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A Critical Evaluation of Automatic Bacterial Colony Counters

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ABSTRACT

Four automatic colony counters (ACC), 3M Model 620 and Artek Models 480, 870 and 880, were evaluated for their precision and accuracy in counting bacterial colonies in pour-plates prepared using raw and pasteurized milk samples. The automatic colony counters were precise, labor saving devices, but not one of the ACC units approached our acceptability criterion that 90% of the ACC counts fall within ± 10% of the corresponding manual count. Some parameters of experimental design and instrument calibration which may significantly influence the response and performance of the automatic counters are discussed.

The plate count remains one of the most basic but tedious techniques employed in dairy microbiology. While many factors influence the Standard Plate Count during the course of pour-plate preparation, technician counting proficiency is regarded as the greatest and most uncontrollable source of error (*2,12*). *Standard Methods for the Examination of Dairy Products* (*1*) states that analysts should be able to duplicate their counts on the same plate to within 5% and the counts of other analysts to within 10%. Recent studies suggest that these restrictions may be unrealistic. Snyder (*12*) reported on an unpublished study conducted by the American Public Health Association in 1975 which found that the variation among technicians counting the same plates was ± 24%. Fowler et al. (*4*) found that analysts could reproduce their own counts only within ± 7.71% and the counts of others within ± 18.19%. Fruin and Clark (*5*) also reported that analysts were within 5% of the true count on only 68% of the plate counts.

The need to improve the precision and speed of the counting technique, coupled with advanced electronic technology, led to the evolution of automatic bacterial colony counters (ACC). The principles of the automatic counting system are described in other papers (*10,12,13*). In 1957, Mansberg (*9*) described the first practical ACC for use with the pour-plate technique. He noted certain limitations and precautions to be observed when using the automated system: the scanning resolution was only 0.5 mm and needed substantial improvement; preparation of pour plates for use with the automatic counter required greater care to ensure even distribution of the inoculum and to avoid scratches and agar defects (using a mechanical rotator was suggested); certain types of colonies, e.g. "spreaders" and "pulls," could not be accurately counted; the counter was sensitive to plate orientation; the molded rim of the petri dish interfered with the automatic counter's ability to enumerate colonies growing on or near the meniscus of the agar where it contacted the sides of the petri dish. This latter interference had to be optically masked, but a correction factor for the masking could be applied.

An improved version of Mansberg's counter was evaluated by Malligo in 1965 (*8*). Using pure cultures of *Serratia marcescens* and *Bacillus subtilis* var. *niger*, Malligo determined that the manual and ACC techniques had the same precision; however, the ACC had a stable bias which could be corrected by interpolating from a linear regression equation derived from the combined data of both pure culture studies. Malligo used spread plates to minimize dispersion of colonies to the outer edges of the agar and thereby reduce the number of colonies developing within the masked area.

Within the past decade, a number of more sophisticated ACC systems have been developed and evaluated. The 3M Brand Automatic Colony Counter, Model 620, was evaluated for counting variability by Packard and Ginn (*11*) and more recently for accuracy by LaGrange et al. (*7*). Packard and Ginn concluded that 3M counts were somewhat more variable than the manual counts. The overall mean values of the 3M colony counts were comparable to those obtained by the manual technique, but averaged 2.33 counts higher. LaGrange et al. reported that the 3M counter tended to underestimate the number of colonies per plate. Both studies suggested the application of correction factors for adjusting the automated counts based on interpolation from linear regression equations.

Goss et al. (*6*) evaluated the Artek 870 colony counter for sensitivity, precision and accuracy by comparing the counts obtained by two technicians with an Artek count on the same plates. Their data showed that surface and subsurface colonies of 0.3-mm size or larger were counted with good precision but that the Artek 870 uniformly underestimated the manual count. A scatter plot of the plate count data on a logarithmic scale indicated a linear relationship between the manual and automated counts.

