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Evaluation of an Automated Colony Counter

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An automated colony counter was found to readily detect surface and subsurface bacterial colonies of 0.3-mm size or greater with a high degree of precision. On a logarithmic scale, counting efficiency consistently ranged from 89 to 95% of corresponding manual count determinations for plates containing up to 1,000 colonies. In routine application, however, automated plate counts up to approximately 400 colonies were selected as a more practical range for operation. The automated counter was easily interfaced with an automated data acquisition system.

One of the most tedious laboratory procedures in practice today is the manual counting of bacterial colonies on petri dishes. Recently, several types of automated colony-counting instruments became available. We have evaluated the performance of one, namely, the bacterial colony counter (model 870) manufactured by Artek Systems Corp., Farmingdale, N. Y. Some of the key parameters which we elected to examine were (i) colony size discrimination (sensitivity), (ii) instrument precision (reproducibility), (iii) accuracy, and (iv) feasibility of interfacing the counter with automated data acquisition systems. The latter point was of prime importance for maximum utilization of the automated counter for our research needs.

The system components evaluated include (i) the colony counter, (ii) a parallel BCD to serial ASCII interface (Digital Equipment Corp., Maynard, Mass.), and (iii) a standard ASR-33 teletype (Teletype Corp., Skokie, Ill.) (Fig. 1).

The main element of the colony counter is an internal high-speed scanning television camera which can detect and record differences in optical density between a bacterial colony and the agar background. A television monitor (Fig. 1, arrow 1) is supplied which visually displays the total area being scanned; an illuminated dot on the video display is automatically superimposed over each surface and subsurface colony which has been counted. A button (Fig. 1, arrow 2) is pressed to record the total count on the digital display (Fig. 1, arrow 3). An adjustment dial (Fig. 1, arrow 4) is also provided to optimize instrument sensitivity for the culture medium in use. Also shown are the plate alignment stops and a culture plate in counting position (Fig. 1, arrow 5).

Capability for electronically monitoring the digital display is provided through an external connector located at the rear of the counter. When the count button is depressed, the contents of the display panel (BCD) are read and converted to serial print characters (ASCII) by the interface. This information is then transmitted either to the teletype or to a remote computer for further processing (switch selectable).

Soybean casein digest agar (Trypticase soy agar, BBL) was used for studies with *Staphylococcus epidermidis* (ATCC 17917) and *Escherichia coli* (ATCC 8739). Skin extract samples obtained by a method previously described (1) were plated in Trypticase glucose extract agar with added lecithin and polysorbate 80 (Letheen agar, BBL). The standard pour plate system used in all studies involved the plating of 1- or 2-ml samples of a bacterial suspension, followed immediately by the addition of melted, tempered agar. Except for preliminary studies, no attempt was made to add a measured volume of agar (estimated to be between 10 and 20 ml). All plates were incubated at 37 C for approximately 48 h except as noted. This incubation period corresponds to that which is routinely employed in this laboratory for the types of specimens and conditions described herein. Manual plate counts usually were performed by two technicians who also counted the same plates on the automated colony counter. All counts were obtained with plate covers removed. Where plates contained 300 to 1,000 colonies, an estimated total count was obtained by the standard method of manually counting a representative defined subarea of the plate and applying a mathematical correction for the total plate area.

In preliminary studies, 100-mm commercial

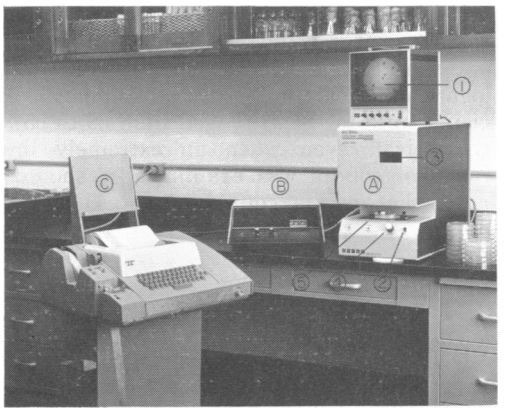

FIG. 1. *Photograph of automated colony counter (A) coupled to data acquisition system (B) and teletype (C). 1, Television monitor; 2, count button; 3, digital display; 4, sensitivity adjustment dial; 5, plate alignment stops and culture plate in counting position.*

plastic disposable petri dishes with and without stacking rings were examined. Because the optical system does not view the peripheral 15% of the total plate area, stacking rings did not interfere with the counting process, provided that plates were properly centered. In addition, several types of culture media and various agar volumes were examined and found to have little, if any, detectable effect on counting efficiency. However, it was noted that occasional artifacts in the agar, such as bubbles or cracks, were sometimes detected by the scanner, producing erroneously high counts. Therefore, plates of questionable quality were routinely rejected on the basis of either visual examination of the plate or the appearance of obvious artifacts on the video display.

