Evaluation of an IUL Flash & Go Automated Colony Counter

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ABSTRACT

An IUL Flash & Go automated colony counter was used to enumerate *E. coli* (ATCC 700728) colonies and its performance was compared with manual counting on spiral plates. A total of 85 plates were analyzed. Linear regression analysis and the log differences between the manual and automated counts were determined. The results were analyzed to evaluate the reliability and accuracy of the colony counter. A correlation coefficient of 0.969, a slope of 0.932 and intercept of 0.25 all indicate a strong, linear relationship. The mean log value difference between the manual and Flash & Go count methods was -0.035. Of the 85 plates counted, 95% of the plates were within 0.15 $\log_{10}$ difference between the manual and Flash & Go automated counts. These results demonstrate that the Flash & Go automated colony counter is an effective, accurate and time saving alternative to the standard method of manual counting.

Keywords: Flash & Go, automated colony counting, cfu, *E. coli*, reproducibility**,** USA

1. INTRODUCTION

Counting of microbial populations in food is a standard procedure of testing food contamination in a food microbiology laboratory. Manual counting of colony-forming units (cfu), grown on Petri plates containing growth media is one of the most tedious, laborious, and time-consuming processes in a laboratory (Putman et al., 2005; Garry et al., 2006). Recently, several types of automated colony counters have been developed to improve efficiency in colony counting. However, there are very few published comparisons between automated and manual counting methods (Fotheringham, 2006). Reliability and accuracy are the crucial parameters to be considered in case of an automated colony counter (Marotz et al., 2001). Automated counting systems are acceptable if the automated counts are within 0.5 $\log_{10}$ of the manual count (Garry et al., 2006). In this study, an IUL Flash & Go (Neutec Group Inc., Farmingdale, NY) automated colony counter was used to enumerate *E. coli* (ATCC 700728) colonies and its performance was compared with manual counting of spiral plates.

The Flash & Go is a small bench-top automatic colony counter for pour plates and any type of spiral spreading. It can count all kinds of colonies on the various media and its high definition color camera connected to an external PC can detect and count colonies as small as 0.07 mm (Anon, 2009). The PC captures and processes the agar plate images and saves results in a MS Excel format. The number of colonies counted and the number of cfu per mL are displayed on the screen.

2. MATERIALS AND METHODS

Low voltage direct current was applied to inactivate *E. coli* (ATCC 700728) on beef surfaces (Mahapatra et al., 2008; Saif et al., 2006). To determine the effectiveness of the process colony counts of surviving *E. coli* were carried out.

Meat sample along with saline solution was stomached and filtered. An Eddy Jet (Neutec Group Inc., Farmingdale, NY) automated spiral spreader was used to spread 50 µL of solution on Nutrient agar plates (100-mm plastic disposable Petri dishes). Inverted plates were incubated at 37 ^{0}C for 24 h. For improving the speed and efficiency of enumerating colonies at our Food Engineering Lab, an IUL Flash & Go automated colony counter (Neutec Group Inc., Farmingdale, NY) was used. The various parameters and process settings of Flash & Go were set based on the suggestions by the manufacturer. One member of staff then counted the true number of colonies on the same plates using a manual counter (Bantex, Model 920A, American Bantex Corp; Burlingame, CA). All counts were obtained with plate covers removed.

2.1 Data Handling Method

A total of 85 plates were randomly selected from the low voltage current experiments. Any plate with a total count less than 20 or greater than 300 was excluded from further analysis (Fotheringham, 2006; Garry et al., 2006). The base 10 logarithms of these colony counts were used for statistical analysis. The results of the manual count were correlated with the results from the Flash & Go count (Mahapatra et al., 2009). The reproducibility (counting precision) of the values obtained with the Flash & Go counter was determined by inserting the same agar plate 20 times in a fixed position (Wilson, 1995). Values ranging from 10^2 to 10^5 cfu/mL were evaluated. Linear regression analysis and the log differences between the manual and automated counts were examined using SAS 9.1 statistical package (SAS, 2003). The manual and automated colony counts were also analyzed using GLM procedures in SAS (2003) with manual count as the independent variable.

