing Practice, is the execution and routine review of a robust environmental monitoring program. This takes the form of collecting environmental samples from surfaces, personnel and the cleanroom air, whether that be active or passive sampling; these samples are collective on nutrient agar plates. The plates are incubated and then reviewed by microbiologists to assess for the presence/absence or quantity of growth. Data is trended in compliance with the regulatory expectations and assessment of the level of control of the environment is executed prior to batch review.

Regulators have become increasingly interested in Data Integrity across the whole pharmaceutical supply chain, and the microbiology laboratory is not without challenge. The raw data is reported from the agar plate, typically into computerised systems, second checking of results has become common place, however there are questions over the value of contemporaneous second checks – defining the time frame required to execute the second check is important, as additional proliferation can occur, but also the subjective nature of microbiology means that one technician's report of 2 cfu could be another technician's report of 3 cfu.

The availability of automated, computerised plate counting technology has increased in recent years, and there are benefits that can be realised, from enhancing process efficiency, reducing subjectivity, and providing an auditable data trail to the original reported result.

INTRODUCTION

The production of products for parenteral administration is a highly regulated activity, supported by following the Good Manufacturing Practices outlined in many regulations and guidance's, including Annex 1 (Manufacture of Sterile Medicinal Products). The level of environmental monitoring performed is dependent on the criticality of the manufacturing that is being supported, for example in high grade clean areas, supporting the manufacture of parenterally administered products many more samples will be collected when compared to a secondary packaging location for an oral solid dosage form. In the sterile manufacturing area, good levels of control largely lead to negative agar plates (i.e., plates with no growth) collected from these locations. Across a single site with three sterile production facilities, this can amount to 30,000 plates per month, each of which will be reviewed by two microbiology technicians. This manual handling takes time and there are also variations in the way individuals count colonies suggesting a more standardized and automated way of measuring is required. In circumstances of growth above alert and action areas, these plates can then undergo further review and characterisation by microbial identification methods.

Microbiology is a science steeped in tradition, much like today, our microbiologist predecessors were visually inspecting agar plates to confirm the presence or absence of microbial growth. Samples are taken from the manufacturing environments to provide assurance that validated conditions are maintained and under control. This leads

to thousands of samples being generated across the global network each month. The plates are incubated, then visually inspected for counting or a check for absence of growth. This is open to personal interpretation and bias and there is limited data integrity associated with the sample that is being reviewed. For example, a plate can be inspected, result reported, discarded and hours later, should an FDA inspector review the original plate, additional proliferation may have occurred since the plate left a controlled environment, or it could be the case that the initial results were simply incorrect because of misinterpretation or transcription errors. In these circumstances, there is no way of demonstrating that the reported result accurately represented what was on the plate at the time of inspection, or that the original reported result was in fact correct. To mitigate this risk, currently good practice guidance's may be created for second checking of plates based on risk to patient safety, in those areas where reporting of an inaccurate result could potentially cause patient harm.

Advances in microbiology automation are now offering sophisticated instruments to assist laboratorians with plate reading. The introduction of image analysis and interpretation using artificial intelligence is promising as it offers a permanent visible record of the agar plates at the time of reading (via the image), and it provides an objective and reproducible assessment for the growth on a given plate (via the instrument report), all while automating the process and decreasing repetitive strain injury risk. Digitising this process also has the advantage of ensuring the data integrity of a given sample is strengthened through compliance with CFR 21 Part 11, which is a large focus for regulators. In this extended proof-of-concept study, we present initial findings into a technology that uses proprietary artificial intelligence algorithms for the detection and counting of organisms commonly encountered in environmental monitoring.

METHODOLOGY

A study to investigate the use of image analysis with artificial intelligence to process, read, and interpret settle plates was conducted using Tryptic Soy Agar plates and the APAS Independence where a novel algorithm for growth detection and counting was developed using images and data from settle plates from non-sterile environments (PC2 laboratory and office spaces). Using these sampling locations was likely to provide a broad range of organisms and fungi to provide better coverage for the initial algorithm classification system. The creation of the algorithm required microbiologist-led annotation (labelling) of images as input data, and then making a series of adjustments to the configuration culminating in a machine learning model which is able to segment images of settle plates into categories that represent organism morphology. This algorithm was then used in traditional linearity studies using *Staphylococcus aureus* (NCTC 10788), *Pseudomonas aeruginosa* (NCTC 12924), and *Aspergillus brasiliensis* (NCPF 2275) to investigate the veracity of the artificial intelligence model when compared to a human-read result. A number of algorithm iterations were tested. A range of 0 – 250 cfu per plate was prepared for each organism and counts from a panel of microbiologists (reference) obtained and compared to the APAS results (test). Correlation co-efficient (r^2) was calculated for each organism. A subjective assessment of settle plate images was also performed where microbiologists inspected plates and APAS assessment to qualitatively examine if the algorithm was successful in detecting growth.

