WITH THE AUTHOR'S COMPLIMENTS.

R.D.

[From the PROCEEDINGS OF THE ROYAL SOCIETY, B, VOL. 86, 1913.]

6.

An Apparatus for Liquid Measurement by Drops ~~and~~ Applications in Counting Bacteria and other Cells and in Serology, &c.

BY R. DONALD, B.SC. (N.Z.), D.P.H. (OXF.).

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, A. Vol. 86]

An Apparatus for Liquid Measurement by Drops and Applications in Counting Bacteria and other Cells and in Serology, etc.

By R. DONALD, B.Sc. (N.Z.), D.P.H. (Oxf.).

(Communicated by Dr. L. Hill, F.R.S. Received November 21, 1912,—Read January 16, 1913.)

(From the London Hospital Bacteriological Laboratory. Dr. William Bulloch, Director.)

To promote drop-measuring in serological and bacteriological work, etc., the writer has devised a simple system of producing uniform pipettes, clean and sterile, which deliver uniform drops of any required size from $\frac{1}{4}$ c.c. down to 1/200 c.c. or less, and has devised also simple forms of constant-pressure apparatus for use with the pipettes.

The fundamental principle of his method rests on the fact that the size of a drop of a given liquid yielded by a clean pipette is determined by the outer circumference of the pipette at the level where the contact-edge of the drop clings round the glass—due allowance being made for the rate at which the drop is detached and the temperature.

The pipettes, freshly drawn out from glass tubing in a Bunsen flame to a nearly cylindrical capillary form, are gauged in a wire gauge and cut off at the required sizes. The gauges used are such as the Starrett Morse Drill and Wire Gauge, which has holes ranging in diameter from 5·79 mm. down to 0·34 mm.

Tubes larger than these sizes may conveniently be gauged by the Columbia vernier slide gauge. Capillary tubes less than 0·34 mm. may be gauged in a wire drawplate.

In gauging, the capillary tube is pushed gently down into the particular gauge hole required and is then cut off—preferably at the upper surface of the plate, so that the dropping-point shall not come into contact with any trace of greasy matter which may remain on the cleaned gauge plate.

To ascertain the size of drop yielded by such a dropping-point an adjustable constant-pressure apparatus was devised. This (fig. 1) consists of a straight tube of 3 to 4 mm. internal diameter and of such a length, *e.g.* 50 or 60 cm., that the free air space within shall be amply greater than the capacity of the pipette employed. The tube is carefully cleaned, washed finally with distilled water, and dried with grease-free cotton wool drawn through on a thread. The ends are opened out slightly funnel-shaped to facilitate the ramming in of an inch or so of pure cotton wool, which is required to retard the passage of air and to prevent effectively the escape of any of a column of pure dry mercury, 20 cm. long, which is introduced to act as a plunger.

The tube is arranged as shown in the figure. The right-hand end is joined by a short rubber tube to the upper end of a pipette, which is supported by a clamp, and the left-hand end, attached to a screw-clamp, can be moved up or down the tall stem of a retort stand.

The stem of the pipette has an internal diameter of 1 mm. or so, and the lower of its two calibration marks is 1 or 2 cm. below the bulb. Then