Fleming and O'Conner (*3*) examined the Artek 480 counter and the Foss Biomatic 16900 electronic counter using raw milk samples. They observed that the automatic counters overestimated the plate counts relative to the manual method, but were more precise than the corresponding manual counts. Fruin and Clark (*5*) evaluated the accuracy of the Artek 480 colony counter by comparing manual and automated counts with the true count obtained by the use of photographs. They commented on a number of errors associated with the experimental design of studies used to evaluate ACC's and the need for a more standardized and sophisticated approach. Lack of awareness of these sources of error, including improper plate orientation, irregularities in agar surfaces, air bubbles in the medium, spreaders and extremely larger surface colonies could influence the results and lead to erroneous conclusions. After these possible sources of error had been eliminated, the data from Fruin and Clark indicated that the Artek 480 underestimated plate counts when the density exceeded 100 colonies per plate.

It is evident from the literature that the results of ACC evaluations were often contradictory, even when similar units were examined by different investigators. Within the past 4 years we examined the performance of a number of ACC units. This paper reports on our evaluation of four ACC systems. Some parameters of experimental design and instrument calibration which may significantly influence the response and performance of the automatic counters are discussed.

MATERIALS AND METHODS

Instrument description and calibration

Four ACC systems, supplied by the manufacturers, were evaluated: 3M Brand Automatic Bacterial Colony Counter (3M Company, New Business Ventures Division, St. Paul, Minnesota, 55101); Artek models 480, 870 and 880 (Artek Systems Corporation, Farmingdale, New York, 11735).

The 3M 620 was equipped with a fixed-position plate support stage designed to allow the petri plates to be "nestled" in the correct position for counting. The masked area and compensation factor were factory-set at 17%. The size threshold was empirically adjusted to exclude all particles less than 0.2 mm in diameter as a "noise" factor was introduced at more sensitive settings. According to the manufacturer, this "noise" factor is created by the inability of the scanning system to distinguish between the background and low density colonies or particles when the medium/colony contrast is minimized at maximum threshold settings.

The Artek 480 had a flat support stage with two adjustable plate-guide arms. The plates had to be centrally positioned and the guide arms secured with tightening screws. The diaphragm was manually adjusted to mask the rim of the petri plate. The correction factor (CF) was calculated, according to the manufacturer's instructions, as the percent difference between the arithmetic mean count of 20 plates (30-300 counting range) determined manually and by the ACC without compensation. For our study, the CF for the Artek 480 was calculated to be + 14%. The Artek 480 was also equipped with a sensitivity threshold dial which was adjusted to obtain a maximum response without introducing a "noise" factor.

The Artek 870 was similar in design and operation to the Artek 480 except that it was equipped with an electronic aperture for controlling the counting area. The CF and sensitivity threshold settings were determined as for the Artek 480.

The Artek 880 was originally equipped with a plate support stage similar to that for the 480, but this was changed to a "nesting" type stage at our request. The CF was calculated as the percent difference between the total area of the plate surface and the actual counting area controlled by the electronic aperture. For our study, a CF of 11% was determined. The size threshold discriminator was set to exclude all particles less than 0.2 mm in diameter. An added feature of the Artek 880 was a separate sensitivity threshold adjustment with two "tuning windows" in the viewing screen. Maximum sensitivity was obtained for each counting series by increasing the threshold until the upper window began flashing and then slowly reducing the threshold until the flashing ceased.

Source of samples and preparation of pour plates

Pasteurized and raw milk samples submitted to the laboratory for routine bacteriological examination were used to prepare pour plates for the comparative studies. Each sample was diluted appropriately in Butterfield's phosphate buffer (*1*) to obtain 30 to 300 colonies per plate. Standard Methods Agar (BBL) was prepared in the Agarmatic Bench-Top Agar Sterilizer (New Brunswick Scientific Co., Inc., New Brunswick, New Jersey) and dispensed in 12-15-ml volumes per pour plate. Plastic petri plates (100 × 15 mm, Fisherbrand Cat. 8-757-13) without stacking rings were used throughout the study. The plates were incubated for 48 ± 4 h at 32 C before counting.

