

## Technical methods

### A disposable counting chamber for urinary cytology

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In the investigation of urinary tract disease it is usual to determine the content of cells and bacteria in specimens of urine received in the clinical laboratory. This is done with varying degrees of accuracy, the main methods used being as follows: 1 An assessment which is based on the microscopy of a drop of uncentrifuged urine in an ordinary coverslip preparation; 2 a similar assessment made on a drop of centrifuged deposit, usually reported as 'cells per high-power field'; 3 a cell-count proper which is carried out on uncentrifuged urine in a haemocytometer chamber, *e.g.*, Fuchs-Rosenthal or Neubauer.

Methods 1 and 2 are not sufficiently accurate (Stansfeld and Webb, 1953; Stansfeld, 1962; Little, 1962), largely due to differences in amounts of urine placed on the slide, leading to considerable variation in the depth of fluid under the coverslip. There is therefore a very imperfect relationship between the figure for 'cells per field' and the actual cell concentration. Other factors, such as the volume of the specimen and the method of centrifugation, may also influence the results.

Method 3 is accurate, perhaps more so than is really needed for routine purposes, since urine itself is a very variable product. Though convenient for the examination of occasional specimens, it is laborious and time-consuming for repeated use. The haemocytometer chambers have to be disinfected and cleaned after use. A laboratory faced with the sudden receipt of large batches of urine specimens from maternity or urogenital clinics needs to keep in use several haemocytometer chambers, and accidental breakages are common and relatively expensive.

This paper describes a compromise technique, the 'three-coverslip method' which, in effect, provides a disposable counting chamber for examining uncentrifuged urine specimens. It is sufficiently accurate for the quantitative assessment of urinary cytology, is quick, cheap, and easy to use, and requires no special equipment.

#### METHOD

**THREE-COVERSLIP COUNTING CHAMBER** This is shown in Figure 1. Two parallel lines are drawn with grease pencil about 1 cm. apart at right angles to the long axis of a 3 in. × 1 in. slide to make a 'compartment', which can be further subdivided with another line, as shown in the Figure, to allow two samples of urine to be set up at the same time, if so desired. On either side of the com-

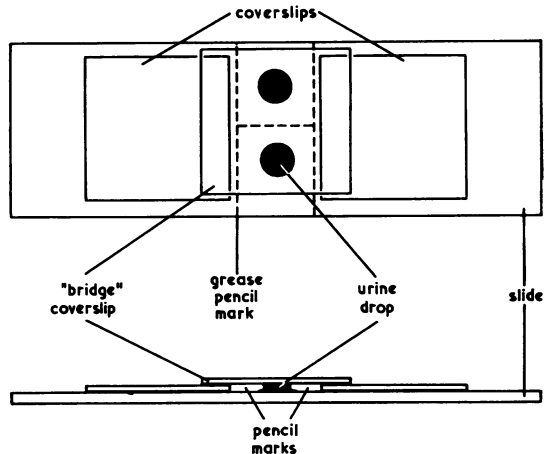


FIG. 1. A 'three-coverslip' preparation.

partment, just outside the grease-pencil marks, two Chance no. 1 coverslips ( $\frac{3}{8}$  in. ×  $\frac{3}{8}$  in. square) are laid. These are anchored to the slide by applying a trace of moisture to the centre of the under side of each. A loopful of well-mixed uncentrifuged urine is then placed in each compartment and a third coverslip dropped on to form a bridge between the first two coverslips. The under side of the third coverslip comes into contact with the urine drops, and the preparation is ready for examination. One special precaution is necessary: the coverslips to be used are checked for distortion by a glance at the image reflected in one face of the coverslip; however, very few coverslips have to be rejected for this reason. The grease-pencil lines prevent migration of the urine droplets under the side coverslips, where they would otherwise be lost by capillary attraction.

The specimen is first examined with the ×10 microscope objective and the average number of pus and/or red cells per field determined after counting the total number of each in 10 separate fields. The number of cells per cubic millimetre is calculated by multiplying the average number per field by a conversion factor obtained as described below. The urine is then examined under the ×40 objective to confirm the nature of the cells counted and to note the numbers and variety of bacteria or other formed elements present. In more cellular specimens, or those containing dense deposits, a sufficiently accurate assessment of the cell content may only be possible by repeating the cell count with this objective and applying the different conversion factor appropriate to it. When the examination has been completed, the entire preparation is discarded, though for economy it is possible to retain the two side coverslips which are uncontaminated.