RESULTS

The data in Table 1 detail the correlation coefficients of the APAS result verses the microbiologist over 4 iterations of APAS PharmaQC Analysis Module. In the first three iterations, the linearity of *Aspergillus brasiliensis* is not as high as the *Pseudomonas aeruginosa* and *Staphylococcus aureus* due to variable size and morphologies exhibited, which

Table 1. Correlation co-efficient for linearity studies over four APAS PharmaQC software iterations

Organism	APAS PharmaQC v1	APAS PharmaQC v2	APAS PharmaQC v3	APAS PharmaQC v4
***Aspergillus brasiliensis* (NCPF 2275)**	0.71	0.67	0.57	0.95
***Pseudomonas aeruginosa* (NCTC 12924)**	0.90	0.93	0.95	0.98
***Staphylococcus aureus* (NCTC 10788)**	0.96	0.98	0.98	1.0

presented challenges with accurate counting using the current counting method of the algorithm. The performance of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, however, demonstrates a very high performance with improved r^2 values over iterations of the algorithm. Notably, the v4 result has superior performance, improving r^2 across all test organisms via the introduction of a novel counting method developed specifically for this application (Figure 1), which also significantly improved *Aspergillus brasiliensis* performance. Subjective assessment of settle plate images used in the development of this algorithm yielded the observation that

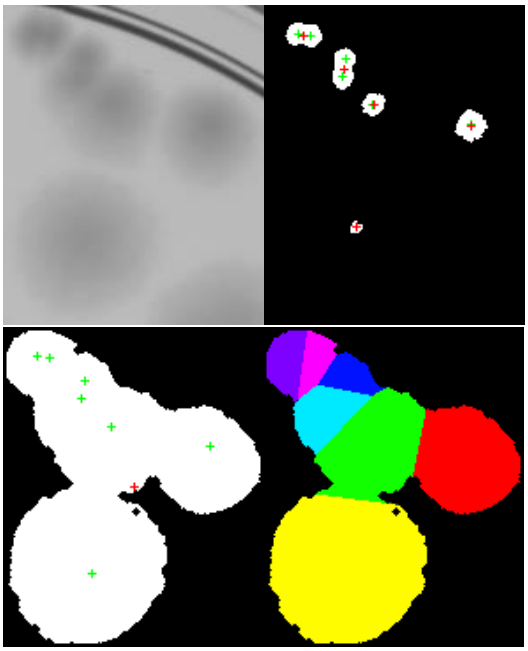

Figure 1. Insight into new counting mechanism on APAS PharmaQC. Features of the original backlit image (top left) are used to drive the determination of colony boundaries, which is ultimately depicted in the top right and bottom images where colony segmentation is evident.

the algorithm was successful in detecting growth 100% of the time when growth was present on the plate. Detailed assessment of this observation is currently planned to be tested more thoroughly in verification and validation activities.

Figure 2 is an example of the TSA plate with *Staphylococcus aureus* and AI-generated classification of growth using the algorithm. These images show that every colony on the plate has been detected using the algorithm and that some areas are also presenting as a false positive detection, which may affect counting accuracy. Future iterations of algorithm development will address this which will improve specificity.

DISCUSSION

Within the QC laboratory environment, such advances can enhance process robustness, providing additional assurance of patient safety. The environmental monitoring program for any pharmaceutical production facility is a key component of the Contamination Control Strategy. Understanding drifts in cleanroom control is based on review and trending of accurate environmental monitoring data. Whilst trained microbiologists can vary in the interpretation of results (1) the adoption of such technologies is a key enabler to standardisation. By automating the simpler processes, the pharmaceutical microbiologist can focus their attention on interpreting and scrutinising results.