Counting procedures

All plates were visually screened to eliminate any plates with colony counts less than 30 or greater than 300, and any showing uneven or rippled agar or scratched plastic surfaces. Each acceptable pour plate was counted manually by three different technicians using Quebec colony counters and hand tallies. Only those plates whose colony counts agreed within 10% of the median manual count were acceptable for comparative counting with the 3M 620, Artek 480 and Artek 870. Only those plates whose manual counts agreed within 5% of the median count were used for comparative counting with the Artek 880. Each plate was counted three times on the automatic counters using 90° rotation between counts.

Performance criteria and statistical analysis

The performance of each instrument was statistically evaluated by linear regression analysis of the scatter plot data obtained from the $\log_{10}$ of the mean counts. The overall regression data and the data from low (30-100), middle (101-200) and high (201-300) counting ranges of each comparative study were subjected to Analysis of Variance (ANOVA). The null hypothesis, Ho: m(Slope) = 0, was applied so that Ho would be rejected and we could assume that a linear regression existed if F was significant (*14*). ACC performance was further evaluated by calculating the percentage of ACC counts deviating by more than ± 10% of the mean manual count. We set for our laboratory a performance criterion of 90% agreement for acceptance of an automatic colony counter.

RESULTS

Figure 1 displays the scatter plot data of a comparison between the manual counts and the corresponding 3M counts. Table 1 presents a statistical

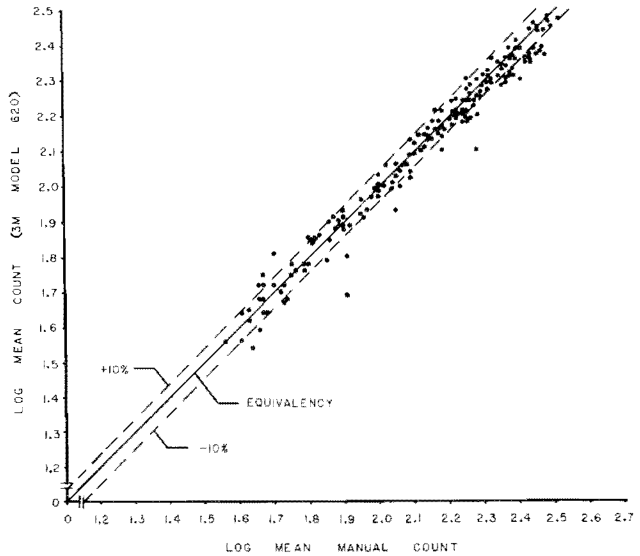

Figure 1. *Scatter plot relating plate counts determined by 3M 620 to manual technique. Linear regression equation: 3M 620 count = 0.069 + (0.960×manual count).*

TABLE 1. *Linear regression evaluation of data comparing colony counts by 3M 620 automatic colony counter with manual counts.*

Parameters	Counting range 30-100	101-200	201-300	30-300
n^1	64	81	28	173
Manual: $\bar{x}_g{}^2$	63.4	147	246	117
3M 620: $\bar{x}_g$	62.5	141	230	113
r^3	0.933	0.914	0.841	0.986
m^4	0.957	0.939	0.791	0.960
b^5	0.071	0.116	0.470	0.069
ANOVA (F)[6]	S	S	NS	S
EMS[7]	0.002	0.001	0.001	0.002
No. unacceptable ACC counts[8]	16	12	14	42
(%)	(25.0%)	(14.8%)	(50.0%)	(24.3%)

[1]n, number of observations.
[2]$\bar{x}_g$, geometric mean.
[3]r, correlation coefficient.
[4]m, slope of regression line.
[5]b, Y intercept.
[6]F, S - significant; NS - not significant.
[7]EMS, error mean square.
[8]Unacceptable arithmetic mean counts by ACC exceed ± 10% of corresponding mean manual count.

