

# A comparison of manual and automated colony counting

by V. Fotheringham

**Counting four different types of serially diluted bacterial colonies on 75 pour plates compared automated and manual counting systems. The results were analysed statistically and showed that the automated counting method produced results that were not significantly different from the manual counting method at the 95% confidence level.**

Disinfectants are used to kill harmful microorganisms in many different applications. They are used in veterinary practice to help to prevent the spread of infectious diseases such as foot and mouth and avian influenza. They are used in food production and catering premises, as well as in the pharmaceutical industry, to prevent contamination of products with harmful bacteria. In the medical area, hospital acquired infections (e.g. caused by *Staphylococcus aureus* MRSA) can be controlled by employing a rigorous regime of cleaning and disinfecting with appropriate bactericidal products [1, 2].

Figures published by the UK Health Protection Agency Communicable Disease Surveillance Center ([www.dh.gov.uk](http://www.dh.gov.uk)) reveal the total number of methicillin-resistant infections contracted in hospitals in England from October 2004 to March 2005 is 3688. The corresponding figure for the same time period in the previous three years is 3651 (2001), 3799 (2002) and 3940 (2003) so there is no sign that the levels of infection are abating. Since MRSA is just one cause of hospital acquired infection, the public has good reason to be concerned about the issue of hospital acquired infections.

Evans Vanodine manufactures disinfectant products used in all of the above areas. To determine their effectiveness against a range of bacteria, colony counts of surviving bacteria post-disinfectant treatments according to European Standard test methods [3,4,5,6] are used. Four species of bacteria are used in stan-

dard bactericidal tests, chosen as representatives of Gram positive and Gram negative bacteria of importance. Colony counts provide the data to calculate log reductions in bacterial numbers. Since these counts determine pass and fail dilutions it is essential to obtain accurate counts. Evans Vanodine used a manual system from IUL (Barcelona, Spain), which consists of a light box and pen, where the pen clicks are recorded automatically as a colony count. More than 100 plates with up to 300 colonies are counted every week. Using this system it could take four microbiologists a working day each to count the plates and transfer the results manually to a computer.



Figure 1. An aCOLyte SuperCount colony counting system.

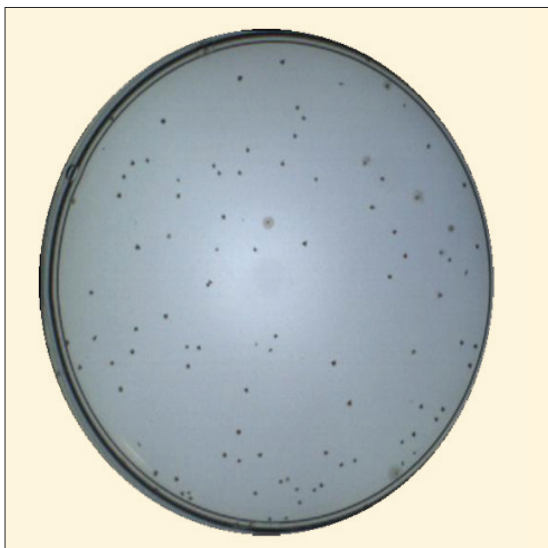


Figure 2. Image of *Staphylococcus aureus* colonies on TSA pour plates produced using the  $\hat{a}$ COLyte system.

This was therefore a labour-intensive method of assessing disinfectant efficacy, which has the potential to create plate reading and keying errors. Since the IUL colony counter does not produce a digital image of the plate alongside its associated colony count, there is also no method for reviewing the raw data to check the results at a future date.

