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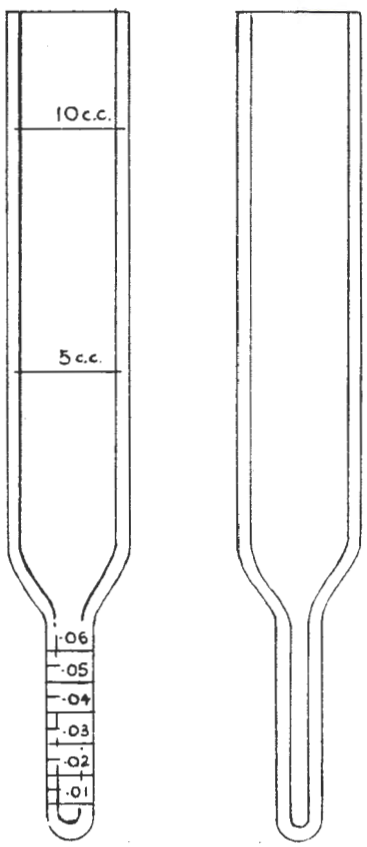
A Rapid Economical Method for Accurate Determination of the Percentage Packed Cells in a Bacterial Suspension.

By G. C. VAN DRIMMELEN, Section Bacteriology, Onderstepoort.

THE FITCH-MODIFIED HOPKINS' TUBE graduated to show good measurements for 0·1 to 0·5 per cent. packed cells is generally used for standardizing the density of Brucella Vaccine. It dispenses with the weighing of the bacterial suspension and the packed cell residue in a centrifuge tube because volumetric determination of the percentage cells is equally useful.

FIGURE 1.



Graduated Fitch-modified Hopkins' tube.

Difficulty in obtaining a sufficient supply of Hopkins' tubes led to the introduction of a very simple, accurate and less expensive method described in this note.

When a bacterial suspension is centrifuged in an ordinary dome-ended centrifuge tube the cells settle to form a disc on the bottom of the tube.

The size of the disc varies with the density of the suspension but, due to the form of the dome, the rate of change of the size of the disc varies inversely with the density of the suspension.

It was found that the variation in diameter of the disc of packed cells in the dome-shaped end of a centrifuge tube could be recorded easily and accurately with discs ranging in size from one-half to two-thirds of the diameter of the centrifuge tube. Below this range the edge of the disc was found to be insufficiently sharp and above this range the variation was too small to avoid slight errors. If a suspension produced a disc of a size outside this range it was adjusted by resuspending the cells and increasing or decreasing the height of the column in the tube.

Since it was adopted at Onderstepoort as an emergency adjunct to the use of Hopkins' tubes, calibration was done by comparison of readings. This calibration by comparison is, however, unnecessary as the percentage packed cells can be calculated directly from the dimensions of the dome-shaped centrifuge tube.

FIGURE 2.

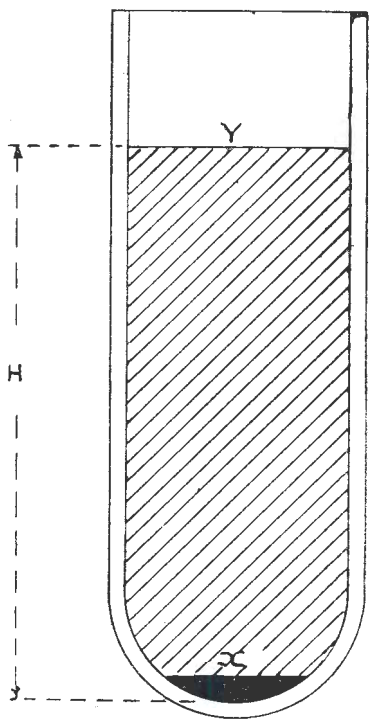


Diagram indicating dimensions of contents of a dome-ended centrifuge tube.

