Evaluation of the ScanStation 100 system for the automated incubation and analysis of pharmaceutical environmental monitoring samples using standard Petri plates

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Introduction

The technical performance of the ScanStation 100 instrument (ScanStation), manufactured by Interscience and provided to us by bioMérieux (figure 1) using standard Petri plates was evaluated in this study. The system we tested is a prelaunch version of the instrument that was operating with algorithm version 1.17; the evaluation was designed to challenge the existing algorithm and to identify any improvements that are needed prior to the commercial launch of the ScanStation 300 system for pharmaceutical use by bioMerieux – expected in 2020.

The ScanStation 300 instrument could form a key part of a fully integrated automated environmental monitoring system that will be able to track plates from their initial use in a pharmaceutical production environment through to the final reporting of results in the Laboratory Information Management System (LIMS).

The ScanStation incubates and reads plates automatically and can change the temperature of incubation during the cycle. Plates are read every hour which enables colonies that grow on the media to be detected as they appear. This is particularly valuable where an incubated plate has confluent growth or is 'too numerous to count' at the end of the incubation period as the true count of isolated colonies prior to overgrowth occurring is reported and the growth development can be seen on the incubation video of each plate.

Figure 1:
ScanStation 100 Instrument

As the system automates the compendial method, the level of qualification required to implement the system is less than with an alternative method. Using the 'reference count' approach described below, we were able to determine the true count of each plate and ensure an accurate comparison between the ScanStation and manual readings that were recorded.

Overall the ScanStation performed well and gave results that were as good as, or better than, the operator readings.

Material and Method

A total of 400 samples were processed through the ScanStation system using algorithm V1.17.

In the first phase of the work, plates were either artificially inoculated with one of five standard pharmacopeia species or three site specific strains onto both 90mm TSA 3P irradiated media and 55mm CountTact 3P irradiated contact plates from bioMerieux (43819 and 43699 respectively). The inoculum levels used were at 5, 25 and 50 CFU, with 5 replicates used per organism / spike level / plate type and plates were incubated in the system at 30-35 ℃ for 24h to 5 days (depending on the strain). Bioball Multishot 550 were used for the pharmacopeial strains (bioMerieux – 56001 - *Aspergillus niger*; 56002 - *Bacillus subtilis subsp. spizizenii*; 56003 - *Candida albicans*; 56007 - *Pseudomonas aeruginosa*; and 56009 - *Staphylococcus aureus*.)

In the second phase of the work, 100 plates were used in the laboratory for environmental monitoring. These plates were incubated at 30-35℃ for 3 days followe d by 4 days at 20-25℃ on the instrument.

The system is able to read the plates through the lids of the plates, there is therefore no requirement to open the plates during the incubation cycle which significantly reduces the risk of cross-contamination.

Determining the 'Reference Count'

The reference count is defined as the most precise numeration one can obtain between 3 different operators and the ScanStation 100. It is obtained as a consensus numeration. At the end of the incubation period, plates were independently read by three operators. The operators then compared their individual counts with the count obtained from the ScanStation and also used the video recording and zoom feature of each plate's growth on the ScanStation system to agree on a true "reference count" for each plate. This enabled the determination of a false positive and false negative rate for individual operators as well as the instrument at the plate result level and at the colony level for the ScanStation.

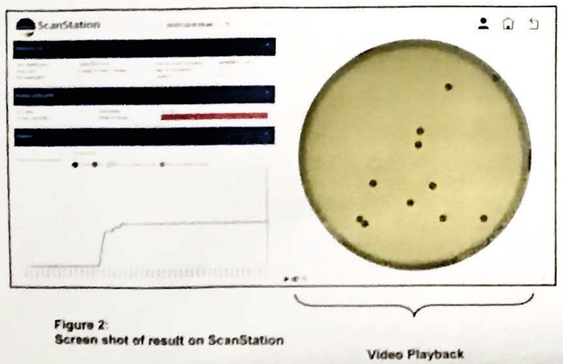


Figure 2:
Screen shot of result on ScanStation

Figure 2 shows a screenshot of the ScanStation's view of the result from a single plate. If used in conjunction with a LIMS system, the instrument will report results that reach an action or alert level as soon as that level of growth has been detected. The ability to 'go back in time' is accessed through the highlighted area ("Video Playback"); this feature allows the inspection of any plate where there is confluent or overgrowth to determine the true number of originating colonies on the plate.

Results

The final reference count result obtained for each plate was plotted against each of the operator and ScanStation results for the pharmacopeial strains, the site specific strains and the environmental monitoring samples (Figure 3). The performance of the operators was comparable to the performance of the ScanStation with all sample types. Of the 400 plates incubated, growth was observed on 278 plates (results shown below); 112 plates were not inoculated and so had no growth, whilst results of 10 plates were excluded from the analysis due to the presence of artefacts on the plate.

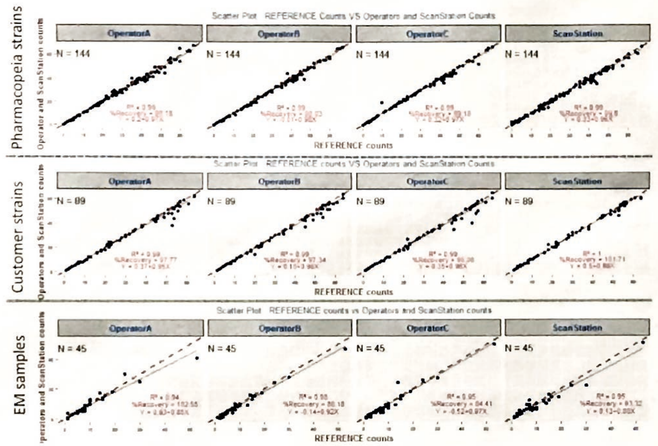


Figure 3:
ScanStation and operator counts plotted against the reference count for each plate

A summary of the results obtained for all samples is shown in Table 1.

