Automated Colony Counting Proves Accurate

by Helen Jolliffe

Introduction

Producing new anti-microbial therapies and vaccines to treat biological terrorism threats such as anthrax and smallpox has become a priority. Since colony counts provide the data on which the efficacy of this type of treatment is based, it is essential to obtain accurate counts in the shortest possible time.

A light box and pen is the method commonly used for colony counting, with the results being manually transferred into a computer. This is both time consuming and labor intensive. It has the disadvantage of allowing plate reading and keying errors to occur. Since this technique does not generate a digital image of the plate alongside its associated colony count there is no procedure for independently auditing the results.

79 plates were counted manually and with the automated system.

Automated colony counters such as the åCOLyte SuperCount (Synbiosis, Frederick, MD) address the difficulties associated with manual enumeration. This product is comprised of a lighting unit with camera and software hosted on a PC running a Microsoft Windows operating system (Figure 1). To overcome the perception that automated systems may produce results that are not as precise as a manual count, the system was extensively evaluated for accuracy and reliability alongside manual counting at Don Whitley Scientific's GLP compliant laboratories.

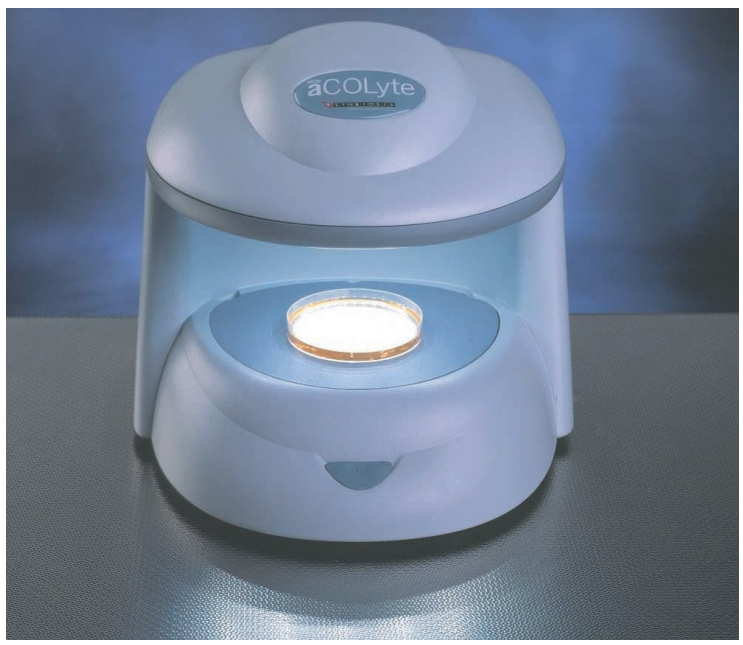

Figure 1. An åCOLyte SuperCount colony counting system.

Method

Operational qualification

The åCOLyte was performance tested to verify the hardware and software. For this purpose a validation kit was created consisting of two paper plates, each with a known number of 'colonies', one representing two sectors of a spiral plate, and the other representing a whole plate.

For each validation plate the following steps were used:

- All dust was removed from the validation plate.
- The validation plate was placed under the camera of the åCOLyte and the image displayed on a computer screen.
- All 'colonies' were placed within the frame boundary.

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)	2.0×10^1-9.4×10^4	2.0×10^1-4.5×10^4	1.3-5.0	1.3-4.7
S. aureus on Nutrient Agar (8 plates counted)	2.5×10^0-9.6×10^2	2.5×10^0-8.4×10^2	0.4-3.0	0.4-2.9
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

Table 1. Comparison of manual and åCOLyte count methods.