3. RESULTS AND DISCUSSION

The results of the manual and automated colony counts were analyzed by linear regression using PROC REG in the SAS system (SAS, 2003) and the regression equation obtained was:

$$\text{Flash \& Go count} = 0.932 * \text{Manual count} + 0.25$$

There was no significant difference ($p > 0.05$) when comparing 85 plates counted using the Flash & Go automated colony counter with manual enumeration method.

Figure 1 shows the counts obtained by manual and Flash & Go counting methods for 85 spiral plates. Automated counting and manual counting methods had a linear relationship with a high correlation coefficient of 0.969. This is consistent with the findings in studies by other researchers.

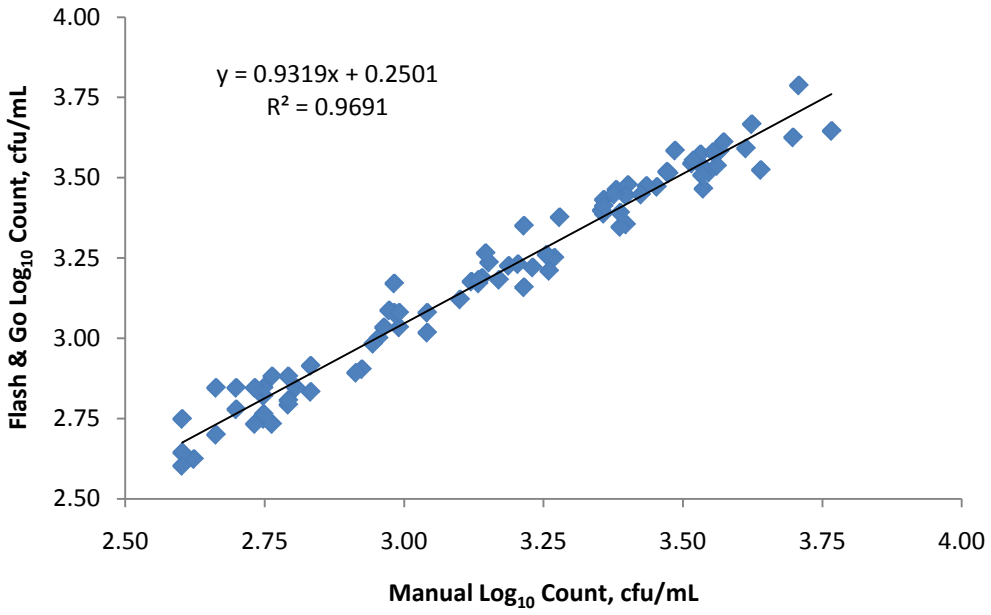

Figure 1. Linear trend line analysis of data comparing manual and automated counts.

Dobson et al. (1999) reported that automated counting and manual counting methods had a similar relationship with a correlation coefficient of 0.99. Similarly, Putman et al. (2005) observed that the automated and manual counts were highly correlated (r^2 = 0.96), but the machine counts were slightly lower than the manual counts.

Figure 2 illustrates the performance limits of the automated versus manual counts. Of the 85 plates counted, 95% of the plates were within 0.15 $\log_{10}$ difference between manual and automated counts.

