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Evaluation of an Automated Agar Plate Streaker

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An automated agar plate streaker was evaluated. The Autostreaker mechanizes the agar plate streaking process by providing storage for plates, labeling and streaking one or more plates for either isolation or quantitation, and stacking in one of several racks for subsequent incubation. Results showed the Autostreaker to produce agar plates with well-separated colonies and accurate colony counts. A total of 1,930 clinical specimens were processed either in parallel with manual methods or solely by the Autostreaker. Technologist acceptance of machine-streaked plates was outstanding.

Solidified growth medium in round dishes has been used for over a century to isolate microorganisms. Only in the last decade have attempts been made to automate the laborious task of inoculating a specimen for microbial analysis on one or more agar plates.

Methods for automated agar plate inoculation include that in which inoculum was spread only after it was manually placed on the agar surface (3) as well as that of Campbell (1) who added inoculum in the form of an Archimedes spiral to a rotating plate. Wilkins et al. (4) described a device for the automatic surface inoculation of rectangular agar trays.

This report describes the in-use testing of a machine that inoculates one or more agar plates for quantitation or isolation and labels and stacks them in one of three racks for incubation.

MATERIALS AND METHODS

Autostreaker description. The Autostreaker (TomTec, Orange, Conn.) (Fig. 1) is designed to automate the entire primary agar plating process. The only manual input is the conversion of the specimen to a liquid phase by the technologist. The specimen is streaked on the required number of plates according to a selected preset program, either for isolation (IM, isolation mode) or for quantitation (CM, count mode). The plates are labeled with an accession number, sorted, and stacked for transfer to the appropriate incubator.

The Autostreaker selects agar plates from a large, self-contained magazine capable of holding over 800 plates. The bottom portion of the magazine is refrigerated to allow storing a several-day supply. The top portion, from which the plates are fed, is maintained at room temperature so that specimens are not streaked on cold plates.

A pin plug board is used to program the Autostreaker. For any 1 of 10 different kinds of agar plates, the machine determines (i) whether the plate is to be streaked for isolation or for count, (ii) the volume of inoculum to be deposited per plate (four preset variable amounts), and (iii) one of three incubator stacks.

The streaking method (patent pending) is unique to the Autostreaker. The agar plate spins at 300 rpm. The specimen is drawn into a soft piece of plastic tubing and expelled onto the agar plate surface. The inoculating tubing is oscillated at 200 strokes per min as it comes in contact with the spinning agar, off-center of the plate. The spinning plate moves under the oscillating tubing so that the specimen being streaked is drawn to the outer edge of the agar plate. After the specimen is processed, the tubing is automatically cut off and discarded. A new length of sterile tubing is expelled for the next specimen.

The controlled and adjustable variables are as follows: (i) the mode of deposit of inoculum, in the center only (IM) or uniformly across the whole plate surface (CM); (ii) the volume of inoculum deposited; and (iii) the time allowed for streaking each section of the agar plate from the middle to the outer edge.

In the IM, the entire inoculum volume is deposited in the center section. Organisms are transferred from the center section to the second and third sections by the overlapping action of the oscillating tube. A heavy concentration of bacterial colonies will occur in the center section, with isolated colonies in the outside ring. For the evaluation of the IM, the center of the agar plate was streaked for 8 s, the middle section was streaked for 4 s, and the outer section was streaked for 2 s.

In the CM, the inoculum is dispensed from the tubing at a uniform rate over the entire plate surface. For the evaluation, 10 μl of inoculum was deposited uniformly over the agar surface.

Study protocol. The Autostreaker was used in three hospital laboratories, each for a different phase of the evaluation. Laboratories included Waterbury Hospital, Waterbury, Conn. (calibration of the instrument); St. Francis Hospital and Medical Center (clinical specimens and time study); and the John Dempsey Hospital of the University of Connecticut Health Center (laboratory evaluation and clinical specimens). The manual agar plate streaking used routinely in each of the three hospitals was considered as the reference method. In general, the streaking procedure followed that described in the *Manual of Clinical Microbiology*

(2). Because of the unique streaking pattern of the Autostreaker, a true blind study was not possible. However, agar plates were evaluated independently as a function of the inoculation method and by two or more microbiologists.