If—

X = diameter (in cm.) of packed cell disc after centrifuging and

Y = diameter of inside of tube in cm.

define—

$$Z = \sqrt{Y^2 - X^2}$$

It can be shown, utilizing the formula for a solid of revolution in the infinitesimal calculus that the volume of the disc of sediment can now be expressed as:—

$$\text{Volume} = \frac{\pi}{24}(Y-Z)^2(2Y+Z).$$

Therefore the percentage packed cells—

$$= \frac{13{\cdot}09\,(Y-Z)^2(2Y+Z)}{\text{Total volume of original suspension.}}$$

Fifty ml. capacity centrifuge tubes with a diameter of 30 mm. were used in the present work. The Brucella vaccine usually produces a disc of 17, 18 or 19 mm. diameter. The size of the disc is measured by looking at the tube from the bottom after pouring off the supernatant and holding a brass (or plastic) plate containing holes with diameters of 5 to 20 mm. between the tube and the eye. Starting from the largest hole, the disc is compared with each until the exact size is determined. All tubes were centrifuged at 2,750 r.p.m. for 75 minutes on a 30 cm. centrifuge head.

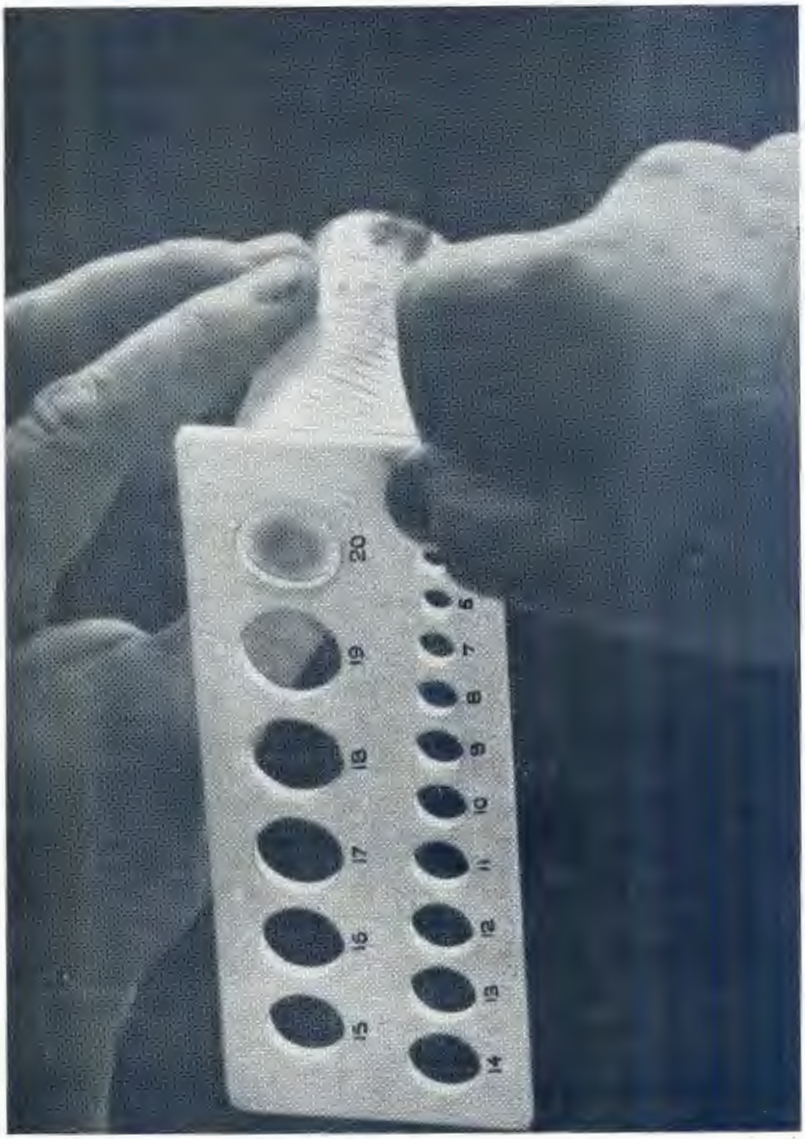
The following figures show the close agreement between the application of this method to a dome-shaped centrifuge tube of 50 ml. capacity, with an internal diameter of 3 cm., and the readings obtained by using a Hopkins' tube. Suspensions with 0·5, 0·6 and 0·8 per cent. packed cells in a Hopkins' tube, yielded discs of 1·6 cm., 1·72 cm. and 1·8 cm. respectively. Disc diameters of 1·6, 1·7 and 1·8 cm. in the dome-shaped tube were calculated to represent packed cell percentages of 0·48, 0·62 and 0·79 respectively.

