Automatic Gram staining by a linear conveyor system

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The examination of a well-stained film of either clinical material or organisms grown in laboratory media constitutes an important part of most investigations in hospital microbiology laboratories. The staining of large numbers of microscope slides, usually by Gram's method, is however time-consuming, and a number of reports have been made on methods mechanising this procedure. Cremer (1968) and Burdash *et al.* (1975) have reported such methods based on a procedure whereby microscope slides were collected into batches and processed as such. We report our experience with an automatic linear conveyor system which does not require batching.

The machine

The original version of the staining machine¹ (Fig. 1) consisted of a longitudinal box 59 × 6 × 5 in (149 × 15 × 12 cm) capable of holding 28 jars of staining reagents as it was designed for histopathological staining. This machine was modified

for staining bacteriological smears by Gram's method (based on Preston and Morrell (1962)) by removing the unnecessary staining chambers and blocking the water jets below each of these. The sequence of reagents for staining the preparations used in the mechanical method are shown in Table 1. The reagents are changed daily. The rate of flow of constant running tap water used throughout was that which filled the jar in 65 seconds.

Table 1 *Order of reagents for the mechanical Gram staining procedure*

Jars	Reagents	Jars	Reagents
1 to 4	Industrial methylated spirit 74 OP	9	Liquor iodi fortis (BP) and acetone
5	Crystal violet solution	10	Flowing tap water*
6 & 7	Flowing tap water*	11	Dilute carbol fuchsin
8	Lugol's iodine	12	Flowing tap water*

*See text for rate of flow

In the manual method the biological material is usually 'fixed' on the microscope slide by gently applied heat. In the mechanical method described here, the biological material on the slide is fixed in alcohol as part of the mechanised procedure, and the heating facility on the machine is used only for

¹Obtainable from Smith Kline Instruments Co Ltd, Welwyn Garden City, Herts.

Fig. 1(a) *The original version of the staining machine, (b) The Honeywell HMS-360-1, a later machine designed for microbiological use.*

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Fig. 2 *Microscope slide holders.*

drying the stained preparations. A similar machine (Honeywell HMS 360-1, Fig. 1) designed for microbiological use is now available from the same source. It has 12 chambers and is $18\frac{1}{2}$ in (47 cm) shorter than the original model.

A standard 13 amp power point, water supply,

and drainage facility are required. Comprehensive specifications of the machine are given in the manufacturer's manual.

Procedure for machine staining

Slides are inserted in a counterweighted holder (Fig. 2) which is then pressed into a moving chain. As the chain rotates each slide (carried by the chain) is immersed into successive reagents in a continuous flow system (Fig. 3). The reagent from each jar is in contact with the preparation for 30 seconds. After the slide has been immersed in the last jar, it is carried on into a heated area for drying and then into a storage or parking area, where it may be left until required for examination. Twenty slides can be stacked in the storage area. Because the system is continuous, no batching is required, and preparations for staining may be put on to the system at will.

RATE OF STAINING

The mechanical procedure for fixing, staining, and washing one slide takes 6 minutes. With the thermostat set at 70°C , an additional 4 minutes is required for drying the slide. However, since the system is a continuous flow arrangement slides may pass through the system to come off stained at a rate of more than eight per minute. By comparison, the manual method of staining used in this laboratory takes $3\frac{1}{2}$ minutes per slide and is not suitable for 'batching' other than in small numbers of two or three slides at a time.

Fig. 3 *A continuous flow system.*

TESTS

A series of tests were carried out to assess the machine and compare its performance with the manual staining method. All microscopic examinations were made with a $\times 100$ oil immersion objective lens and $\times 8$ oculars.

