Handy Helper

Application Note #08

How to validate an åCOLyte automated colony counter to ensure accuracy in a quality control environment

Introduction

Colony counts provide the data on which the effectiveness of essential products such as anti-microbial drug treatments or bactericidal disinfectants is based. Therefore, when quality controlling these products, it is vital to accurately enumerate colonies.

Traditionally, a light box and pen is the method used for colony counting. As the results are manually generated and transferred into a computer, this is a time consuming task requiring constant staff input, which can result in both plate reading and keying in errors occurring. Additionally, manual counts do not produce a digital image of the plate at the same time as the colony count, so there is no method of obtaining documentary evidence to independently verify and audit the results of each count.

To overcome these technical difficulties associated with manual enumeration, Synbiosis offer a range of automated colony counters including the åCOLyte SuperCount, an affordable system that can automatically and precisely count more than 500 colonies in one second. The system features LED lighting and a camera integrated to software hosted on a PC (Figure 1) and provides GLP compliant full colour image display and storage, as well as instant reporting of results.

Before åCOLyte or any other automated colony counter can be used in a quality control environment, the system has to prove it will provide results that are comparable to the current method being used as part of the standard operating procedure (SOP). This application note presents a method of evaluating the performance of åCOLyte compared to manual counting and a protocol for validating åCOLyte on a day to day basis to ensure consistency of results.

Figure 1: An åCOLyte SuperCount colony counting system

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Methods

1. Performance Qualification

To compare the åCOLyte with a manual counting method, a set of 50-100 test plates should be counted over a period of one-two months. This number of plates is required for comparison so the results obtained are statistically significant. Plate out the bacteria commonly counted in the laboratory onto the recommended media by the method stated in the laboratory's SOP. The åCOLyte can read plates prepared by spiral, pour or spread plating methods and so can accommodate most SOPs.

Using the laboratory's standard manual counting method, enumerate and record the colony counts for these plates. Then count the same plates with the åCOLyte system by placing each plate in the illuminated plate holder to create an image on screen. Click the SuperCount icon to automatically count and record colony numbers.

Express the mean counts from both the manual and åCOLyte counts as log mean values. To facilitate accurate analysis, all results with a count of '0' should be excluded, as log 0 cannot be calculated. Statistically calculate the agreement between log manual and log åCOLyte counts for each plate type using Microsoft Excel Data Analysis (or similar software) and the paired T-test. Using the Two-Tailed T-test, compare the 'P' value obtained with the T critical Two-Tailed value at 5 %. If the 'P' value is less than or equal to the T critical Two-Tailed value at 5 % then there is no significant difference between manual and automated colony counting at the 95% confidence level.

Case Studies

Using this performance qualification method, two independent validation studies of åCOLyte SuperCount have been carried out by Don Whitley Scientific Limited (Shipley, UK), a major supplier of equipment for microbial applications and Evans Vanodine International (Lancashire, UK), a leading hygiene chemicals manufacturer.

Case Study 1

In the Don Whitley Scientific Limited validation study, spiral plates were prepared using a Don Whitley Scientific automatic spiral plater of the following bacteria:

1. *Escherichia coli* and *Staphylococcus aureus* on Plate Count Agar, Columbia Blood Agar or Nutrient Agar
2. *Enterococcus faecalis* on Slantez and Bartley Agar
3. A mixed population of unidentified organisms on Plate Count Agar.

All plates (79) were counted both manually with a light box and pen and automatically with the åCOLyte SuperCount. The results are listed in Table 1.

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Table 1: Comparison of bacteria on spiral plates counted manually and with the åCOLyte SuperCount.

