Cross contamination of cytological smears, with automated staining machines and bulk manual staining procedures

With a specific study of the problems of the Cytotek¹ and the Shandon Elliott² staining machines

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SUMMARY Further development of an individual staining machine is to be strongly encouraged but meanwhile, using bulk stainers, frequent changing of wash fluids and staining solutions, particularly leading up to and following the haematoxylin pot, is essential to reduce the risk of cross contamination. Certain smears, such as from semen or from serous fluids where malignancy is suspected or known, must be stained on separate racks. In some laboratories it is the rule not to stain semen or serous fluids in bulk staining machines at all and this may have to become the rule everywhere until we are provided with safe individual slide stainers.

The occasional occurrence of malignant cell cross contamination in our bulk staining machines has forced us to investigate the problem and it was decided to compare certain varieties of staining procedures in the form of a comparative trial. The choice of instruments was made to compare an individual slide stainer with that of a bulk staining machine and to compare it with a bulk staining system operated by hand.

In the Cytotek machine, marketed as an individual slide stainer, the slides are passed face down over a series of metal plates (platen), and the staining solutions are run between the slide and the individual plates. Preliminary studies of the Cytotek staining machine in a routine setting had shown that cross contamination was a problem, and it was decided to assess the extent of this problem using albuminised 'receptor' slides as in the method devised by Barr *et al.* (1970). A comparison is made with an automated bulk staining machine in routine use in the laboratory (Shandon Elliott) and with bulk hand

staining where a similar quantity of slides in a carrier was subjected to the somewhat erratic shake and plunge of the cytotechnician.

Material and methods

The three procedures were tested by two experiments.

CROSS CONTAMINATION EXPERIMENT A

1 Cytotek

Fifty routine cytological smears were stained by the Cytotek Automatic Staining machine, being 10 sputa, 10 centrifuged deposits from urine, 20 gynaecological smears (either vaginal or cervical smears), and 10 centrifuged deposits from various body fluids. The smears were prepared according to the Papanicolaou technique and wet fixed in alcohol. In the case of smears from urine, albuminised slides were used. Between each cytological specimen smear was placed a clean albuminised¹ blank slide prepared by a no-touch technique. The preparations were racked in the following sequence—blank-sputum-blank - urine - blank - cervical - blank - vaginal - blank-fluid-blank; the sequence was then repeated. A number of albuminised blank slides were placed before and after the run of slides. The slides were

¹Glycerin albumin obtainable from Raymond A. Lamb.

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¹Cytotek is made by Ames Co, Division of Miles Laboratories Ltd, PO Box 37, Stoke Poges, Slough, Buckinghamshire, UK

²Shandon Elliott is made by Shandon Southern Instruments Ltd, Camberley, Surrey, UK

then stained by the Papanicolaou schedule as arranged by the Cytotek template.

2 *Shandon Elliott*

A similar sequence of cytological smears and albuminised blank receptor slides were bulk stained using a Shandon-Elliott 23 stage staining machine in routine use. Vertical staining racks were used, with 46 slides in two rows of 23 slides. Albuminised blank slides were placed between the cytological smears.

3 *Manual*

The same sequence of smears and albuminised slides were bulk stained using the same vertical staining racks, but the pots were placed on the bench and the procedure was carried out manually.

In all three staining methods fresh staining solutions were used at the beginning of each experiment. At the end of the experiment these solutions were measured in volume and a measured aliquot was filtered through a millipore filter. In the case of the Cytotek an aliquot of the mixed effluent was examined. Each of the millipore filters was stained in a second Shandon Elliott machine with a modified Papanicolaou stain schedule and examined for cell content.

All the slides processed in the experiments were screened by one of us (J.M.G.) and checked by the other authors. Occasional distorted squames, usually anucleate and orangeophilic, and usually single but occasionally in clusters, were seen in all slides and even in slides processed in the Cytotek machine before any specimens had been presented. These were not regarded as evidence of cross contamination and were assumed to be derived from air or skin contact. Well preserved cells, nucleated, often in clusters or non-squames, were regarded as evidence of cross contamination. Usually the cells were recognised as coming from a processed cytological smear.

CROSS CONTAMINATION EXPERIMENT B

Cross contamination by spermatozoa and adenocarcinoma cells in serous fluid has always been considered to be a particular hazard and, because of this, a second experiment was carried out using five semen smears and five pleural fluid smears containing adenocarcinoma.