Colony counts. Colony counts were performed on 1,500 prepared liquid specimens over a 4-month period to obtain a comparison of colony counts on the Autostreaker and by a manual method.

Control organisms with obvious differences in colony morphology (*Serratia marcescens* [pigmented], *Escherichia coli*, hemolytic *Staphylococcus aureus*, and *Staphylococcus epidermidis*) were prepared in suspensions of 1.5×10^7 to 3×10^7 colony-forming units (CFU) per ml. The inocula were individually standardized by using the nephelometer of the Autobac (Pfizer Diagnostics, Groton, Conn.). From these individual suspensions, a stock inoculum was made consisting of 1 to 2 parts *S. marcescens*, 2 to 8 parts *E. coli*, 20 parts *S. aureus*, and 20 parts *S. epidermidis*.

The stock inoculum was diluted to 1×10^4, 1×10^3, and 1×10^2 CFU/ml and added to the Autostreaker cups. Amounts of 1, 5, and 10 μl were streaked in both CM and IM. Each test included control plates streaked manually with a 10-μl calibrated loop.

For a given volume of inoculum dispensed and a given method of streaking (IM, CM, and manual), all colony counts performed in either IM or CM were averaged. The mean, standard deviation, and coefficient of variation (CV) were determined. The CV was employed for purposes of comparing the accuracy of the Autostreaker versus the manual semiquantitative loop method.

Clinical specimens: (i) urine. A small quantity (1.5 to 2 ml) or urine was poured into the sample cup. The machine was programmed to streak a blood agar plate (BAP, 7.5% sheep blood) with 5 μl of urine for quantitation (CM) and a MacConkey agar plate for isolation (IM). A 10-μl platinum loop was used to streak agar plates manually.

All urine specimens were refrigerated from the work of the previous days to test the deterioration of urine samples sitting in the machine awaiting processing. Those specimens with either significant growth of bacteria ($>1 \times 10^4$ CFU/ml) or a mixture of skin contaminants were reprocessed after a delay on the machine carousel of 0 to 4 h.

After parallel testing, the Autostreaker was used to streak 750 clinical urine specimens in place of the manual method. To provide Autostreaker colony counts comparable to the manual method, 10 μl of urine was dispensed per agar plate.

(ii) Throat cultures. A program was established with Pediatrics Associates of New Haven, Conn. to provide duplicate swabs from 144 patients suspected of streptococcal pharyngitis. One swab was inoculated manually, the other was inoculated by machine. The swab was twirled briefly in a sample cup containing 1.5 ml of modified Stuart transport medium. A 15-μl amount of sample was streaked on BAP and a chocolate agar plate. After duplicate testing, an additional 127 specimens were processed solely by the Autostreaker.

(iii) Sputum cultures. A total of 163 clinical sputum specimens were processed, of which 152 were performed in parallel with the conventional method. Sputum was removed from its container with a swab and added to 1.0 ml of sputolysin (Calbiochem, La Jolla, Calif.) in a capped, sterile test tube (13 by 100 mm). The contents were mixed vigorously and transferred to the Autostreaker sample cup. BAP, chocolate agar plate, MacConkey agar, and mannitol salt agar were inoculated.

(iv) Genital specimens. A total of 30 genital specimens were processed, 16 in parallel with the conventional method. All specimens were received on swabs, and transferred to modified Stuart medium. A 15-μl portion of fluid was inoculated on BAP, chocolate agar plate, and Thayer-Martin agar.

(v) Other swab-type specimens (wounds, ear, eye, etc.). The same specimen preparation procedure was used for these swab specimens as for throat cultures.

(vi) Fecal cultures. A fecal sample was removed from its container with a swab and added to the sample cup containing modified Stuart medium. A total of 52 specimens were processed, 8 in parallel with the conventional method on BAP, MacConkey agar, Hektoen agar, and mannitol salt agar. The contents of the sample cup were poured into Selenite F broth after processing.

