Technical methods

Use of polylysine-coated slides in preparation of cell samples for diagnostic cytology
with special reference to urine sample

O A N HUSAIN, J A MILLETT, AND J M GRAINGER
Cytology Department, Charing Cross Hospital, Fulham Palace Road, London W6

After some years of seeking a simple but effective method to lay cells on slides in an evenly dispersed monolayer for automated cell scanners we managed to utilise what we believe to be the electrical charges on the cell and slide surface to achieve this end. The machine scanners required additional techniques to disrupt cell clusters and prevent reaggregation of cells, and these are detailed in our previous publication.[1] These are not necessary for visual screening.

It is well known that cells acquire a negative charge in solution, and we sought to increase this on the one hand and, on the other, to induce a positive charge on the slide surface. After much experimentation we achieved some success with the latter by the use of the cationic polymerised amino acid polylysine, which attaches as a monolayer to the negatively charged glass surface, leaving excess positive charge available for the attraction of cells.[2] The latter, moreover, appear to remain discrete, if not to repel each other under gentle sedimentation forces of 1 *g*, to result in a fairly well-dispersed monolayer. Whatever the form of attachment achieved, it appears strong enough to withstand the gentle 'wash-off' of the fluid after 2-5 minutes' sedimentation and the rigours of the subsequent staining schedule.

We thought that such a technique would be useful in a number of different ways in routine cytology practice where cells are in solution to start off with, as in the case of urine, or where, to produce better results, cells could be put into suspension—not necessarily dispersed—and then laid down more evenly and flat with less curling or overlap of the cytoplasm or even nucleus. We have found that cyst fluids lend themselves readily to such techniques, which result in more even cell spreads without the accompanying debris, and this aspect has been pursued by Duguid *et al.*[3] The handling of buccal smears for nuclear sexing was a further problem needing improvement, as it is often found that squames and even their nuclei appear distorted or folded over and as such do not allow Barr body counting. We present evidence of such improvements in studies on urine and buccal cell preparations.

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Material and methods

1 URINE

We attempted two forms of trial:

(A) to test various slide surfaces for cell adhesion utilising the direct laying down of cells from urine; and

(B) to compare the polylysine method as in (A) above to the routine methods of preparation for urine samples considered ideal for the other slide surfaces.

At least 160 ml of urine (2-3 hour output) was necessary for both trials to be performed.

TRIAL A

The following slide surfaces were investigated: 1, polylysine; 2, glycerine albumin; 3, gelatin; and 4, plain glass.

Preparation of slide surfaces

1 An aqueous solution of polylysine (0·1%) is spread evenly over a precleaned glass slide with a Pasteur pipette and is left to dry before use. Slides must be coated daily but the polylysine solution can be stored for some weeks at 4°C.

2 A thin film of glycerine albumin is smeared on to a clean glass slide just before the application of the cell sample.

3 A thin aqueous solution (0·5%) of gelatin is flooded on to the slide surface and left to dry.

4 Precleaned slides (Chance-Propper Ltd, Smethick, UK) were used throughout the trial.

Method

Shake sample vigorously 20 times to ensure even cell dispersion.

Spin down 80 ml of the urine (5 minutes, 1200 rpm).

Resuspend cell pellet 1 vol:5 vols with saline.

Using an Eppendorf pipette, spread one 100 μl dose over an area of approximately 2·5 cm² on each of the four slide surfaces.

Leave to settle for 5 minutes, covered by a petri dish to avoid evaporation and dust contamination.

Gently wash off any excess cells with saline into a beaker.

Fix slide in 74 OP alcohol.

Pass 'wash-off' sample through a millipore filter, taking care to rinse beaker thoroughly to ensure collection of all cells present.

Assessment
Random area cell counts were made starting at prefixed focal points within the area of spread and counting 20 consecutive microscope fields (using the X40 objective) from each point, thus making 100 fields in all. These counts were made on the four smear surfaces and could be considered as matching areas. Ten cell samples from urothelial cancer cases and 14 samples from non-malignant cases were used to evaluate the differential cell retention on the different slide surfaces. A further differential cell count was made on each of the slides and its paired millipore wash-off sample in order to assess any preferential loss or gain of cell type.

TRIAL B
The method in trial A was used for polylysine, and for the other three surfaces the routine procedure was performed, taking the cell button from a spun deposit and making a heavily cellular smear on the slide which was then allowed almost to dry before the smear was placed into fixative.

Assessment
The comparative assessment of each slide surface was then attempted but as total cell counts were found to be far too laborious, an overall qualitative assessment was made.

2 BUCCAL SMEARS
Buccal smears from five women and five men were prepared using both the direct smear method by spatula on to a slide, which is immediately wet-fixed in alcohol, and also by the new polylysine method as follows:

Method
The inside of the mouth is scraped with a plastic spatula and the saliva and cells are shaken off into approximately 4 ml saline solution. This is repeated until the saline is cloudy with cells. The sample is then centrifuged (5 minutes, 1200 rpm) and the deposit concentrated to a 1 in 5 suspension, which is spread on to a polylysine-coated slide. After 2-5 minutes the excess fluid is washed off gently with saline. The smears are placed in alcohol fixative for 30 minutes, then allowed to dry, and stained by Cresyl Fast Violet.

