Automated Slide Staining Machine

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A machine is described which can perform the Gram stain. Comparison of slides stained by machine versus hand revealed no difference in reproducibility or accuracy. In addition to providing clean, dry, uniformly stained slides, the machine saves 24 sec per slide when compared with a hand staining technique.

Microbiology is the least automated of the disciplines in the clinical laboratory. Several procedures in microbiology lend themselves to mechanization but little has been achieved. A Gram staining apparatus, the Shandon-Eliott slide staining machine, has been developed (1) but has not been widely distributed. We describe here a machine which simply and reliably automates the Gram staining procedure.

MATERIALS AND METHODS

Slide staining machine. The slide staining machine is a patented (A. N. Pedersen, U.S. Patent 3,507,292, 1970) device for transporting slides through a number of troughs containing staining solutions. After heat fixation of the specimen, the slide is inserted in a wire carrier which is then connected to a conveyor chain. During transport, slides are carried at a slight angle. When contact is made with the “downstream” wall of a trough, the angle of carriage permits the slide to be lifted above the trough wall and to pass to the succeeding jar. The angle of carriage during passage from one trough to another also permits the staining solutions to drain from the slide. A counterweight on the slide holder minimizes the force required to raise the slide.

Figure 1 is a drawing of part of the slide staining machine. It shows three of the staining troughs and two slides, in their carriers. Slide 1 has been forced to rise up upon contact with the wall of a trough. Slide 2 is passing through the staining solution in a trough.

Troughs containing staining solutions are followed by one or more troughs of running water for rinsing the slides between stains. This continuous water flow is controlled by a solenoid valve that automatically is activated by starting the machine. The water enters the lower section of the trough via tubing and exits through a notch in the upper side wall.

At the completion of staining, the slide carriers are moved off the conveyor onto a rail. There they accumulate in front of an air blower which dries them. Accordingly, the operator of the apparatus need only be concerned with the input to the apparatus; the output end can be ignored until a large number of slides accumulate.

Figure 2 is a picture of the staining machine in actual operation.

Stains. To perform the Gram stain with this machine, the solutions are arranged in troughs as follows: (i) crystal violet, (ii) running water, (iii) running water, (iv) iodine, (v) running water, (vi) acetone alcohol, (vii) acetone alcohol, (viii) acetone alcohol, (ix) running water, (x) safranin, and (xi) running water. Optimal Gram staining is achieved by immersing the slides in each trough for 15 sec. Since staining time with this machine is determined by the width of the trough, each trough is of identical width. Total staining time is 2 min and 45 sec.

The compositions of these solutions are as follows. 1. Crystal violet contains 2 g of crystal violet, 20 ml of 95% ethanol, and 80 ml of 1% ammonium oxalate. 2. Lugol's iodine (1% aqueous iodine) contains 1 g of iodide, 2 g of potassium iodide, and 100 ml of deionized water. 3. Acetone alcohol contains 40% acetone and 60% alcohol. 4. Safranin contains 0.5 g of safranin and 100 ml of deionized water. The stain solutions are changed daily, and their troughs are cleaned thoroughly with acetone alcohol.

Reproducibility studies. Four slides from each of 50 specimens (sputa, urine, etc.) were stained: two by machine and two by hand. They were then examined microscopically with an oil immersion lens. Slides were graded on a scale of 16 for perfect correlation. They were read as 0 to 4+ in quantity for the four categories of bacteria (gram-positive cocci and rods; gram-negative cocci and rods). Gradings were based on the number of bacteria seen per oil immersion field: 1+, <1 bacterium seen; 2+, 1 to 5 bacteria seen; 3+, 5 to 30 bacteria seen; 4+, >30 bacteria seen. One point was deducted for each lack of correlation. Results represent the mean scores for 50 specimens. All slides were coded and read in a “blind” manner by four different observers.