summary of the 3M linear regression data obtained for three arbitrary counting ranges, 30-100, 101-200, 201-300, and overall, 30-300. All the correlation coefficients (r) were significant ($a = 0.05$), indicating that the counts obtained by the 3M were meaningfully related to the corresponding manual counts. Analysis of the regression coefficients (m or slope) by ANOVA showed that there was a linear relationship between the two counting techniques overall; however, the F value was not significant in the 201-300 counting range indicating a non-linear relationship in this range. Although the overall arithmetic mean count obtained by the 3M 620 fell within ± 10% of the mean manual count, 24.3% of the individual 3M counts exceeded the 10% limit of the corresponding manual count.

Figure 2 shows the scatter plot data of a comparison between $\log_{10}$ Artek 480 and manual counts. Table 2 is a statistical summary of the linear regression data displayed in Fig. 2 and the data from a second study comparing the manual counts with those obtained by the 3M 620. The correlation coefficients for the manual versus the Artek 480 were significant ($a = 0.05$); however, analysis of the regression coefficients by ANOVA for each counting range and overall were not significant, indicating that the relationship between the manual and Artek 480 counts was not truly linear. Furthermore, although the overall arithmetic mean count obtained by the Artek 480 fell within ± 10% of the arithmetic mean manual count, 47.7% of the individual mean Artek counts fell outside the ± 10% limit for the corresponding manual counts. The results for the 3M 620 in this study were similar to those in Table 1.

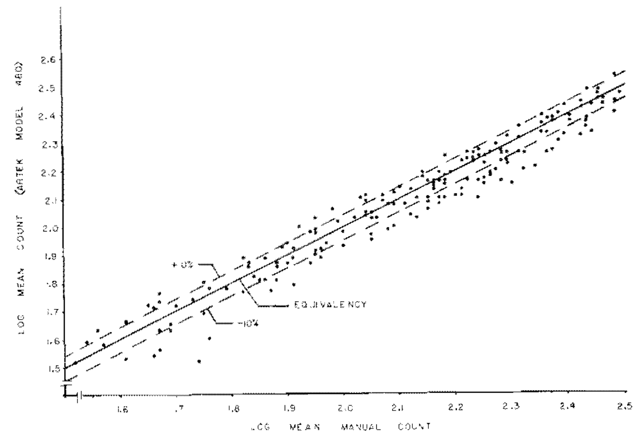

Figure 2. *Scatter plot relating plate counts determined by Artek 480 to manual technique. Linear regression equation: Artek 480 counts = 0.148 + (0.924 × manual count).*

Fig. 3 illustrates, by scatter plot, the relationship between the corresponding Artek 870 and manual counts. Table 3 presents a statistical summary of the data from the Artek 870 study as well as data from additional studies of the 3M 620 and Artek 480 with the same manual counts for comparison. The correlation coefficients for the Artek 870 versus the manual counts were all statistically significant. Analysis of the regression coefficients by ANOVA illustrated that a linear relationship existed between the automated and manual counts within the individual arbitrary counting ranges but not overall. A comparison of the arithmetic mean counts indicated close agreement between the Artek 870 and manual technique but 36% of the individual counts by the Artek 870 varied by more than 10% of the corresponding manual counts.

The statistical conclusions for the 3M 620 data in this study were similar to those presented for the data from Tables 1 and 2, except that a non-significant F value was calculated for the 30-100 colony count range in addition to the previously observed 201-300 range. Also the number of individual counts by the 3M unit which varied

TABLE 2. *Linear regression evaluation of data comparing colony counts by 3M 620 and Artek 480 automatic colony counters with manual counts.*