Assessment
Barr body counts were performed on slides prepared by both methods and a comparison was made. The number of non-readable cells identified during these counts were also noted.

Results

1 URINES

TRIAL A
Tables 1 and 2 depict the detailed average cell counts for each cell type for the benign and malignant cell samples respectively. It can be seen that the polylysine slides have a higher total number of cells present throughout. The numbers of different cell types present were investigated statistically. The findings in the differential cell retention counts show that in the 24 cases put together there is a significant increase in the number of transitional cells on the polylysine slides compared with the plain glass ($P < 0.025$ in a Student's t test), the gelatin

Table 1 *Average cell content for 14 normal urine cases*

Slide surface	*Polymorphs*	*Histiocytes*	*Transitional cells*	*Squamous cells*	*Atypical cells*	*Non-polymorph cell total*	*Total*
Albumen	336·4	1·93	29·57	0·5	0·5	32·5	368·9
Polylysine	1389·6	45·71	395·4	47·36	8·86	497·36	1886·9
Gelatin	123·9	4·78	20·71	2·07	0·43	28	151·86
Plain glass	1251·2	12·64	82·64	1·71	0·79	97·78	1349·7

Table 2 *Average cell content for 10 malignant cases*

Slide surface	*Polymorphs*	*Histiocytes*	*Transitional cells*	*Squamous cells*	*Atypical cells*	*Non-polymorph cell total*	*Total*
Albumen	886	2·1	112·3	0·4	43·2	158·9	1043·9
Polylysine	3670·9	23·6	866·8	12·5	554·1	1446·5	5117·4
Gelatin	177	0·7	37·5	4·1	30·8	73	250
Plain glass	1544·2	0·7	189·9	1·8	68·6	260·7	1804·9

(P < 0·001), and the albumin (P < 0·005) preparations.

With atypical cells, the retention of such cells in the 10 malignant cases was found to be significantly greater on the polylysine slides than on the plain glass slides (P < 0·001), the gelatin slides (P < 0·025), and the albumin slides (P < 0·005).

Another interesting finding was the polymorph to non-polymorph cell retention on the various slide surfaces. Here again, the advantage of the polylysine slide to plain glass was significant (P < 0·001), to the albumin slide (P < 0·001), and to the gelatin slide (P < 0·001).

Differential cell loss

Table 3 shows the correlation coefficients between slide surface and millipore differential cell counts.

It will be observed that overall the polylysine slide surface gives a much more consistent performance in preserving the differential cell pattern.

Table 3 *Correlation coefficients between differential cell counts of each slide surface and its corresponding millipore (24 urine cases including 10 malignant)*

Cell types	*Polylysine*	*Albumen*	*Gelatin*	*Plain*
Polymorphs	0·85	0·70	0·73	−0·71
Histiocytes	0·80	−0·06	0·34	0·90
Transitional	0·87	0·91	0·66	0·75
Squamous	0·89	0·92	0·20	0·33
Atypical	0·64	0·35	0·62	0·62

TRIAL B

Only qualitative assessment was possible due to the large cell numbers involved and the often large denuded areas of slide, especially on the plain slide and gelatin surface preparations which made random field counts inaccurate. Improved cell dispersion with good cellular presentation were found utilising the polylysine method.

2 BUCCAL SMEARS

Table 4 shows that the Barr body counts on the five case samples do not significantly vary between the two forms of preparation but there was a significantly higher proportion of readable cells using the polylysine method (69%) than using the direct spread (40%).

Table 4 *Barr body counts in buccal smears of five women*

	Direct spread method	*Polylysine method*
F1	15%	19%
F2	17%	12%
F3	9%	9%
F4	15%	18%
F5	5%	6%

Discussion

URINE CYTOLOGY

Preparation techniques in urine cytology can be criticised on many grounds. The standard Papanicolaou techniques give rise to uneven spreading and, for various reasons, sometimes indifferent morphology. Furthermore, owing to (*a*) difficulty in obtaining a distinct centrifuged deposit and, more important, (*b*) the poor adherence of the deposit on the glass slide, often only a small fraction of the deposit remains on the processed stained smear. Filter preparations are expensive for a mass screening process and are rapidly obscured by red cells or leucocytes. The cytocentrifuge favoured by some does have the same criticism of a significant cell loss during processing as well as the danger of contamination of the apparatus. Also it gives only a small area of cells, so that a number of slides must be prepared for each sample.

It can be seen that, utilising the polylysine method, a much more consistent and representative cellular preparation results. Similar findings utilising cyst fluids, buccal smears, and other cell suspensions have been equally satisfactory in our and other hands.

The essential precautions are that the polylysine solution is preserved at 4°C, that the slide coatings should be prepared daily, and that the area over which the cells are spread on a slide should preferably be limited. We use a felt-tip pen to define that area.

References

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Requests for reprints to: Dr O A N Husain, Cytology Department, Charing Cross Hospital, Fulham Palace Road, London W6, UK.

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