With increased regulatory scrutiny on data integrity, technology in this space can provide an auditable data trail,

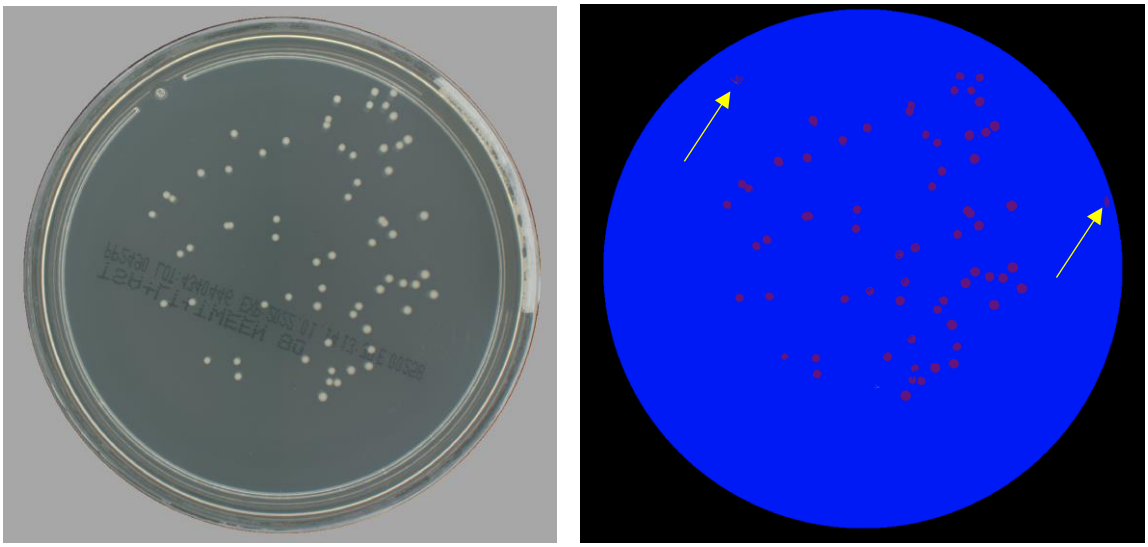

Figure 2. Left – TSA plate with S. aureus as imaged by APAS. Right – Computer generated image with colonies identified in red, agar in blue. Yellow arrows are areas of false colony detection on the injection mould (ten o'clock) and label interference (three o'clock).

providing clear evidence of the time the sample was reviewed, who was involved in the handling of the sample, and directly capturing data in an electronic reporting system. Certainly, most automated systems, including APAS PharmaQC, can provide these audit trails. Where deviations occur, it is possible to review images of the plates remotely, to further enable an understanding of the root cause – for example if samples collected on the previous environmental monitoring run had low levels of bioburden detected in a Grade C area, the identification may not have been obtained, however if this drift continued upwards it would be possible to review those images preceding an alert or action level failure. Whilst it would not currently be possible to identify to a species level using the image, similarities in colony morphology could support root cause analysis and batch disposition assessments.

The standardisation, process robustness and reduction of data integrity risks are clear, but in addition to this, the QC laboratory can become a more rewarding place for the pharmaceutical microbiologist to work. By using technology to automate the routine activities, there are productivity benefits, where the microbiologist can find themselves involved in more interesting activities, such as trending and reviewing broader data sets, to provide a more comprehensive understanding of the microbial quality assurance.

The major pharmacopeia's provide outlines of how to validate an alternative microbiology method (USP< 1223>, Ph Eur. 5.1.4 and PDA Tr. 33) it is not clear if the use of an automated system to read

Figure 1. High level workflow of the APAS in a QC laboratory

plates would be considered an alternative microbiology method or simply automation of an existing method. One benefit of the validation framework outlined, is the ability to obtain a data set that provides unequivocal data to support non-inferiority of the automated method when compared to the traditional ways of working. Furthermore, the data set will facilitate response to questions doing routine regulatory audits within the GMP setting.

CONCLUSIONS

The APAS and algorithm detailed here show great promise to address key challenges in the QC laboratory. Further work is planned to refine the algorithm and continue to challenge the APAS system through verification and validation procedures where significant workflow benefits can be availed by the automated triaging and reporting of those plates with no growth (Figure 3).

REFERENCES

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DISCLOSURES

Author has left AstraZeneca.

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