Parameters	Counting range			
	30-100	101-200	201-300	30-300
n[1]	60	71	22	153
Manual: $\bar{x}_g$[2]	62.0	146	249	113
3M 620: $\bar{x}_g$	63.4	141	234	111
r[3]	0.913	0.892	0.866	0.982
m[4]	0.857	0.891	0.831	0.924
b[5]	0.266	0.222	0.378	0.148
ANOVA (F)[6]	S	S	NS	S
EMS[7]	0.003	0.001	0.001	0.002
No. unacceptable 3M 620 counts[8]	23	10	10	43
(%)	(38.3%)	(14.1%)	(45.5%)	(28.1%)
Artek 480:				
$\bar{x}_g$	59.9	138	227	107
r	0.858	0.793	0.671	0.965
m	0.875	0.861	0.878	0.946
b	0.209	0.273	0.253	0.086
ANOVA	NS	NS	NS	NS
EMS	0.005	0.003	0.004	0.004
No. unacceptable Artek 480 counts	31	31	11	73
(%)	(51.7%)	(43.7%)	(50.0%)	(47.7%)

1,2,3,4,5,6,7 and 8 - See Table 1.

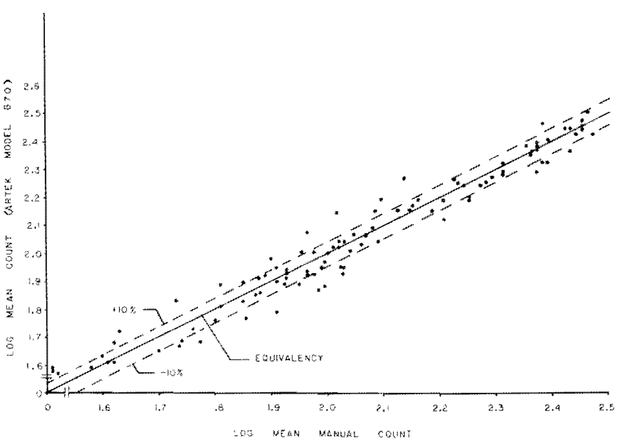

Figure 3. *Scatter plot relating plate counts determined by Artek 870 to manual technique. Linear regression equation: Artek 870 count = 0.475 + (0.936 × manual count).*

by more than 10% of the manual counts increased to 37.3% although the overall mean counts remained comparable.

Similarly, the data for the Artek 480 presented in Table 3 indicated a change in machine performance as a non-significant F value was determined for all three counting ranges but not overall. The overall arithmetic mean counts remained comparable to the mean manual counts, but only 37.3% of the individual counts exceeded ± 10% of the corresponding manual counts, a drop of 12% compared to the results in Table 2.

Figure 4 displays the scatter plot data of the comparison between the $\log_{10}$ Artek 880 and manual

TABLE 3. *Linear regression evaluation of data comparing colony counts by 3M 620, Artek 480 and Artek 870 automatic colony counters with manual counts.*

Parameters	Counting range			
	30-100	101-200	201-300	30-300
n[1]	26	25	24	75
Manual:				
$\bar{x}_g$[2]	57.8	140	249	124
3M 620				
$\bar{x}$[3]	60.7	137	233	122
r[4]	0.955	0.962	0.762	0.991
m[5]	0.916	0.856	0.810	0.918
B[6]	0.170	0.300	0.427	0.166
ANOVA (F)[6]	NS	S	NS	S
EMS[7]	0.002	0.001	0.001	0.001
No. unacceptable 3M 620 counts[8]	13	5	10	28
(%)	(50.0%)	(20.0%)	(41.7%)	(37.3%)
Artek 480:				
$\bar{x}_g$	60.5	138	245	124
r	0.953	0.900	0.663	0.987
m	0.893	0.837	0.857	0.943
b	0.201	0.343	0.335	0.120
ANOVA	NS	NS	NS	S
EMS	0.002	0.002	0.002	0.002
No. unacceptable Artek 480 counts	13	8	7	28
(%)	(50.0%)	(32.0%)	(29.2%)	(37.3%)
Artek 870:				
$\bar{x}_g$	60.9	144	242	126
r	0.951	0.847	0.668	0.986
m	0.903	0.811	0.771	0.936
b	0.526	0.751	0.869	0.475
ANOVA	NS	NS	NS	S
EMS	0.002	0.003	0.002	0.002
No. unacceptable Artek 870 counts	12	7	8	27
(%)	(46.2%)	(28.0%)	(33.3%)	(36.0%)

1,2,3,4,5,6,7 and 8 - See Table 1.