Time factors. Time studies were performed at a 750-bed community hospital to determine potential labor savings. The primary plating process was defined as that part of handling the incoming specimen that the Autostreaker mechanizes, i.e., selecting and sorting agar plates from the refrigerated storage, labeling the plate, inoculating, streaking, sorting, and stacking for transfer to an incubator. That part of the specimen-handling process, such as accession logging, slide preparation, and inoculating tubed media, was considered to be common to both manual and automated methods.

Technologists were timed performing various phases of the process. The streaking exercise depended on several factors such as size of loop used and how many times it was flamed and cooled. Separate tests were conducted on loop cooling times. Various loops were heated to redness, and the time required to cool to touch was recorded.

Contamination potential. The Autostreaker has a plastic hood that covers the working area. When there are no samples in the instrument, two ultraviolet lamps are automatically turned on. They provide a calculated radiation level at 253.7 of 200 $\mu W/s$ per cm^2 on the streaking area. To test the effectiveness of the ultraviolet system, 15 BAPs were inoculated with 10 μl of *S. aureus* and *E. coli* (1×10^7 CFU/ml) and exposed to the ultraviolet lamp for 1, 2, 5, 10, and 15 min.

All testing was conducted with agar plates (Baltimore Biological Laboratory) supplied by BioQuest, Cockeysville, Md.

RESULTS

Figure 1 shows the Autostreaker. Figure 2 shows those components of the Autostreaker in sequence that hold the specimens, label and streak the agar plates, and sort for incubation.

Table 1 summarizes the data on 1,500 laboratory-prepared specimens containing four different microorganisms. Based on mean colony count of all specimens streaked, the Autostreaker was less accurate when dispensing 1 μl (CV = 50%) than when dispensing 5 or 10 μl (CV = 21 to 22%, IM) of inoculum. The IM was slightly more precise than the CM (CV = 21 to 22%, IM versus 23 to 26%, CM). This difference is not statistically significant. The CV of the calibrated loop delivery was 37%. On all machine-streaked plates, the four microorganisms were present in approximately equal numbers and well-separated so that distinctive colonial morphology was easily recognized.

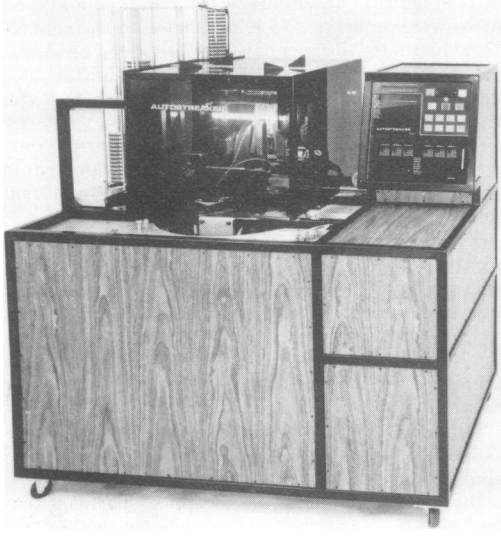

FIG. 1. *The Autostreaker, an automated agar plate processing machine.*

Table 2 presents the variety of clinical specimens processed in this study. Of the 818 specimens, 42% were tested in parallel with the conventional method.

Of the 319 urine specimens processed in parallel, 68 (21%) had a colony count >100,000 CFU/ml; 102 (32%) failed to grow on either MacConkey agar or BAP; 149 (47%) had colony counts of either questionable significance (1×10^4 to 1×10^5 CFU/ml) or representative of contamination ($<10^4$ CFU per ml). Of this group of 149 specimens, 87 showed microbial growth only on the BAP. If those agar plates with less than five colonies were ignored because of statistical inaccuracy, then there was complete agreement as to biological types of microorganisms recovered by both methods. There was complete quantitive agreement with those specimens considered positive and those of questionable significance. In general, the machine-streaked plates showed more uniform colony