**CHAMBER DEPTH** Knowledge of the absolute depth of urine in a 'three-coverslip' preparation is not required for

calculating the number of cells per cubic millimetre of urine, but the depth has to be sufficiently consistent from one preparation to another. The average depth of the layer of urine is equal to the mean of the thicknesses of the two side coverslip on which the central bridge rests. The specification of the manufacturers<sup>1</sup> for the no. 1 gauge coverslip indicates that they are between 0.13 and 0.16 mm. in thickness, and this was confirmed by measuring a large number of coverslips with a micrometer gauge (about 4% were as much as 0.17 mm. thick). The average depth of the chambers on this basis is therefore within the range 0.14 to 0.16 mm.

**CALIBRATION FACTORS** In most laboratories receiving specimens of urine for examination of cells there is likely to be one particular microscope which is generally used for that purpose. For this technique the microscope must first be calibrated to obtain a simple factor for converting 'cells per field' to 'cells/c.mm.'. This can conveniently be done by diluting a sample of blood 1 in 10,000 and 1 in 100,000 (v/v) with physiological saline, and determining the red-cell count on both diluted samples with a Fuchs-Rosenthal or other haemocytometer chamber. The counts should be in the ranges 200-500 and 20-50 per c.mm. respectively. Both samples are then set up in a three-coverslip preparation and examined in turn with the  $\times 10$  and  $\times 40$  objectives: in each case the total number of cells in 10 microscopic fields (or more) is determined and the average number of cells per field is calculated. This process should preferably be repeated several times and the mean of the results determined. The necessary conversion factor (N) for each objective is obtained from the calculation

$$\frac{\text{cells per c.mm.}}{\text{average cells per field}} = (N).$$

The whole number nearest to the value of N so obtained can be used as a conversion factor with sufficient accuracy. It was found in this Department that for two binocular microscopes (a Baker with  $\times 5$  eyepieces and a Watson Bactil with  $\times 6$  eyepieces) the factor for the  $\times 10$  objective was 5 and for the  $\times 40$  was 100, and it is probable that these convenient values will be found to apply to most microscopes of similar pattern or can be arrived at by adjustments to the eyepiece magnification<sup>2</sup>. It would be unsafe, however, to assume these factors to apply: they must be determined experimentally.

Table I illustrates the correlation which exists between estimates in three-coverslip preparations and those made in a conventional haemocytometer chamber (Fuchs-Rosenthal). A series of dilutions of a specimen of blood was made as shown in Table I, and the red-cell concentration in each dilution determined by both methods. It can be seen that the counts in the three-coverslip preparations vary from those in the haemocytometer by only 10% or less and thus there is a correlation adequate for clinical purposes.

<sup>1</sup>Messrs. Chance Brothers, Ltd., 29 St. James's Street, London, S.W.1.

<sup>2</sup>This gives a 20-fold difference between the two objectives; the theoretical difference based on diameters of magnification would of course be 16 (4<sup>2</sup>).

TABLE I  
COMPARISON OF RED CELL COUNTS ON DILUTED BLOOD SAMPLES IN A FUCHS-ROSENTHAL CHAMBER AND IN THREE-COVERSLIP PREPARATIONS<sup>1</sup>

Blood Dilution	Three-coverslip Count		Haemocytometer Count (R.B.C./c.mm.)
	(R.B.C./Field)	(R.B.C./c.mm.)	
10,000	120	600	550
30,000	38	190	183
100,000	11	55	55
300,000	4	20	18
1,000,000	1	5	6

<sup>1</sup>Microscopic objective,  $\times 10$ , eyepieces  $\times 5$ ; conversion factor N ('cells per field to 'cells per c.mm.') = 5

#### DISCUSSION

Three-coverslip preparations have been in use in this Department for some 18 months and have been found convenient and satisfactory substitutes for standard types of haemocytometer in the routine examination of urine specimens; they may therefore be justifiably referred to as disposable counting chambers. Their use in place of the technique described above as method 2, combined with a semi-quantitative 'minimal loopful' method of culture similar to those described by Gillespie, Linton, Miller, and Slade (1960), O'Sullivan, FitzGerald, Meynell, and Malins (1960), and McGeachie and Kennedy (1963), has led to a far closer correlation between the results of cytology and culture of specimens of urine; this confirms the findings of McGeachie and Kennedy. The convenience of the three-coverslip method is in fact greater than is implied above since only a minority of urine specimens require a full quantitative assessment. The vast majority can be placed in one of two categories, either with cells within normal limits or obviously purulent, which can be ascertained at a glance.

#### SUMMARY

The construction and use of a disposable cell-counting chamber is described. The technique is intended primarily for the assessment of the cell content of urine. The chamber is made from an ordinary microscope slide and three coverslips. Used in conjunction with a microscope calibrated by a simple method, it permits the estimation of urinary cell concentrations in terms of number of cells per cubic millimetre. The accuracy of the results obtained in this way is comparable to that achieved with conventional haemocytometers, and is adequate for the purposes described. The chief advantages of a disposable chamber lie in the saving of time and labour, since the disinfection and cleaning necessary when using conventional haemocytometers are obviated.

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