To improve the speed and efficiency of enumerating colonies at Evans Vanodine, automated counters were assessed as they have been shown to provide productivity benefits in other areas of quality control such as vaccine manufacture [7]. One automated colony counter, the Synbiosis  $\hat{a}$ COLyte SuperCount (Cambridge, UK) was chosen as suitable for this type of bacterial count as it combines a lighting unit with camera and software hosted on a PC running a Microsoft Windows operating system [Figure 1]. Since the Microbiology Laboratory at Evans Vanodine is UKAS accredited for disinfectant testing, the system could not be routinely used as part of the standard operating procedure for colony counts until the method had been validated as providing comparable results to the IUL colony counter.

### Materials and methods

In order to compare counts obtained with the  $\hat{a}$ COLyte SuperCount and with the manual IUL

colony counter, pour plates were prepared of the four bacterial species, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae* and *Escherichia coli*. The bacterial cultures were serially diluted in Tryptone Buffered Saline and pour plates with Tryptone Soya Agar (TSA) were prepared. Using the IUL counter five laboratory personnel counted and recorded the colony numbers on these plates (only plates with 300 or fewer colonies were used). One member of staff then counted the same TSA plates with the  $\hat{a}$ COLyte system. Counting using the  $\hat{a}$ COLyte SuperCount involves placing a plate in the illuminated plate holder to create an image of the plate on screen. The colonies are enumerated by clicking the SuperCount icon and the numbers of colonies counted in the chosen area is recorded on the  $\hat{a}$ COLyte display panel. A calculated number of colonies on 100% of the plate is also recorded.

Mean counts from both the manual and automated counts were expressed as log means and the agreement between these log means was examined statistically using a Microsoft Excel program for a paired T-test.

### Results

Figure 2 is an example of a typical plate image that the  $\hat{a}$ COLyte system generates. It was not possible to use the  $\hat{a}$ COLyte for counting all test plates. Bacteria that form large, uneven or spreading colonies were counted as multiple colonies and adjustment of the light could not balance this effect. However, since the  $\hat{a}$ COLyte SuperCount can be used manually by clicking the colonies on the screen, numbers can also be deleted by this means to correct counts.

The agreement between manual and  $\hat{a}$ COLyte colony counts for the four species of bacteria is shown in Table 1. The log mean cfu/mL was examined statistically using Microsoft Excel Data Analysis with the paired T-test (T-test: Paired Two samples for Means).

The null hypothesis stated there was no mean difference between manual and  $\hat{a}$ COLyte results. Using the two-tailed test, the alternative hypothesis stated there was

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

Table 1. Comparison of manual and automated colony count methods.

a significant mean difference between these methods. The results of the statistical analysis are presented in Figure 3.

For a difference to be identified between manual and aCOLyte 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%. Since this is not the case [see Figure 3] there is thus no significant difference between manual and automated colony counting in this study.

### Discussion

There are very few published comparisons between automated and manual colony counting methods. However, the earliest report in 1974 showed that a crude automated colony counter could detect surface and subsurface bacterial colonies of 0.3-mm diameter or greater with a good degree of precision. On a log scale, counting efficiency consistently ranged from 89 to 95% of corresponding manual count determinations for plates containing up to 1,000 colonies [8]. More recently another study showed automated counting and manual counting methods had a linear relationship with a correlation coefficient of 0.99, so there was no significant

difference between the two methods of enumeration [9].

These two studies are consistent with the finding in this investigation which demonstrated that there was no significant difference when comparing 75 plates counted using the aCOLyte system with a manual enumeration method. This is satisfactory evidence that an aCOLyte can be used as an alternative to manual counting of colonies on pour plates.