False results. The slides read in the study of reproducibility were also evaluated for false results. Slides stained by manual or machine techniques were evaluated separately and within the two groups for absolute discrepancies, i.e., the frequency with which one slide of a pair showed no organisms, whereas the companion slide was positive for bac-
Detection of contamination of staining solutions. All stain solutions as well as the troughs of running water were cultured for bacteria on three separate occasions with a pour plate technique with Trypticase soy yeast (TSY) agar. The stain solutions were also deliberately contaminated with $10^8$ cells of *Pseudomonas aeruginosa*, *Enterobacter* species, *Streptococcus fecalis*, and *Candida albicans* and were subsequently cultured to study their capacity to support microbial growth.

Expenditure of time in gram staining. During a portion of two separate days, the total time spent on Gram staining slides by hand was determined. On two other days, a similar calculation was made for the time spent in attaching the slides to and removing them from the slide carrier and for engaging the carrier on the conveyor.

RESULTS

Table 1 indicates the reproducibility of the hand and machine techniques for Gram staining and compares the techniques with each other. The readings of the two slides stained by the machine are as comparable as those for the two slides stained by hand. Discrepancies were similar when slides stained by hand were individually compared with slides stained by the machine.

Table 2 is an attempt to study the potential for false smears resulting from acquisition or loss of bacteria on a slide while it is being moved through the Gram staining machine. There were 21 readings in which all four slides were negative for bacteria. Twelve of those specimens were positive on culture, usually with light growth. On two occasions, both of the slides stained by the hand technique were positive, whereas both of the machine-stained slides were negative. The converse situation was not encountered. There were four readings when only one slide, of four, was positive; two
VOL. 23, 1972

SLIDE STAINING MACHINE

Table 1. Reproducibility

| Determination | 1*                  | 2                  | 3                  | 4                  |
|--------------|---------------------|-------------------|-------------------|-------------------|
| Machine      | 14.48 ± 2.06 (362)* | 14.08 ± 1.69 (352)| 15.13 ± 1.22 (378)| 14.68 ± 1.31 (367)|
| Hand         | 14.44 ± 1.98 (361)  | 13.66 ± 2.06 (342)| 14.54 ± 1.50 (364)| 14.88 ± 1.67 (372)|
| Machine 1* versus Hand | 14.64 ± 2.08 (366) | 13.46 ± 2.26 (337)| 14.33 ± 1.55 (359)| 14.84 ± 1.68 (371)|
| Hand 1       |                     |                   |                   |                   |
| Machine 2* versus Hand 2 | 14.48 ± 2.06 (362) | 13.79 ± 2.34 (345)| 14.25 ± 1.80 (357)| 14.76 ± 1.94 (369)|

* Four columns represent the results of four readers.
* Four slides from each of 50 specimens (sputa, urine, etc.) were stained: two by machine and two by hand. Slides were graded on a scale of 16 for perfect correlation. They were read as 0 to 4+ in quantity for the four categories of bacteria (gram-positive cocci and rods, gram-negative cocci and rods). One point was deducted for each lack of correlation. Results represent the mean scores for 50 specimens. All slides were coded and read in a “double-blind” manner. Data presented include mean, standard deviation, and, in parentheses, the total score recorded out of a possible 400 for perfect correlation.
* For each of the 50 specimens, one slide stained by hand was compared with one slide stained by machine.

Table 2. False readings

| No. of readings | Hand 1 | Hand 2 | Machine 1 | Machine 2 | Culture results | Type of culture |
|-----------------|--------|--------|-----------|-----------|----------------|----------------|
| 21              | 0*     | 0      | 0         | 0         | 12/21 +        | Sputum         |
| 2               | +      | +      | 0         | 0         | 2+ Oral flora  | Sputum         |
| 2               | 0      | 0      | +         | +         | 2+ Staphylococci| Sputum         |
| 2               | 0      | 0      | 0         | +         | 1+ Klebsiella  | Sputum         |
| 1               | 0      | +      | +         | 0         | 1+ Proteus     | Sputum         |
| 1               | 0      | +      | +         | 0         | 2+ Staphylococci| Wound          |
| 1               | 0      | +      | +         | 0         | 2+ Staphylococci| Wound          |