The sequence of slides was—semen-blank-adenocarcinoma-blank-semen-blank, etc.

As in experiment A, the staining fluids were examined for cell content, the slides being screened by one of us (J.M.G.) and checked by the other authors, the criteria of cross contamination being the same.

SPECIFIC PROBLEMS RELATING TO EACH STAINING MACHINE

An opportunity was taken while the experiments were proceeding to note other problems associated with the automated staining machines.

Results

CROSS CONTAMINATION EXPERIMENT A

With the Cytotek machine 36 of 62 albuminised blanks (excluding blanks at the beginning and end of the experiment) were found to show cross contamination (58%), although this was very largely composed of squamous clusters (Figs. 1 and 2). The contamination was usually in the form of scrape streaks as if the contaminating material was scraped off a preceding slide and on to the leading edge of a subsequent slide (Fig. 3). Eleven out of the 50 routine smears showed cells from another smear. In one sputum smear suspicious cell clusters were derived from a preceding fluid (fig. 4). With the Shandon Elliott machine 21 of the 58 blanks showed cross contamination (38%) and 2 of the 50 specimen smears were recognised as being contaminated with cell material from another slide.

Using bulk staining by hand, 37 of 60 blank slides showed cellular contamination (62%). One specimen smear showed contamination. A comparison of these results is seen in Table 1. In the bulk staining

Fig. 1 Cross contaminating cluster of squamous cells found on an albuminised blank slide and derived from a gynaecological smear. $\times 480$

Fig. 2 Cross contaminating cluster of adenocarcinoma cells on a blank albuminised slide derived from a serous fluid smear. $\times 882$

methods, most of the contamination occurred in the second and third baskets processed, suggesting that the contamination rate is higher when the staining solutions have already been used.

CROSS CONTAMINATION EXPERIMENT B

The results are given in Table 2. With bulk staining nearly all the blanks were contaminated with spermatozoa. Contamination by adenocarcinoma clusters was common, and in the manual bulk staining four out of five semen smears showed adenocarcinoma clusters. Most of the contaminating cells and spermatozoa appear to be concentrated at the edge of a smear, suggesting that it is only at these edges that swirl effects permit circulation of cells and not between closely positioned smears in a rack.

EXAMINATION OF STAINING FLUIDS

Millipore filters taken from samples of wash and staining fluid showed that the earlier wash fluids and solutions contained large numbers of all types of cells, but after the haematoxylin wash the fluids contained much smaller numbers of cells. The haematoxylin staining solution contained large numbers of spermatozoa in experiment B (Fig. 5). These findings could be interpreted as suggesting that the cells that will come off will do so in the

Fig. 3 Cross contamination in the Cytotek machine. The dots on the slide on the right mark squamous clusters on the leading edge of the slide and are derived from the preceding slide (left).

earlier pots, but it has been found that rapid dehydration tends to pull off cells more than if the dehydration is gradual.

SPECIFIC PROBLEMS RELATED TO THE STAINING MACHINES

In the Cytotek:

- 1 The resin coating of the slides at the end of staining dries slowly and tends to cause sticking of the slides (Fig. 6) and this may be another source of contamination. There was no effective hot air drying element in the machine examined and this was no doubt a remediable fault.
- 2 Broken slides cannot be gripped at both ends on the trailer limbs.
- 3 If the smear is put on the wrong side it is not stained.
- 4 The machine leaves an unstained band at both ends due to the limited width of the platen.
- 5 The supply of fluids for each reservoir is not visible and may result in the exhaustion of a pot without warning.

Fig. 4 *Suspicious cell clusters in a sputum derived from a preceding fluid. $\times 630$*

The Shandon Elliott machine suffers from the fault that an unstained band may result if only a few slides are loaded into a basket when the fluid levels are adjusted for a full load, the influence of evaporation being another variable. This requires regular changing or topping up of fluids, or an overall increase in the size of the pots. The occasional jump of the schedule in missing a pot or too rapidly traversing it is a fault presumably in the micro-switch timing mechanism to which such machines are vulnerable and may well be due to the double-decker principle, which we understand is being phased out.