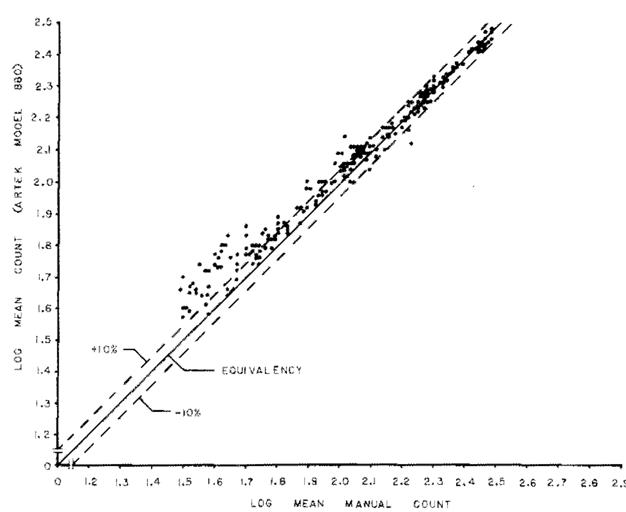

Figure 4. *Scatter plot relating plate counts determined by Artek 880 to manual technique. Linear regression equation: Artek 880 count = 0.304 + (.866 × manual count).*

counts. This figure clearly indicates a closer agreement between manual and Artek 880 data with plate counts of more than 100 colonies per plate. The statistical summary of the data in Table 4 confirmed this observation. A non-significant F value was calculated for

Table 4. *Linear regression evaluation of data comparing plate counts by Artek 880 automatic colony counter with manual counts.*

Parameters	Counting range		
	30-100	101-200	30-300
n[1]	105	130	235
Manual: $\bar{x}_g$[2]	54.6	163	100
r[3]	0.941	0.982	0.990
m[4]	0.826	0.913	0.866
b[5]	0.375	0.203	0.304
ANOVA (F)[6]	NS	S	S
EMS[7]	0.002	0.001	0.001
No. unacceptable ACC counts[8]	70	18	88
(%)	(66.7%)	(13.9%)	(37.5%)

1,2,3,4,5,6,7 and 8 - See Table 1.

related counts in the 30-100 range but not for the 101-300 range or the overall data. Eighty-eight out of 235 (37.4%) of the individual arithmetic mean counts exceeded ± 10% of the manual counts; however, 79.1% of these counts occurred in the 30-100 range and all were underestimated values.

DISCUSSION

The determination of the "true count", against which the performance of the ACC is measured, is critical to every ACC evaluation. Fruin and Clark (*5*) suggested that an evaluation based on a simple comparison between ACC and manual counts was not valid due to the recognized imprecision of replicate technician counts. In our investigation, certain precautions were taken to obtain pour plates with precise counts for comparative counting purposes. Plates which contained "spreaders" or "pulls", air bubbles, scratches or other artifacts were excluded because such plates were subject to individual interpretations which the ACC's were incapable of making. Furthermore, we used three different analysts for counting and rejected any plate whose counts could not be reproduced within 10% or 5% as previously described. Care was also taken to ensure that a representative number of "true count" plates in the low (30-100), middle (101-200) and high (201-300) plate count ranges were included in each evaluation.