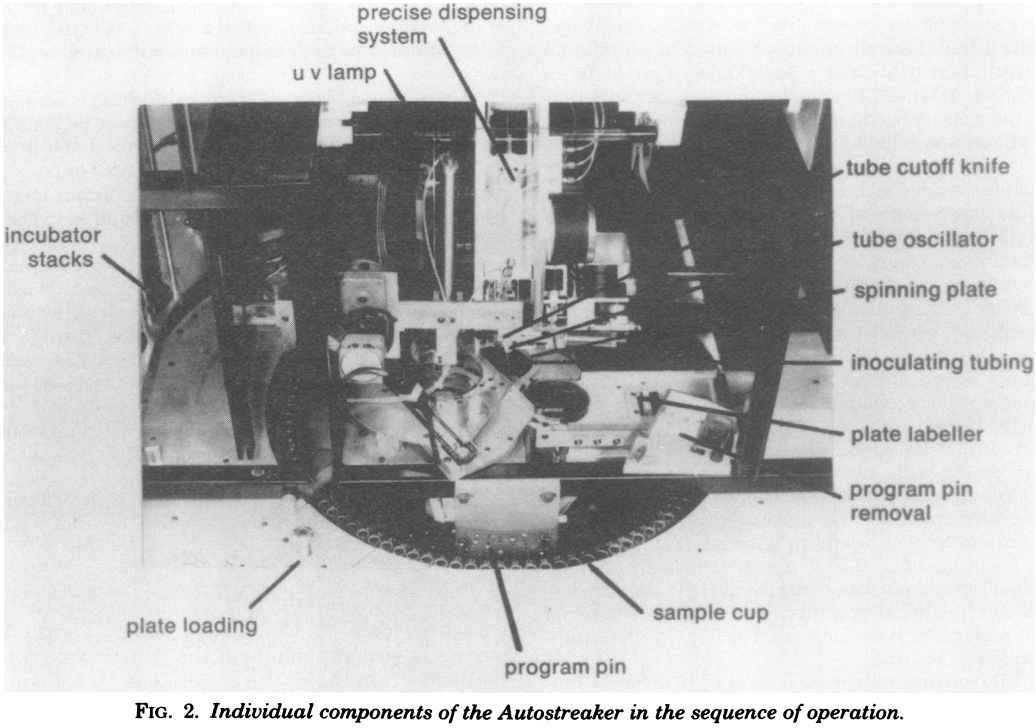

FIG. 2. *Individual components of the Autostreaker in the sequence of operation.*

distribution with consistently better isolation of colonies.

The Autostreaker plates were more easily evaluated on specimens containing $\geq 10^5$ CFU/ml, even in the heavy areas of initial inoculation. The colonies were better separated. These plates enabled the microbiologist to make a more accurate judgement as to the type of organism present and the relationship of various microbial types to the total plate count.

After duplicate testing, the Autostreaker was used to process 750 clinical urine specimens on a routine basis in place of the manual method. Technologist acceptance of the Autostreaker plates was excellent. Their comments and subjective judgements on plate quality confirmed those heard and observed in the duplicate testing phase of the project.

Of the routine urine specimens inoculated by the Autostreaker, 9.2% had colony counts greater than 1×10^5 CFU/ml, 42% were between 1×10^4 and 1×10^5 CFU/ml, and 35.7% were either sterile or had $<10^4$ CFU/ml.

Results of the specimen deterioration study revealed no change in colony count in the 2-h waiting period. However, at the end of 4 h, significant overgrowth of the specimen was observed (from 20 to between 150 and 200 additional colonies per plate in 4 h.

TABLE 1. *Accuracy of the Autostreaker compared to a conventional semiquantitative streaking method on 1,500 specimens*

μl Dispensed	Mode	Mean CFU[a]	CV (%)
1	IM	24	50
	CM	22	64
5	IM	54	21
	CM	47	23
10	IM	31	22
	CM	27	26
10-μl Calibrated loop (manual)		62	37

[a] Mean CFU per volume dispensed.

A total of 144 throat cultures were processed in duplicate. Of the specimens, 39% had normal throat flora and 59% contained hemolytic colonies of which 27% were beta-hemolytic streptococci resembling group A by a bacitracin susceptibility test.