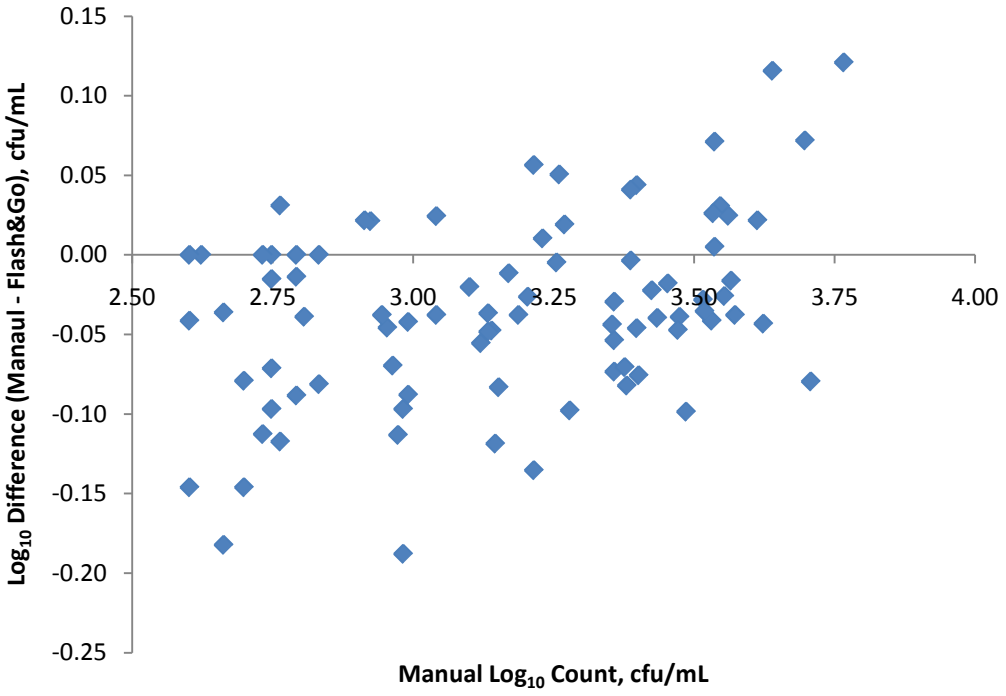

Figure 2. Performance limits demonstrating the mean log value differences between the manual and automated.

The reproducibility (counting precision) of the values obtained with the Flash & Go counter is shown in table 1.

Table 1. Reproducibility of Flash & Go automated colony counter results at a range of bacterial concentrations

Mean log count, cfu/mL	Standard deviation	Variance
2.81	0.02143	0.00046
3.43	0.00482	0.00002
4.95	0.01095	0.00012
5.27	0.06320	0.00399

The standard deviations obtained for repeatedly counted plates were low. The mean standard deviation for the Flash & Go colony counter for values ranging from 10^2 to 10^5 cfu/mL was 0.025 and the variance was less than 0.00399.

The mean $\log_{10}$ value difference between the manual and automated count methods was -0.035. This small, negative dispersion could be because of poor contrast between background agar and colonies or overlapping of colonies (Garry et al., 2006). Occasional bubbles or cracks in the agar might have produced erroneous counts. Table 2 shows the mean $\log_{10}$ value differences at a range of colony counts i.e. in the range of 20-50, 50-100, 100-150, and 150-200 (number of colonies present on the agar plat during manual count). The Flash & Go automated counts were slightly higher than the manual counts. But the discrepancy between manual and automated counts appears to decrease gradually as the number of colonies present on the plate increases.

Table 2. Mean $\log_{10}$ value differences between manual and automated counts at a range of colonies

No. of colonies per plate (Manual count)	No. of plates	Mean $\log_{10}$ differences (Manual count-Flash & Go count), cfu/mL
20-50	26	-0.049
50-100	23	-0.042
100-150	17	-0.037
150-200	15	-0.013

4. CONCLUSIONS

The IUL Flash & Go automated colony counter is a suitable alternative to the standard method of manual counting. A correlation coefficient of 0.969, a slope of 0.932 and intercept of 0.25 all indicate a strong, linear relationship between the automated and manual methods. Log differences were within 0.15 $\log_{10}$ of the manual count for 95% of all plates analyzed, which is well within the standard of 0.5 $\log_{10}$. There was no significant difference when comparing 85 plates counted using the Flash & Go automated colony counter with manual enumeration method. The use of automated colony counter increased the overall efficiency in our research operations by significantly reducing the time devoted to conventional manual plate count and made our tasks easier for data manipulation, analysis and interpretation.

5. ACKNOWLEDGEMENTS

The funding by the Cooperative State Research, Education and Extension Services (CSREES), USDA is gratefully acknowledged.

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