The aCOLyte automated colony counter will therefore be used in the Evans Vanodine Microbiology laboratory by trained members of staff to count colonies under the following

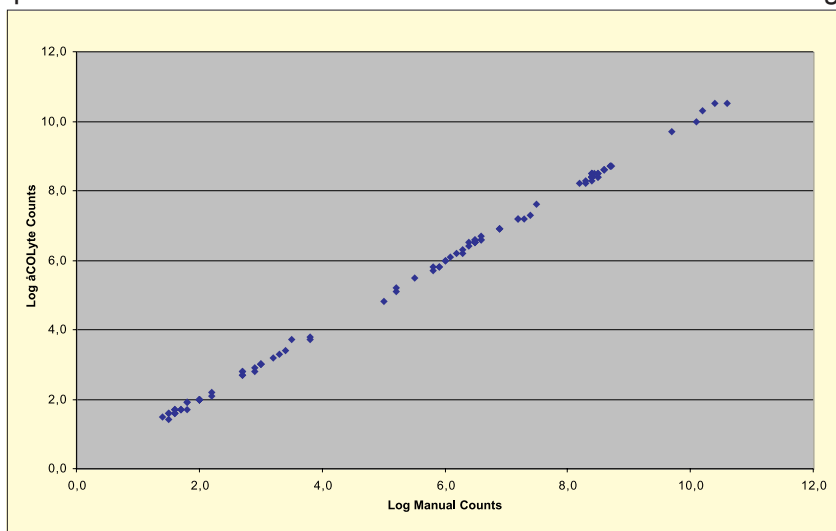


Figure 3. Graphical representation comparing manual and aCOLyte counts.

conditions. Firstly weekly comparison checks are carried out with each trained microbiologist counting the same plate. One plate is chosen with between 15 and 300 colonies from one of the week's test plates and is counted by each tester, manually on the IUL counter and on the  $\hat{a}$ COLyte. The log mean  $\hat{a}$ COLyte count must be equal to the log mean manual count 0.2. Secondly plates with mould colonies, spreading or merging colonies or plates with lumpy agar or agar with large bubbles will not be counted using the 'SuperCount' facility.

### Conclusion

The  $\hat{a}$ COLyte is an affordable system which when used at Evans Vanodine has reduced the amount of time spent counting colonies by 50 percent each week. Since  $\hat{a}$ COLyte is being routinely used to test the bactericidal activity of disinfectants, many of which will be used in hospitals, it is imperative the system is as accurate as a manual count. This study clearly shows automated counting using an  $\hat{a}$ COLyte SuperCount does not compromise precision and because the system has been tested with four commonly found species of bacteria on pour plates this ensures microbiologists at Evans Vanodine will have greater confidence in results generated by the  $\hat{a}$ COLyte SuperCount. This research also indicates the  $\hat{a}$ COLyte SuperCount is ideally suited for quality testing any types of bactericidal disinfectants, where speed and accuracy are crucial factors in helping get these products to the market.

### References

1. Beckford-Ball J, Hainsworth T. The control and prevention of hospital-acquired infections. *Nursing Times* 2004; 100: 28-9.
2. Rutala WA, Weber DJ. Surface disinfection: should we do it? *J Hosp Infect* 2001; 48: 64-8.
3. Anonymous. Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field (Phase 2, Step 1). British Standards Institute Directive. 2000; EN 1656.
4. Anonymous. Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of

bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas (Phase 2, Step 1). British Standards Institute Directive. 1997; EN 1276.

5. Anonymous. Chemical disinfectants and antiseptics, products for hygienic and surgical handrub and handwash. British Standards Institute Directive. 1997; EN 12054.

6. Anonymous. Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial domestic and institutional areas. British Standards Institute Directive. 2001; EN 13697.

7. Putman M, Burton R, Nahm MH. Simplified method to automatically count bacterial colony forming unit. *J Immunol Methods* 2005; 302: 99-102.

8. Goss WA, Michaud RN, McGrath MB. Evaluation of an automated colony counter. *Appl Microbiol* 1974; 27: 264-7.

9. Dobson K, Reading L, Scutt A. A cost-effective method for the automatic quantitative analysis of fibroblastic colony-forming units. *Calcif Tissue Int* 1999; 65: 66-72.

### Author

Valerie Fotheringham,  
Chief Microbiologist,  
Evans Vanodine International.  
e-mail: microlab@evansvanodine.co.uk