Because subsurface colonies which result from the use of the pour plate technique would be expected to be smaller than their surface counterparts, the exact capabilities of the optical system in this regard were of paramount importance to us. To determine the minimal detectable colony size, pour plates were prepared from two dilutions of an overnight broth culture of *S. epidermidis* to yield one set of plates with a density of approximately 50 colonies and another set with approximately 25 colonies. Ten randomly selected plates from each dilution set were counted both manually and automatically after either 18, 24, 48, 72, or 96 h of incubation. In addition, the minimal diameters for each of three representative subsurface colonies from each plate were measured with an ocular micrometer for each dilution at all incubation intervals.

The results of this study showed that automated colony count and subsurface colony size were correlative (Table 1). A plot of the ratios of automated to manual counts for each plate against the mean subsurface colony diameter for the corresponding plate showed that colonies less than 0.3 mm were not readily detected by the automated instrument (Fig. 2). For colony diameters of 0.3 mm, however, the detection capability of the automated counter increased abruptly and, thereafter, remained unchanged for colony sizes greater than 0.3 mm. Similar results were obtained in a related study with *E. coli*.

The counting precision (reproducibility) of the automated counter was found to be proportional to the number of colonies for pour plates of bacterial mixtures obtained from skin extracts. Repetitive automated counts (10 to 20) on single plates in fixed position varied by approximately 3, 2, and 1% for colony densities of 30 to 100, 200 to 300, and 600 to 1,000, respectively. When the same plates were repositioned in the instrument prior to each repetitive count, the automated counts varied by approximately 12, 4, and 3% for the above-cited colony density ranges. The adverse influence of plate orientation on instrument precision, particularly at lower colony densities, probably reflects the interplay of several inherent factors, such as variable colony distribution at the peripheral edge of the optically scanned area, and the linear (directional) nature of the optical scan itself. The increased precision observed at

TABLE 1. *Effect of incubation time on colony counts of Staphylococcus epidermidis*

Incubation time (h)	Dilution set	Mean colony count[a]		Mean colony diam[b] (mm)
		Manual	Automated	
18	1	35	3	0.178
	2	17	1	0.172
24	1	51	6	0.241
	2	17	1	0.243
48	1	52	30	0.308
	2	22	14	0.308
72	1	45	32	0.322
	2	22	17	0.381
96	1	50	40	0.422
	2	21	17	0.422

[a] Mean of 10 replicate plates for each time period and each dilution.

[b] Overall mean of 3 subsurface colonies from each of 10 plates for each dilution set.

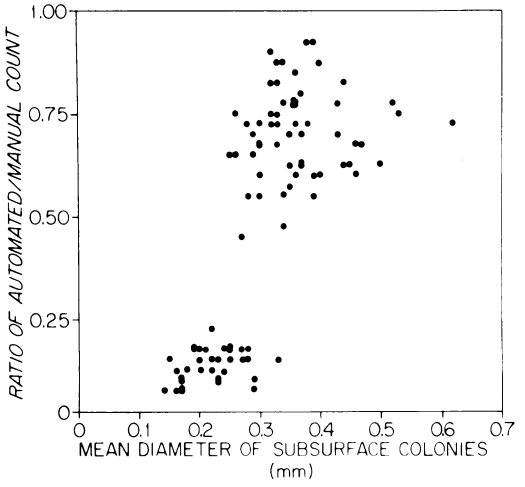

FIG. 2. *Relationship of counting efficiency to subsurface colony size.*

higher colony densities for both fixed and repositioned plates is of little practical advantage. It is shown below that increased precision for higher automated counts is gained at the expense of accuracy.

To this point, we had satisfied ourselves that the automated colony counter was an adequately precise instrument and could readily detect colonies as small as 0.3 mm. Our major concern, however, was instrument accuracy relative to manual count determinations. To examine this parameter, pour plates prepared from skin extract samples and containing mixed bacterial populations were counted manually and automatically. A graphic presentation of the relationship of the two counting systems clearly demonstrates a nonuniform bias in the automated estimate of counts (Fig. 3). It will be noted that the discrepancy became more apparent as the manual count increased much above 100 colonies. Secondly, the spread in automated counts also became greater with higher manual colony counts. Several inherent factors, such as increasing superposition and proximity of colonies, undoubtedly contributed to the increasing bias and variation observed in automated counts for plates with higher colony densities.