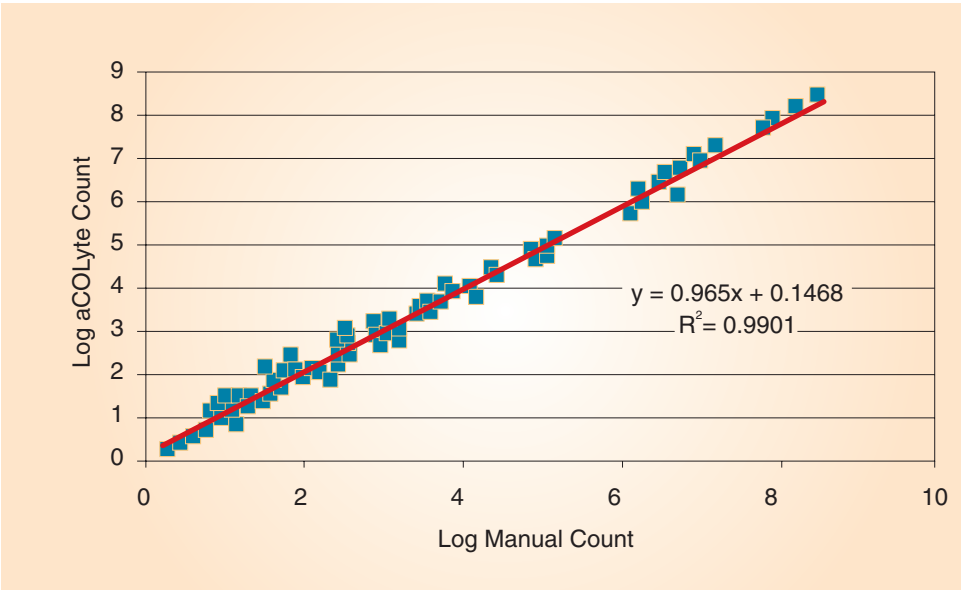

Figure 2. Statistical analysis of åCOLyte and manual count data using a T-Test.

• The colonies on the validation plate were counted by clicking the SuperCount icon.
• Each plate was counted three times by the åCOLyte.

Performance qualification
The åCOLyte was 'suitability' tested over a period of approximately one month. During this period the system was compared with manual counting to enumerate a range of bacterial colonies on opaque and clear agar plates. All bacteria were serially diluted and spiral plated using a Whitley Automatic Spiral Plater (WASP) (Don Whitley Scientific, Shipley, UK).

The following plate types were counted:
• Pure cultures of Escherichia coli, serially diluted and plated onto Plate Count Agar, Columbia Blood Agar or Nutrient Agar.
• Pure cultures of Staphylococcus aureus, serially diluted and plated onto Plate Count Agar, Columbia Blood Agar or Nutrient Agar.
• Pure cultures of Enterococcus faecalis serially diluted and plated onto Slanetz and Bartley Agar.
• A mixed population of unidentified organisms from raw minced beef serially diluted and plated onto Plate Count Agar.

Results

Operational qualification
To prove that the åCOLyte is working correctly the number of colonies counted should be 48 in sector 3a of the two-sector spiral plate and 40 in sector 4b for the whole frame spiral plate. The results of the Operational Qualification (results not shown) demonstrated that the system consistently worked correctly, thus allowing routine performance checks to be specified for the instrument.

Performance qualification
Over the month, 79 plates were counted manually and with the automated system. The mean cfu/ml for all the different bacteria and plates types counted are listed in Table 1.

The agreement between manual and automated colony counts for each plate type shown in Table 1 was examined statistically using Microsoft Excel Data Analysis. To facilitate analysis, all results giving a count of '0' were excluded, as log 0 cannot be calculated. The remaining data were analyzed using the paired T-test (T-test: Paired Two samples for Means). The analysis was performed using a two-tailed test, with a test value of 0 for the mean difference in log cfu between the two count methods. Thus, the null hypothesis stated there was no mean difference between manual and automated results. Using the two-tailed test, the alternative hypothesis stated there was a significant mean difference between these methods. The results of this analysis are presented below in Table 2 and Figure 2.

For a difference to be identified between manual and automated results at the 95% significance level, the 'P' value obtained in the Two-Tailed T-Test would have to be less than or equal to the T critical Two-Tailed value at 5 %. Thus, no significant differences were identified in the analysis shown above.

	Variable 1	Variable 2
Mean	3.33	3.36
Variance	4.63	4.36
Observations	79	79
Pearson Correlation	1.00	
Hypothesized Mean Difference	0	
Df	75	
t Stat	-1.19	

Table 2. Statistical analysis of åCOLyte and manual count data using a T-Test.

Discussion

Initial Operational Qualification was completed satisfactorily and verified the hardware and software to be working correctly as the image was captured and colonies counted accurately. Having demonstrated the accuracy of the automated system the counting of validation plates was adopted as the daily check. The daily check data also demonstrates the system is reliable, as the same results were achieved consistently.

Performance Qualification was undertaken to evaluate the åCOLyte for counting of different types of bacterial colonies spiraled onto both opaque and transparent agar plates. Comparison of 79 plates counted using the system with manual enumeration showed there is no significant difference between these counting methods. This is satisfactory evidence that an åCOLyte can be used as an alternative to manual counting of spiral plates.

Conclusion

This study clearly shows automated counting does not compromise precision. The fact that the system has been extensively tested with a number of commonly found bacteria spiral plated onto a range of agars ensures microbiologists using an åCOLyte can be confident in their results. This research also indicates the system would be suited for testing anti-microbial therapies, where speed and accuracy are crucial factors in helping get important new treatments to market.

About the author

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More information about automated colony counting and the products referenced in this article is available from:
Synbiosis Ltd.
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