Description	Mean cfu/ml manual count	Mean cfu/ml åCOLyte count	Log mean cfu/ml manual count	Log mean cfu/ml åCOLyte count
E. coli on Columbia Blood Agar (13 plates counted)	1.7×10^{0}-1.3×10^{5}	1.7×10^{0}-$1.2\ 10^{5}$	0.2- 5.1	0.2-5.1
E. coli on Nutrient Agar (7 plates counted)	5.0×10^{0}-3.9×10^{3}	5.0×10^{0}-3.1×10^{3}	0.7-3.6	0.7- 3.5
E. coli on Plate Count Agar (8 plates counted)	4.0×10^{1}-1.2×10^{5}	4.7×10^{1}-9.8×10^{4}	1.6-5.1	1.7-5.0
E. faecalis on Slantez & Bartley Agar (10 plates counted)	3.3×10^{0}-9.8×10^{4}	3.3×10^{0}-6.0×10^{4}	0.5- 5.0	0.5-4.9
S. aureus on Columbia Blood Agar (13 plates counted)	0 - 7.9×10^{4}	1.3×10^{1}-7.2×10^{4}	0.2- 3.1	0.2- 3.3
S. aureus on Plate Count Agar (8 plates counted)	2.0×0^{1}-9.4×10^{4}	2.0×10^{1}-4.5×10^{4}	1.3-5.0	1.3-4.7
S. aureus on Nutrient Agar (8 counted)	2.5×0^{0}-9.6×10^{2}	2.5×10^{0}-8.4×10^{2}	0.4-3.0	0.4-2.9
Bacteria isolated from raw minced beef on Plate Count Agar (12 plates counted)	1.6×10^{6}-8.8×10^{6}	5.5×10^{5}-3.0×10^{8}	6.1-8.5	5.7-8.5

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Case Study 2

In the Evans Vanodine International study, pour plates were prepared of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae* and *E.coli* on Tryptone Soya Agar. The plates (75), were counted both manually with an IUL light box and pen system (Barcelona, Spain) and automatically with the åCOLyte SuperCount. The results of the comparison are shown below in Table 2.

Table 2: Comparison of bacteria on pour plates counted manually and with the åCOLyte SuperCount.

Description	Mean cfu/ml manual count	Mean cfu/ml åCOLyte count	Log mean cfu/ml manual count	Log mean cfu/ml åCOLyte count
Pseudomonas aeruginosa (40 plates counted)	9.9×10^{1}-3.9×10^{10}	1.1×10^{2}-3.3×10^{10}	2.0-10.6	2.0-10.5
S. aureus (7 plates counted)	5.3×10^{1}-3.8×10^{8}	5.5×10^{1}- 4.4×10^{8}	1.7-8.6	1.7-8.6
Enterococcus hirae (14 plates counted)	3.6×10^{1}-3.7×10^{8}	3.8×10^{1}-3.8×10^{8}	1.6-8.6	1.6-8.6
E. coli (14 plates counted)	9.6×10^{1}-5.5×10^{8}	9.8×10^{1}-5.6×10^{8}	2.0-8.7	2.0-8.7

In both the Don Whitley Scientific and Evans Vanodine International studies, the 'P' value obtained in the Two-Tailed T-Test is less than the T critical Two-Tailed value at 5 %, showing that there is no significant difference between manual and automated colony counting in either study.

2. Operational Qualification

To check the hardware and software of åCOLyte are working precisely on a day to day basis, a validation plate should be counted at regular intervals. A validation plate can be created using either Petri dishes or paper plates marked with a known 'colony' number (between 30-300). For validating the operational performance of åCOLyte, Synbiosis recommends the following steps:

1. Remove all dust from the validation plate.
2. Place the validation plate under the camera of the åCOLyte to display the image on the åCOLyte's computer screen.
3. Ensure all 'colonies' are within the frame boundary.
4. Click on the SuperCount icon.
5. Automatically count each plate three times to ensure results are consistent from count to count.

Synbiosis recommends that such validation plates be adopted as a daily check which will give confidence in the åCOLyte's reliability as the same results should be achieved from day to day.

Conclusion

Validating the åCOLyte automated colony counter is a straightforward process as the performance qualification studies carried out by Evans Vanodine International and Don Whitley Scientific show. The results of their studies demonstrate that the system can count a range of bacteria prepared by spiral or pour plating on clear and opaque media just as accurately as manual counting methods but in a fraction of the time required using these manual methods. Additionally, checking the daily performance of the åCOLyte system is simple using the Synbiosis five-step operational qualification method.

When åCOLyte has been validated, it is perfect for use in a quality-testing environment. The system generates results more than 200 times faster than a manual count yet is comparable in accuracy. Further advantages of using åCOLyte include the elimination of errors caused by manual subjectivity and manual data transcription, as well as improved GLP compliance provided by time/date recording and image archiving. This means quality control laboratories can reduce the time spent on colony counting by up to 90 percent, while still having confidence in their data thus ensuring that products being tested for bactericidal activity reach the market more rapidly.