The method was statistically checked by taking measurements of sixty specimens all representing different dilutions and concentrations of a batch of vaccine. The measurements were taken without previous knowledge of the degree of concentration or dilution. Four primary dilutions were made and twelve secondary concentrations and dilutions of each of these and of the undiluted vaccine were used.

The standard for each of the twelve secondary concentrations and dilutions was derived by reducing the values calculated for the percentage packed cells of the primary dilutions to those of undiluted vaccine and assuming the value calculated for the undiluted vaccine as correct.

In other words the data were all reduced to a common denominator and it only remained to test whether they differed significantly from the assumed standard. This was done by subtracting the standard from each observation taking the mean of the five differences for each of the twelve secondary concentrations and dilutions; and applying Fisher's "t"-test to these means to see whether they differed significantly from zero. The results are shown in Table 1 where S.S. denotes a highly significant divergence.

FIGURE 3.



Brass or plastic plate with 5 mm. to 20 mm. diameter holes.

TABLE 1.

Percentage Secondary Dilution or Concentration.	Difference from Standard.		
	Mean.	Variance.	"t".
120	—·093	·0078	2·986
110	—·062	·0052	2·430
100 (undiluted)	·077	·0048	3·157
90	·104	·0242	1·892
80	·243	·0381	3·668
70	·153	·0320	2·416
60	·396	·0409	5·540 SS.
50	·458	·0800	4·581 SS.
40	·534	·0137	12·914 SS.
30	·539	·0693	3·789 SS.
20	·539	·0422	7·287 SS.
10	·658	·1936	4·230 SS.

With four degrees of freedom at P = ·01. "t" = 4·604.

From Table 1 it follows that densities of 60 per cent. to 120 per cent. of that of the undiluted vaccine in the batch used, gave measuremnts which were essentially the same. This represents packed cell percentages from 0·3 to 1·0 per cent which compares favourably with the range of the Hopkins tubes.

The measurements of the Hopkins' tube are stated to represent 12×10^9 Brucella organisms p.ml. for 0·54 per cent. packed cells as read by the calibrated end of the tube. On this basis the following table is used for determining the bacterial count of the Brucella vaccine at Onderstepoort by the present method:—

TABLE 2.

Diameter of Disc Sediment in 50 ml. Dome-shaped Centrifuge Tube.	Percentage. Packed Cells.	Brucella Organisms per ml.
1·0 cm.	0·07	$1·56 \times 10^9$
1·1 cm.	0·10	$2·22 \times 10^9$
1·2 cm.	0·14	$3·20 \times 10^9$
1·3 cm.	0·20	$4·44 \times 10^9$
1·4 cm.	0·27	$6·04 \times 10^9$
1·5 cm.	0·36	$8·09 \times 10^9$
1·6 cm.	0·48	$10·58 \times 10^9$
1·7 cm.	0·62	$13·76 \times 10^9$
1·8 cm.	0·79	$17·60 \times 10^9$
1·9 cm.	1·00	$22·27 \times 10^9$
2·0 cm.	1·25	$27·98 \times 10^9$

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