In contrast, when the plate count data were transformed to logarithms, a linear relationship was clearly evident between the automated and manual counts on a logarithmic scale (Fig. 4). Most importantly, the bias was uniform over the counting range examined. This bias was not unexpected because, as previously noted, the optical system only scans approximately 85% of the total plate area.

In a number of independent studies (Table 2), the automated count was found to be consistently predictive of the manual count on a logarithmic scale. In each study, counting efficiency on a log basis ranged from 89 to 95% of theoretical, even when an extremely broad range of counts was examined. It should be noted that manual counts for plates containing

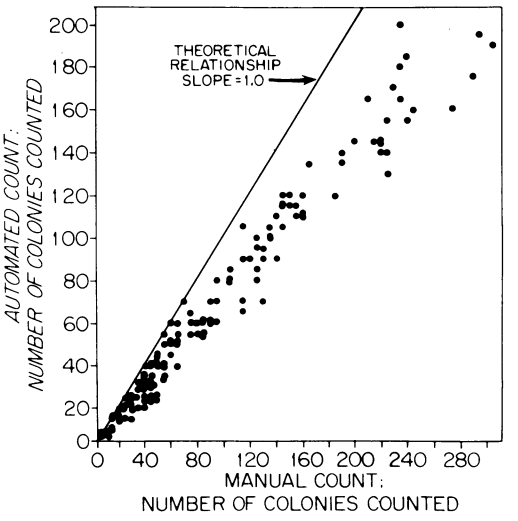

FIG. 3. *Relationship of automated count to manual count.*

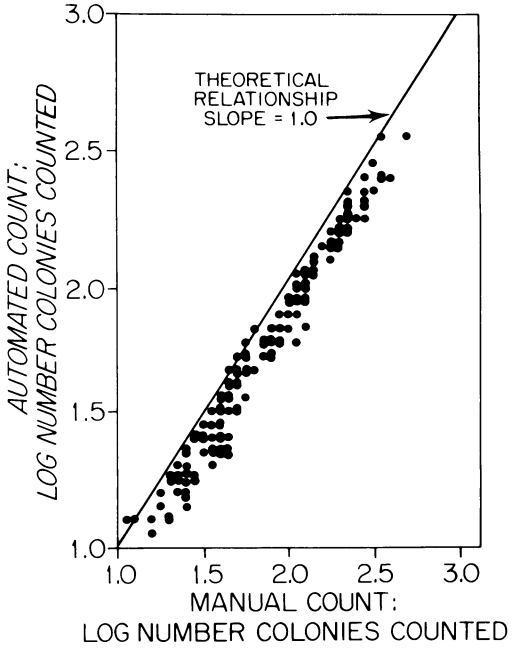

FIG. 4. *Relationship of log automated count to log manual count.*

TABLE 2. *Summary of evaluation studies with automated colony counter*

Expt no.	Specimen	No. of plates counted	Range of manual counts	Technician	% Counting efficiency ± SE[a]
1	Skin microflora	165	4-514	1	95 ± 1
2	Skin microflora	67	9-1000	1	89 ± 2
3	Skin microflora	75	7-1000	1	92 ± 2
				2	92 ± 2
4	*Staphylococcus aureus*	30	15-800	1	94 ± 2
				2	95 ± 2
5	*Escherichia coli*	21	50-800	1	89 ± 2
				2	94 ± 2

[a] Based on least squares estimate of slope for log automated counts relative to log manual counts. Maximum theoretical slope is 1.0 (100% efficiency). SE, standard error.

300 to 1,000 colonies were estimates based on counts of representative subareas of such plates. Nevertheless, on a logarithmic basis, the automated counting efficiency for these high-density plates was similar to that observed for plates containing an actual manual count of fewer than 300 colonies. Because comparatively fewer plates were examined in the range above 300 colonies, and since those examined represent estimated rather than actual manual counts, we have elected in routine application to select automated count data up to approximately 400 colonies as a more practical range for operation. The latter range is readily attainable with standard decimal dilution of samples.

In summary, with respect to sensitivity, precision, and accuracy, our experiments suggest that the automated colony counter is an extremely useful development in microbiological instrumentation. We currently have the counter interfaced to a data acquisition system which provides computer-compatible output. Interfacing was easily accomplished with commercially available hardware. The automated counting system has increased the overall efficiency in several of our research operations by significantly reducing both the time devoted to plate counting and the number of data manipulation tasks previously needed to render results in a form amenable to analysis and interpretation.

Although the automated counter system has potential application in a number of microbiological areas, it is recommended that its suitability for particular circumstances be evaluated with respect to types of samples and culture systems to be employed.

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