Discussion

There appear to be a number of problems with the Cytotek machine, including the risk of cross contamination. In cases where cross contamination is of squamous clusters, this may be of limited importance but the cross contamination with adenocarcinoma clusters may lead to a false-positive diagnosis. We agree with Barr *et al.* (1970) that the contaminating clusters of adenocarcinoma may be in a different focal plane of the microscope but this is not neces-

sarily so. Cross contamination in the Cytotek machine appears to be due mainly to the mode of fluid feed through the centre of a platen which is triggered off only when a slide is well on to the platen. This means that a strong capillary traction occurs in the initial stages of the slide passage and serves to scrape off the ridges of a smear onto the leading edges of the platen. However, some scraping and trailing of cell fragments seems inevitable with the design of the machine which depends on the smeared slide being passed along face down over metal plates. This principle may be satisfactory in the routine staining of thin blood films, but it is less so with the irregular ridged cytological smears such as cervix or sputa, or thicker clumps on a marrow smear.

We have found cross contamination to be a problem in the Shandon Elliott machine and also with manual bulk staining, particularly with semen and adenocarcinoma smears (Table 2). Semen smears in most laboratories are stained individually on a staining rack but we also suggest that cross contamination with bulk staining could be minimised by changing the pre- and post-haematoxylin wash fluids or filtration of them after every run. The higher

Fig. 5 Spermatozoa in the haematoxylin staining solution in Experiment B, using the Shandon Elliott Millipore filter preparation. $\times 150$

Table 1 Cross contamination rates using albuminised 'receptor' slides and routine smears (experiment A)

	Contamination of albuminised blanks	Recognisable contamination of 50 routine smears
Cytotek	36/62 (58%)	11
Shandon Elliott	21/58 (38%)	2
Manual bulk staining	37/60 (62%)	1

contamination with manual bulk staining may well be related to more vigorous action and this should be reduced.

The Shandon Elliott machine and Haematek have been evaluated for the staining of blood films and bone marrow with the Romanowsky stains and found to be satisfactory (Moss, 1968; Hoffmann, 1972). Cross contamination was not noted but these haematological smears are usually a thin and even monolayer, and prepared by the dried on methods rather than wet fixed where cells are poorly adherent to the glass surface. Automatic slide staining is used in the microbiology department for gram stains, and

consideration has been given to the possibility of a false positive through cross contamination. Trials to assess this risk have been made by Ryan *et al.* (1973), Drew *et al.* (1972), and Cremer (1968). The latter author used a Shandon Elliott machine. Bacterial cross contamination has not been found, but preparations were either heat fixed or air dried.

Almost all of the automatic staining machines used in cytology are bulk staining devices (Pickett, 1973). Cross contamination can always be considered a possibility with bulk staining, and it is felt that a suitable individual slide staining machine might eliminate this. An individual single slide staining machine for bacteriology smears has been described by Wilkins and Mills (1975). In 1964, one of us (O.A.N.H.) produced a design of an 'individual slide' staining machine in a paper given to the British Society for Clinical Cytology. The principle suggested then was to feed the staining solutions from the reservoirs on to horizontal slides on moving circular or linear racks with the cells uppermost when each subsequent fluid would wash off the previous one.

Table 2 Cross contamination rates using albuminised receptors and five semen and five fluid smears containing adenocarcinoma (experiment B)

	Contamination of albuminised blanks				Contamination of smears	
	By spermatozoa		By adenocarcinoma		Spermatozoa on fluid smear	Adenocarcinoma on semen smear
Cytotek	3/20	15%	4/20	20%	-/5	1/5
Shandon Elliott	9/12	75%	3/12	25%	5/5	-/5
Manual bulk staining	11/11	100%	10/11	91%	5/5	4/5

Fig. 6 View of Cytotek machine showing sticking and tumbling of slides at end of staining. $\times 160$

Bulk staining machines have practical advantages in large laboratories with a large turnover of gynaecological smears but the cross contamination should be minimised by filtering or changing the wash fluids and staining solutions, particularly leading up to and following haematoxylin, after every run.

The Cytotek machine has a number of inherent problems which have yet to be solved. It might, however, have advantages in the economical and automatic staining of small numbers of slides, particularly after normal working hours.

Manufacturers are once again urged to develop a track single slide non-contaminate automated staining machine, particularly for the increasingly varied cytological smears being prepared in a routine modern laboratory.

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