In general the Autostreaker plates were more easily evaluated. The heavily inoculated area in the center of the plate was diffused more than the solid mass of growth seen when the swab was applied to the plate manually. This facilitated observing the various organisms present and their relationship to the total microbial mass. Isolation of colonies around the perimeter of the plate was excellent.

A total of 127 clinical throat specimens were then processed routinely by the Autostreaker. As with the urine specimens, technologist acceptance of the plates was excellent. They once again confirmed the conclusion that the plates provided more information on the total ecology of the specimen. Of the throat cultures streaked by machine, 10.6% were positive for beta-hemolytic streptococcus (group A).

Of the 152 sputum specimens processed, there was complete microbiological agreement between the types of colonies recovered by both methods. It was often a problem on the manually streaked plates to spread out the primary area of inoculation due to the adhesive nature of the specimen. A typical manually streaked plate showed a heavy mass of colonies in the initial quadrant, but relatively few isolated colonies in the other quadrants. This problem was accentuated when comparing them to the Autostreaker plates, which showed excellent separation of colonies even in the heavily inoculated center area.

Results of other swab-type specimens, such as wound exudate, genital, eye, nasopharyngeal, and feces, revealed good correlation between manual and Autostreaker plates as to both the types of organisms present as well as their relative numbers. Consistently, the Autostreaker

TABLE 2. *Summary of clinical specimens processed by conventional methods and by the Autostreaker*

Specimen type	In parallel	Autostreaker	Total	% of total
Urine	319	750	1069	55
Throat	144	127	271	14
Group A *Streptococcus* screen		25	25	1
Tracheal aspirates	15	17	32	2
Genital	16	30	46	2
Wounds	142	27	169	9
Feces	8	44	52	3
Staphylococcus screen	8	69	77	4
Ear	4	4	8	0.5
Eye	10	8	18	1
Sputum	152	11	163	8

plates were superior to the conventionally streaked ones.

Figure 3 shows representative agar plates streaked in both IM and CM. The separation of colonies in the IM is apparent as is the uniform spreading of the organisms over the plate in the CM.

A number of diluents were tested but the survival rate of microorganisms was higher in agar-free modified Stuart transport medium. Experiments revealed that group A beta-hemolytic streptococci at an initial inoculum concentration of either 1×10^3 or 1×10^4 CFU/ml suffered only a slight diminution of colony count over a 2-h period. Similar tests using *Neisseria gonorrhoeae* plated on chocolate agar indicated a two-