Test for contamination of preparations

These tests were carried out to determine the rate of contamination of the microscopical preparations during the mechanical staining process with reagents that had been in use for a working day. Smears of chick amniotic fluid were made on glass slides and stored unfixed at -40°C . At the end of each working day, for four days in each of four weeks, four slides were processed in the machine and examined microscopically for the presence of organisms. Determining the plane of the object in each smear was facilitated by the presence of red blood cells (in the amniotic fluid), which averaged about 15 per field. Fifty fields per slide were examined. Organisms were not seen in any of the 64 preparations. Staining deposit in small amount was, however, seen on five of the slides. The adequacy of the system to detect contamination was itself examined by deliberately contaminating jars of stain with various organisms (*Escherichia coli*, *Staphylococcus albus*, and *Candida albicans*). Tests using amniotic-fluid-coated slides showed that contamination could be detected only after inoculation of the jars with the highest concentration used (1 ml of an overnight broth culture into 100 ml stain). Tenfold dilutions of this inoculation were not detectable as contamination. On the other hand, the amniotic-fluid-coated slides were five to 10 times more sensitive at detecting contamination than uncoated slides.

Tests to compare mechanical and manual methods of staining

Qualitative test Smears from 21 miscellaneous

clinical specimens were made in duplicate. One set of smears was stained on the machine, and the matching set was stained manually. Reference numbers were then obscured, the slides were randomised, and each slide was examined microscopically for 4 minutes for the presence or absence of organisms. The results (Table 2) show agreement between 17 pairs of smears. There are, however, differences between four pairs of smears; organisms were seen in two pairs stained mechanically but were not seen in the matching manually stained smears. On the other hand, organisms were seen in two pairs stained manually and not in the matching smears which were mechanically stained.

Table 2 *A qualitative comparison between mechanical and manually stained preparations*

No. of specimens	GPC		GPB		GNC		GNB	
	Mech	Man	Mech	Man	Mech	Man	Mech	Man
8	+	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	+	+
2	+	+	-	-	-	-	+	+
<i>Differences</i>								
2	+	-	-	-	-	-	-	-
1	-	+	-	-	-	-	-	-
1	-	-	-	+	-	-	-	-
Total 21	17 pairs showing no difference, 4 showing difference							

GPC = Gram-positive cocci; GPB = Gram-positive bacilli; GNC = Gram-negative cocci; GNB = Gram-negative bacilli; + organisms seen; - organisms not seen.

A semiquantitative test Smears from two specimens of sputum were made in duplicate. A preparation from each sputum was stained by the manual method and the matching pair by the mechanical method. A preliminary examination showed that one specimen contained Gram-positive cocci, Gram-negative cocci, and Gram-positive bacilli. The other specimen contained Gram-positive cocci

Table 3 *A semiquantitative comparison between manual and mechanical staining methods*

Sputum	Organism	Method	Number of organisms seen				No. of fields examined
			Nil/field	Small/field	Moderate/field	Large/field	
A	Gram-positive cocci	Manual	0	8	20	82	110
		Mechanical	0	15	31	64	110
	Gram-negative cocci	Manual	38	57	0	0	95
		Mechanical	25	66	4	0	95
B	Gram-positive bacilli	Manual	9	79	17	0	105
		Mechanical	17	58	30	0	105
	Gram-positive cocci	Manual	21	15	45	14	95
		Mechanical	11	9	71	4	95
	Gram-negative cocci	Manual	20	52	18	0	90
		Mechanical	4	60	25	1	90

Figures indicate number of fields

Table 4 Comparisons between heat and alcohol fixation (no. of organisms seen in 10 fields)

Fixation		Preparations												
		E. coli					Staph. albus					N. catarrhalis		
		1	2	3	4	5	6	7	8	9	10	11	12	13
Manual (heat)	Range	31-83	50-150	45-160	60-182	43-232	50-203	107-221	90-308	60-316	82-343	55-291	122-380	125-285
	Mean	50	93	102	111	128	121	180	201	178	201	157	252	218
Mechanical (alcohol)	Range	25-90	40-120	55-149	63-131	76-262	79-261	85-90	202-370	143-310	76-330	73-393	73-292	180-240
	Mean	63	78	91	102	146	159	134	287	233	219	161	171	208

and Gram-negative cocci. Semiquantitative counts for each type of organism separately were then made on each preparation, and approximately 100 fields were counted on each occasion. A substantial difference between the two methods was not found (Table 3).

Tests on preparations from cultures Preparations made from cultures for Gram staining differ from preparations from clinical material because of the absence of natural protein 'fixative' in the former. It was therefore necessary to determine whether the method of fixation in the mechanised method would be adequate for specimens lacking proteinaceous material.

