Technical methods

Use of dry fixative slides for exfoliative cytology

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Fixatives which are in general use in laboratories for diagnostic cytological work all have certain disadvantages. Ether-alcohol mixtures or iso-propyl alcohol have to be available in the clinic or consulting room and it is usual for the smears to be placed with polythene containers filled with fixative for transport to the laboratory. Spillage is often a hazard and there are objections to the smell as well as to the inflammable properties of ether. The aerosol type sprays are expensive and in our experience require skill in manipulation if good results are to be obtained.

Amies and Garabedian (1965) have described the preparation of fixative slides for the diagnosis of *Trichomonas vaginalis* and stated that these might be useful in the field of diagnostic cytology. We decided therefore to give these slides a trial for cervical and vaginal smears and have now been using them for several months in parallel with ether-alcohol fixed slides. They are equally satisfactory as regards fixation and staining, using the standard Papanicolaou technique. The slides are prepared, several hundred at a time, by dipping 3×1 in. glass slides into the following freshly prepared solution:

Ethanol 30% v/v aqueous solution	..	100 ml.
Mercuric chloride		5.0 g.
Sodium acetate		1.0 g.
Sucrose		5.0 g.

The slides are drained and allowed to dry in special racks. Precautions must be taken in the preparation of the slides to avoid any risk of dermatitis to the operator. (These slides may soon be available commercially.) Smears are made in the usual way on to the prepared slide and allowed to dry.

We thought it worthwhile drawing the attention of others concerned in this field to what we think is an important advance in technique which may well affect the whole policy of cytology centres.

We should like to express our appreciation of the technical assistance of Miss S. Hepple and Miss Olivia Balaskas of the Cytology Department, The Vincent Square Laboratories, Westminster Hospital, S.W.1.

REFERENCE

Amies, C. R., and Garabedian, M. (1965). *J. clin. Path.*, 18, 27.

A mixer for slide cell agglutination¹

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The classical method for testing red cells for agglutination against antisera on a slide is either to rock the slide manually or to examine it in a specially designed rocking rack with background illumination. The disadvantage of the latter is that the rocking motion is in one direction only; when the slide is held in the hand the rocking is usually in a rotating manner. In a transfusion laboratory many procedures are carried out on slides and although sensitization may take place without agitation enhancement of agglutination is always achieved by mixing the cells, usually by some form of rocking. If this is carried out manually considerable technical time may be wasted. We have therefore designed a simple machine which reproduces as nearly as possible the rotating rocking motion produced by the majority of workers when carrying out the technique manually. The components used in the assembly of this machine are readily obtainable and its construction is well within the competence of the majority of workshops. If manufactured in this manner its cost is relatively small. It has been found invaluable for ABO slide grouping of red cells which we carry out as a routine screening procedure and for the final stage of the direct and indirect antiglobulin test. We have also found it useful in the performance of mixed cell antiglobulin tests. For tests using red cells we

¹A working drawing with the specification of the components used is available from the author.

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Simplified technique for counting the number of bacteria in urine and other fluids

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The reluctance to carry out diagnostic catheterization of the bladder because of the danger of introducing infection has increased reliance on mid-stream and 'clean catch' specimens. It must be accepted that such specimens are contaminated to a greater or lesser degree and the problem resolves itself into distinguishing between mere contamination and bacteruria significant of infection. Several investigators have examined urine quantitatively and found that contamination rarely exceeds 10^5 /ml. so that bacterial counts in excess of this are more likely to be significant (Kass, 1955 and 1956; Sanford, Favour, Mao, and Harrison, 1956; Macdonald, Levitin, Mallory, and Kass, 1957; Jackson, Griebble, and Knudsen, 1958; and Monzon, Ory, Dobson, Carter, and Yow, 1958).

A disadvantage of conventional quantitative techniques is that they are too time consuming for use in the routine diagnostic laboratory. Thus to be useful in a busy laboratory methods of quantitative culture must be simple, quick, and of a degree of accuracy sufficient to enable 10-fold differences in viable count, particularly over the range 10^3 /ml. to 10^6 /ml., to be detected.

The aim of the present paper is to compare a modification of the Miles and Misra method with a standard loop technique which fulfils the above criteria.

METHODS

The Miles and Misra modified technique was carried out

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A mixer for slide cell agglutination—concluded

have found the tile described by Mollison (1961) to be the most satisfactory; for leucocytes, tissue cells, or mixed cells we use hollow glass slides.

A MIXER FOR SLIDE CELL AGGLUTINATION DETAILS OF CONSTRUCTION

The mixer consists of a dural tray mounted on a Tufnell baseboard. The mechanism is enclosed by a cover piece of painted sheet steel. The driving mechanism is attached to the tray centrally and consists of a shaft mounted on a Rose spherical bearing passing through a universal jointed centre shaft and connected to a synchronous motor by a v-pulley.

REFERENCE

Mollison, P. L. (1961). *Blood Transfusion in Clinical Medicine*, 3rd ed., p. 409. Blackwell, Oxford.

on suspensions of the common bacterial urinary pathogens, namely, *Esch. coli*, *Streptococcus faecalis* (enterococcus), a *Proteus* species, *Klebsiella aerogenes*, *Staphylococcus pyogenes*, and a coliform bacillus forming small colony, as described in Mackie and McCartney's 'Handbook of bacteriology'. Counts were done on blood agar with 5% digest broth, with the exception of the *Proteus* species which was counted on Oxoid MacConkey agar. All counts were read after overnight incubation at 37°C.

The standard loops were made by using S.W.G. 28 Nichrome wire which was wrapped around a metal bar of 3 mm. external diameter. The loops were calibrated in the following manner:

1 The average pick-up volume was determined by weighing a ¼-oz. bijou bottle before and after removal of 100 loopfuls of fluid vertically. This was repeated 10 times with each loop and five more loops made in the same way were similarly tested.

2 The average delivery volume was calculated by using a solution of potassium acetate (2g./ml.) instead of water. Loops were dipped into the solution and discharged on to small pledgelets of dry sponge on aluminium planchettes which had been previously weighed. These were then dried overnight over calcium chloride and the weight of deposited potassium acetate obtained by difference. By calculation the volume of solution discharged was obtained. Potassium acetate was used because of its high solubility and low viscosity.

By these methods the average pick-up volume and delivery volume of the standard loops was found to be $0.0043 \text{ ml.} \pm 0.0009 \text{ ml.}$

Well-dried 3½-in. blood agar plates and Oxoid MacConkey plates in the case of *Proteus* were inoculated with varying concentrations of the above-mentioned organisms in the following manner (Fig. 1).

FIG. 1. Inoculation of plate. Standard 3 mm. diameter loop of S.W.G. 28 wire (carries and delivers $0.0043 \text{ ml.} \pm 0.0009 \text{ ml.}$); charged and spread over a sector of the plate 'W' by 40 to and fro strokes (sterilized); drawn four times from 'W' into 'I' (sterilized); drawn four times from 'I' into 'II' (sterilized); drawn four times from 'II' into 'III' (sterilized).

A well of segment length 3 in., comprising 17% of the total plate area, was marked out using a wooden template, and inoculated with one loopful of uncentrifuged undiluted urine, the loop being withdrawn from the sample vertically. The well was stroked until no visible fluid remained (about 40 times). After sterilization of the loop four parallel lines were drawn out from the well and this procedure was continued until three sets of four lines had been made round the plate. After overnight incubation at