* Zero (0) indicates no bacteria seen.
* Plus (+) indicates if any bacteria were seen.

of these were hand-stained slides and two were machine-stained slides. Also, there were two instances when the only negative reading of the four matched slides was a machine-stained slide and one when the negative slide was stained by hand. In all instances of discrepancy, cultures were positive. To evaluate further the potential for false “positivity,” cultures of trough fluids were obtained. The specimens were negative, except on one occasion when the safranin solution contained 10* Pseudomonas species per ml. This was traced to contaminated deionized water which was used for the preparation of the stock solution. After correction of this problem, daily culture of the safranin solution has been negative over a period of 3 months. Deliberate contamination of the stain solutions with three different species of bacteria and C. albicans revealed that only the safranin solution was capable of supporting bacterial growth. Furthermore, only gram-negative bacteria were able to survive.

Table 3 reveals the time saved in the clinical microbiology laboratory by the use of this slide staining machine. The period of time studied represents only a part of the entire day’s output, but on the average it takes slightly more than 2.5 times as long to stain by hand as by

Table 3. Technologist time

| Determination | Slides | Time          | Avg/slide (min) | Total daily avg (min) |
|---------------|--------|---------------|-----------------|-----------------------|
| Hand          | 42     | 26 min 47 sec | .64             | 34                    |
|               | 72     | 44 min 31 sec | .62             |                       |
| Machine       | 52     | 13 min       | .25             | 13                    |
|               | 48     | 11 min       | .23             |                       |
machine. Even for the relatively small number of slides stained, an average of 21 min was saved per day.

**DISCUSSION**

The described machine is a simple and inexpensive apparatus for staining slides. The studies reported here indicate that slides stained by machine are equivalent in reproducibility to those stained by hand. The reproducibility within the methods is matched by reproducibility between the two techniques.

There is a theoretical possibility of acquiring bacteria in the staining solutions because of contaminated solutions per se or via transfer of bacteria from previously processed slides. The reproducibility data suggest that this does not occur, at least not more so than in the hand techniques. When the question of "false positivity" was more carefully analyzed (Table 2), it appeared that the problem is not more frequent with machine staining. Cremer (1) also studied this question with the Shandon-Elliot staining machine. No transfer of bacteria from positive unfixed slides to negative ones occurred during either the Gram or auramine stain. Our cultures of the staining solutions were also reassuring in this regard. It is important, however, to note the precaution of using fresh stain solutions daily and for cleaning of the troughs, particularly the one containing safranin, with acetone alcohol. With its demonstrated potential for contamination, daily culture of the safranin solution should be continued.

There are several advantages of such a machine. One can extrapolate from the time saved in our laboratory to other workloads, but in a year we would save at least 128 hr of a technologist’s time. Since each slide is stained in a uniform manner, there is much greater consistency in the appearance of bacteria. In contrast, hand staining of batches of slides leads to unequal exposure to stains and inconsistent staining. To enhance uniformity of staining, especially decolorization, with the machine, thin smears should be made.

Manually stained slides often have residual crystals of stain on both sides which require removal before reading. The machine consistently produces clean, uniformly stained, dry, and ready to read slides.

Also, the availability of such a machine encourages the laboratory and the house staff to perform direct Gram stains on all appropriate specimens for bacterial culture.

Although we have demonstrated the application of this machine to the Gram stain, it should also be suitable for other procedures such as the auramine-rhodamine, Kinyoun, Wright, and hematoxylin-eosin stains.

Negotiations are currently underway with a leading medical equipment manufacturer for the production and distribution of this apparatus.

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**LITERATURE CITED**

1. Cremer, A. W. F. 1968. Automatic slide staining in clinical bacteriology. J. Med. Lab. Technol. 25:387-390.