Despite these precautions, not one of the ACC units approached our acceptability criteria that 90% of the ACC counts fall within 10% of the corresponding manual count. In separate studies, 24.3% (Table 1), 28.1% (Table 2) and 37.3% (Table 3) of the 3M 620 counts exceeded the 10% limit; 47.7% (Table 2) and 37.3% (Table 3) of the Artek 480 counts were unacceptable; 37.3% (Table 3) of the counts by the Artek 870 were in excess of the 10% limit; and 37.5% (Table 4) of the Artek 880 counts were unacceptable. The inability of these ACC counts to meet our acceptability criteria occurred in spite of the overall mean counts of each ACC being comparable to the mean manual counts.

Analysis of variance of the linear regression data for each study revealed other serious deficiencies of the ACC systems. A non-significant F value, indicating a non-linear relationship between the ACC and manual counts, was calculated for different counting ranges for each unit. This finding indicated that ACC counting accuracy was not just a function of population density, but reflected the influence of other parameters. The inherent problem of having to mask the rim of the petri dish and consequently determine the correct compensation for bacterial colonies excluded from the counting area has been a major concern. Applying correction factors based on statistical considerations was originally recommended by Mansberg (*9*). Malligo (*8*), Fruin and Clark (*5*) and Van Reusel et al. (*13*) suggested that the corrected counts for the ACC be determined by extrapolation from a linear regression equation derived from ACC versus manual counts over a series of counting ranges. Every ACC examined in our laboratory, however, employed a simple multiplicity factor to correct the ACC count. The 3M 620 had a fixed masked loss of 17% with a corresponding built-in correction factor. The Artek 480, 870 and 880 had adjustable camera diaphragms or electronic apertures with flexible compensation factor dials. Correction of the ACC counts based only on uniformly applied multiplicity factors, regardless of the calculation method used, is predicated on false assumptions. This simple approach assumes that the response of the ACC is just a function of plate population density when, in fact, the ACC count is also influenced by colony distribution, plate orientation, medium/colony contrast, instrument calibration and the intrinsic resolution of the scanning system (*13*).

The low count range (30-100) data from the Artek 880 study were re-evaluated (data not shown) using ACC counts reduced by 11% (compensation factor). The percentage of Artek 880 counts which fell within ± 10% of the corresponding mean manual values increased from 33% (compensated) to 61% (without compensation). The improved accuracy of the low range counts, without compensation, illustrated the significance of colony distribution. With a small plate population, the probability of colonies developing in the masked area was low and thus a compensation factor was not required. However, the inability of the Artek 880 to accurately count 40% of the low range plates despite the removal of the compensation factor reflected the influence of the other, previously described, parameters. In addition, the confidence limits for the slope of the regression line calculated for the re-evaluated data did not include 1, indicating a lack of equivalency between the Artek 880 and the corresponding manual counts. A representative from the Artek Corporation also discovered that the upper tuning window on our Model 880 was too finely set at the factory. Consequently, noise factors and dust particles on the base plate were being sporadically counted, particularly with low range count plates, resulting in irregularly elevated counts.

Our scatter plot data showed an unbiased distribution of the ACC counts about the line of equality with the exception of the low range counts using the Artek 880. Similar distribution patterns have been demonstrated only by Van Reusel et al. (*13*). Reports by other investigators indicated that the ACC counts were either uniformly greater than the manual counts (*4,10*) or always underestimated the manual count (*3,5,6*). Such contradictory results suggest not only variation in experimental design, but also failure on the part of the manufacturers to provide proper guidance, direction and precautionary controls in instrument calibration to the electronically naive operator.

The Artek Systems Corporation has recently introduced the Artek Compu-Print 700, a card-programmable calculator with a print-out base, which can be integrated with the Artek 880 counter to provide statistically corrected counts automatically. The value of this additional unit for improving the performance of the Artek 880 counter, particularly in the lower counting range, needs to be assessed.

Commercial ACC systems are a distinct improvement in electronic circuitry, optics and aesthetic design over the original unit described in 1957. Despite these improvements, many of the deficiencies inherent in the ACC system described by Mansberg have not been resolved. Our study concludes that ACC systems compromise accuracy for speed and precision. Although ACC systems do not have human foibles, their performance is greatly influenced by human factors.

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