Strains of *E. coli*, *Staph. albus*, and *Neisseria catarrhalis* were picked from agar plates and diluted in phosphate buffered saline, and duplicate preparations on microscope slides were made with a Pasteur pipette. One set of preparations was fixed by heat by passing a slide with the film upwards three times through a bunsen flame until it was just too hot to touch with the back of the hand; preparations were not charred. After heat fixation the slides were placed in slide holders and introduced to the machine at the jar containing crystal violet solution for the staining (but not fixation) part of the mechanised process. The alternative slides were placed on the machine without heat fixation at the beginning of the mechanical process, that is, they were fixed and stained on the machine. After randomisation both sets of slides were examined microscopically, and the results of these tests are shown in Table 4. It can be seen that considerable overlap occurs in the range produced by the two methods; the value of the mean was higher on six occasions by the manual method and on seven by the mechanical method, indicating no overall tendency for one method to give higher counts than the other.

Discussion

The workload in pathology continues to grow

relentlessly, and the microbiology laboratory attracts its due proportion of this increase (Spencely *et al.*, 1979). It is important that the pressure of this increasing workload does not result in curtailment of valuable diagnostic procedures such as microscopic examination of suitable clinical material stained by Gram's method. A mechanised procedure, therefore, which is capable of producing stained films of adequate quality, seems to be a most advantageous asset in the clinical laboratory.

The automatic staining machine described here has been in routine use by 40 members of the staff in our laboratory over a period of 18 months. Between 50 and 60 slides per day have been stained (approximately 25 000 slides). These have been of a very high and reproducible quality, and the machine has met with complete user acceptance.

It is difficult to estimate the saving in labour, but as the machine has been so acceptable it suggests that a significant advantage was being obtained by the user. Precise measurements have not been made on the quantity of staining reagents used, but we have achieved a substantial fall in the consumption of these not inexpensive items over the period under observation, suggesting that the saving might be considerable.

Faults encountered were extremely trivial during the 18 months covered by this report. On one occasion the conveyor chain required adjustment for slackness. On another, the O-ring on one of the nozzles that fits into a reagent jar was damaged; this resulted in the jar rising and obstructing slides on the chain drive. During the 18-month period 30 slide holders were broken and had to be replaced.

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A method of highlighting the macroscopic patterns of congenital cystic kidneys

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The cut surface of a congenital cystic kidney can be graphically displayed by a simple impregnation technique using barium sulphate, a method that has been used for the study of emphysematous lung. The specimen so treated is valuable for pattern recognition and morphometric studies of cystic kidneys.

Morphological studies of pulmonary emphysema have benefited greatly by many refined laboratory innovations during the last 20 years; among these are Gough's (1968) technique of paper-mounted sections and Heard's (1960) techniques of perfusion and barium sulphate impregnation.

The barium sulphate impregnation technique is particularly useful in 'pattern recognition' of the gross appearance of the cut surface of the lung. After repeated sequential treatment of the specimen in barium nitrate and sodium sulphate solutions, the walls of distended air-spaces in emphysematous lung become coated by precipitated barium sulphate and are conspicuously displayed. Simplicity and effectiveness are the two most appealing features of this technique.

Cyst formation is a common morphological feature of the many different varieties of renal dysplasia and polycystic disease of the kidney, and characterisation of the individual types of these anomalies is often possible by gross examination of the cut surfaces of the kidney (Kissane and Smith, 1967). For this purpose we have found that the barium sulphate impregnation technique has many advantages. The spongy nature of many of these kidneys created by the innumerable cystic spaces of different sizes bears some resemblance to emphysematous lungs. By the use of a technique similar to that described by Heard (1960) for the studies of lungs, an equally striking specimen of the cut surface of a cystic kidney also can be produced by barium sulphate impregnation (Figure). The specimen so prepared is particularly valuable for teaching purposes and for morphometric studies.

The sagittal section of the kidney is first fixed in phosphate-buffered neutral 10% formaldehyde, either by total immersion of the specimen or by a

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