	R^2	Slope [95% Confidence Interval]	Bias [95% Confidence Interval]
ScanStation	0.99	0.97 [0.95;0.98]	0.23 [0.05;0.44]
Operators	[0.99;1]	0.96;0.97	-0.03;0.15

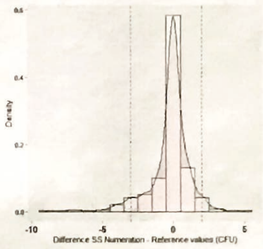
Table 1:
Performance summary at plate level for ScanStation and operators compared to reference count

Further analysis of the results was performed at the colony level to determine which colonies were not detected by the ScanStation (false negative) and events that were reported by the system as colony growth, but were false positive results. This approach of identifying all false negative and false positive events at the colony level was not possible on the operator results as a final count per plate was recorded as the result; however a false negative and false positive rate for an operator could be reported where the final reference count was either respectively less or more than the reference count. In total, by using the reference count approach, the total number of confirmed colonies that were isolated during the study was 6,182.

In analysing the false events on ScanStation, we were being stricter in our assessment of the performance of the ScanStation compared to operator counts. Table 2 shows the False Negative rate for operators and the ScanStation at the colony level from V1.17 of the algorithm (the operator false negative rate was derived from those plates where the operator recorded a lower number of positive events compared to the reference count). Operator false negative readings were often due to the centre of colonies being close together and being read as 1 cfu, whereas the ScanStation correctly identified the origin of both colonies. The false negative rate demonstrated by the ScanStation was not significantly different to the results obtained from operators A and B (Chi-squared test). Overall, the false negative rate for all operators and the ScanStation using the reference count approach was between 3% and 4% of the total number of true colonies that grew on the plates. When the results of plates with counts of <=10cfu were analysed, there is no significant difference between the results obtained from any of the operators or the ScanStation (Chi-squared test) – data not shown.

Operator	Operator Count	True Colonies	False Negative # (FN)	FN %	FN 95% CI
A	6006	6182	229	3.70	[3.26; 4.20]
B	5986		218	3.53	[3.09; 4.02]
C	6028		188	3.04	[2.64; 3.50]
ScanStation	6077		244	3.95	[3.49; 4.46]

Table 2:
False negative results at colony level for ScanStation and operators compared to reference count



The differences in the count of plates with growth between the ScanStation and the reference count were plotted as shown in the histogram in Figure 5 – note that plates with no growth are not included in this analysis.. Using the 5 and 95 percentiles to determine the values at which 95% of the observation may be found, it can be seen that the agreement between the ScanStation and the reference method following this method is strong with 95% of the results included in the interval -3 to +2 cfu per plate. Notably on 58.5% of the plates, the difference in count is zero (i.e. the counts were the same by both reference method and the ScanStation

Figure 4:
Distribution of differences between ScanStation and Reference Count

The differences in count on plates with growth between the ScanStation and reference count are plotted in Figure 5 for plates with less than 10cfu growth. The 5th and 95th percentiles show that ca. 90% of the counts are within 1 cfu of each other with 70% of plates having the same result from both the reference and ScanStation.

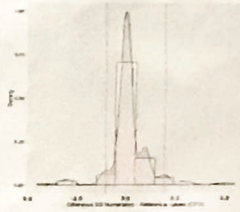


Figure 5:
Distribution of differences between ScanStation and Reference County for <10cfu Samples

One outcome of the work we performed was to improve the performance of the detection algorithm on the Environmental Monitoring samples. An example of the improvement is shown in Figure 6 where the circled colony close to the edge of the plate was not detected by Algorithm 1.17 but is now detected using Algorithm 1.27. The effect of this development will be to improve further the agreement between ScanStation and the reference method as shown in Figure 4.

Figure 6:
Algorithm improvement – colony at edge of plate now detected using Algorithm 1.27

Conclusions

The results obtained in this study using the pre-launch algorithm version 1.17 demonstrated that the instrument gives results that are comparable with standard manual reading of plates and so it would be a suitable candidate for automating the incubation and reading of standard Petri plates. The revised algorithm version 1.27 further improves the detection of growth at the colony level.

With the connectivity features of the system, it lends itself to be used either in a central laboratory or in a decentralised mode with instruments located at various sites through a facility, for example, close to the production area

The kinetic reading of plates and the availability of images from each plate at each hour of incubation allows the viewing of plates with overgrowth to see the origin(s) of growth and the ability to export pictures at various stages of the incubation cycle.

During the course of the evaluation, no difficulties were encountered in using the system, it was able to detect colonies growing on the edge of the plate and managed mixed populations of organism growth. All plates with growth were detected by the system.

Disclaimer: *Pfizer, in describing experiments and studies conducted at its Grange Castle site using the ScanStation 100 ('the equipment'), along with findings / outcomes from such studies, is not in any way endorsing the equipment, nor is it speculating as to what benefits, if any, may accrue from use of the equipment.*