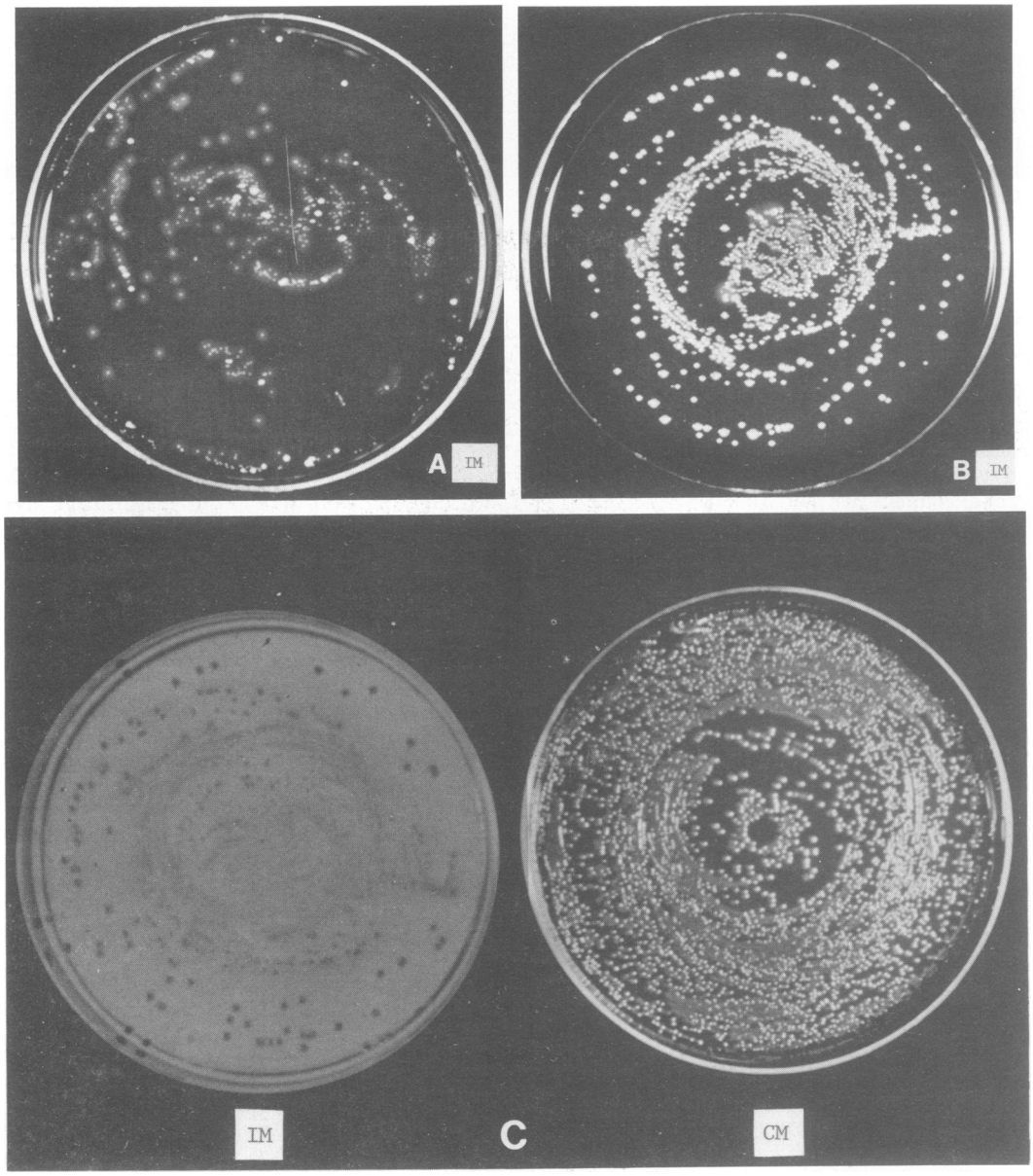

FIG. 3. *(A) BAP streaked for isolation (IM). Beta-hemolytic streptococci were predominant in this throat culture. (B) BAP streaked for isolation (IM). S. aureus was predominant in this wound culture. (C) Urine specimen from which two agar plates were streaked: MacConkey agar for isolation (IM) and blood agar for quantitation (CM). The specimen contained E. coli >100,000 CFU/ml.*

fold reduction in numbers in 1 h and an additional twofold reduction in 2 h.

Results of the time study indicated that the mean time for administrative specimen handling exclusive of streaking the plate was 45 s (range, 30 to 110 s depending on specimen type). The streaking time depended on several factors, such as loop size and the number of times it was flamed and cooled. A 25-gauge nichrome loop required 15 s to cool to touch, and a 20-gauge, 10-μl platinum loop required 30 s to cool to touch. With the nichrome loop, the average manual streaking time per plate was 30 s.

The comparable sample handling time using the Autostreaker was 21 s for liquid samples and 32 s for specimens collected on a swab, regardless of the numbers of plates streaked.

Results of the test to determine the effectiveness of the ultraviolet light in the streaking portion of the machine suggested that the potential for contamination was minimal. Agar plates containing a heavy inoculum of either *E. coli* or *S. aureus* exposed for varying times under the ultraviolet source showed no growth after a 1- to 2-min exposure.

DISCUSSION

The Autostreaker can automate the entire agar plate streaking process. It is a labor-sparing machine that provides results at least equivalent to manual methods. By self-programming, a laboratory can "fine tune" the Autostreaker to process each specimen as the microbiologist desires. In addition to providing a refrigerated storage area for plates, the type of solid media can be chosen for each specimen and the appearance of the colonies on the plate can be controlled by adjusting the amount of inoculum dispensed, the time required to streak the plate, and the pattern of streaking. Such flexibility allows a specimen to be processed for quantitation as well as for analysis of mixed colony types.

Certain specimens received in the clinical microbiology laboratory must be processed for colony count as well as for isolation. Traditionally, quantitative urine colony counts have been done either by the agar pour plate method or by uniformly streaking the surface area of the plate with a pipette or platinum loop calibrated to deliver a certain volume of fluid. Many studies have confirmed the reliability of both methods. The Autostreaker streaks a plate quantitatively by precisely delivering a known amount of fluid on the plate surface and then uniformly spreading it. Test results indicated that colony counts on prestandardized inocula were more accurate by machine than by hand if the volume dispensed by machine was 5 μl or greater. Dispensing volumes of 1 μl resulted in unacceptable variation. A reason for this may be that the delivery mechanism is a positive displacement system within the tubing. However, due to the small volume being dispensed, surface tension at the tube outlet has an effect on the actual delivery volume. If nothing were to touch the end of the tubing, the expelled liquid would form a drop that would accumulate until the drop weight just exceeded the surface tension on the tubing orifice. However, on the Autostreaker the tubing is not hanging free, but in contact with the agar surface. As soon as sufficient liquid is expelled to touch the agar surface, the balance is "wicked away." Thus, the actual amount of liquid transferred to the agar surface will vary slightly from the theoretical displacement volume. This effect is difficult to detect in the IM where volumes in excess of 1 μl are being dispensed at one time in the center section of the plate. However, in the CM, there is intermittent transfer of liquid to the agar surface. This effect is minimized by the longer times of streaking used in CM, because it allows the tubing to spread potential colony clumps over a larger area.

The rendering of specimens to a liquid phase before loading in the Autostreaker is a departure from usual practice. Although the ability of the machine to process plates rapidly with better colonial distribution than by hand was shown in the early phases of the study, the biological similarity of the liquified specimens versus specimens directly inoculated required study.

Initially, phosphate-buffered saline was used as a "transport" medium. However, rapid loss of group A beta-hemolytic streptococci and *N. gonorrhoeae* was observed. Although death of these two organisms was also seen in the modified Stuart medium, it was markedly diminished in the time period (0 to 2 h) that the specimens might await processing. In those specimens in which it was suspected that dilution of the swab contents in the "transport" medium might reduce the number of bacteria below the levels of detection, the inoculum volume was increased to compensate for dilution.

At no time during the 6-month period of machine use was contamination observed on agar plates that could be attributed to the machine. In addition to streaked plates that were sterile after incubation, hundreds of Trypticase soy agar plates were dry processed (no inoculum) to test a variety of machine parameters. In no case were these plates cross-contaminated.

Although this study was primarily concerned with the microbiological capabilities of the Autostreaker, knowledge of the potential labor-saving benefits is essential. The time study revealed that the administrative handling of the specimen

consumed an average of 45 s. The time required to streak a specimen manually was a function of the number of agar plates used. At a 30-s mean streaking time per plate, per-specimen times ranged from 36 s for urine to 121 s for feces.

Exclusive of the 45-s administrative time, technologists consumed from 21 to 32 s to load a specimen on the Autostreaker. The total machine time per specimen is of concern only when it becomes the limiting factor in "through-put." At the present time, the machine processes approximately 85 plates per h (680 plates per 8-h shift). On subsequent models, the cycle times can be compressed so that the mean time to streak a plate is 30 s or less (960 plates per 8-h shift).

In actual laboratory conditions the Autostreaker resulted in an average time savings of 53% (range, 42% for urine cultures to 73% for fecal cultures).

A potential disadvantage of the Autostreaker is its lack of provision for (i) inoculation of tubed media and (ii) rapid processing of anaerobic specimens. Laboratory experience dictated that critical specimens such as cerebrospinal fluid or oxygen-protected specimens for anaerobic culture be processed manually.

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