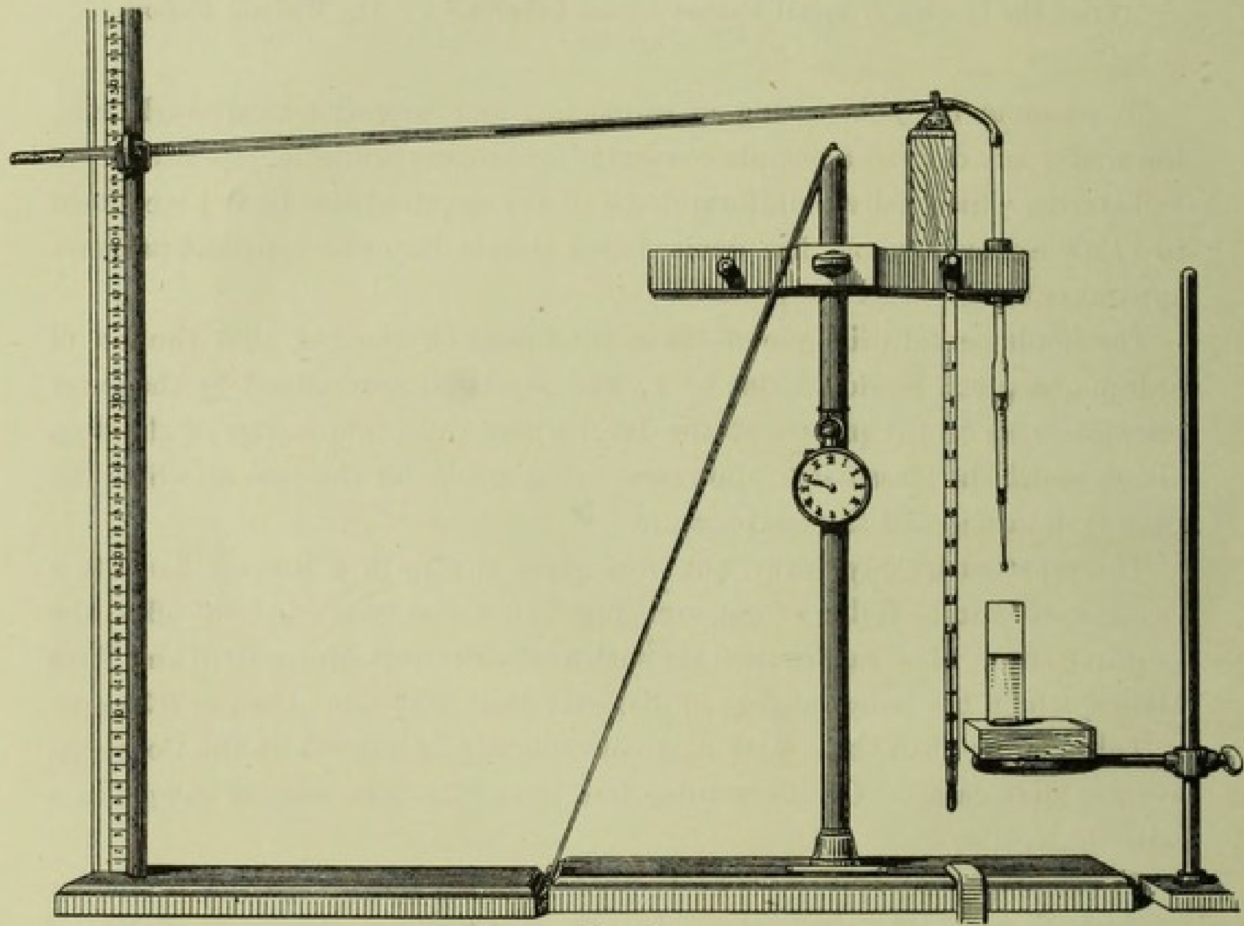

FIG. 1.

1 or 2 cm. below this the pipette is joined by a short piece of cleaned bicycle valve-tubing to the upper end of the capillary dropping nozzle. For special work the junction may be made by means of a sleeve of good cork or by grinding or fusing the nozzle on to the pipette point.

The liquid friction in a small dropping nozzle ought to be such that the head of mercury employed may be great enough to render negligible the loss of 2 or 3 cm. head of water as the pipette empties. The acceleration of the falling mercury down the well-throttled sloped tube is negligible.

To carry out a dropping experiment the mercury is first brought to the right-hand end of the tube, or, if the dropping nozzle has great friction, to within 1 or 2 cm. of the end. The vessel of liquid is raised to cover the end of the nozzle. The left-hand end of the mercury tube is then depressed till the pipette is nearly full. Next it is gently raised until the upper meniscus of the liquid rests at the upper calibration mark. Finally the vessel of liquid is lowered.

Now the end of the mercury tube is raised till the drops fall at the required standard rate, say one per second. The height required for the dropping-point in use is marked by a sliding ring. Then the pipette is refilled, the mercury tube is at once elevated to the required point, and the drop-count at the uniform standard rate is observed.

If necessary, the mercury tube may be slightly lowered at the last drop to allow estimation of the fraction of a drop.

The drop-counts for distilled water thus found are given in the following table. The fourth column contains the quotient drop-weight in mgrm./diam. in mm., *e.g.* for Morse gauge 80, 1000/131/0·340 = 22·45. These quotients are seen to form a fairly uniformly falling curve as the dropping-point increases in size.

Drop-count. Distilled Water.

Morse gauge No.	Diameter.	Drop-count (20° C.) per 1 c.c.	$\frac{\text{Wt. in mgrm.}}{\text{Diam. in mm.}}$
	mm.		
80	0·340	131·0	22·45
79	0·366	122·0	22·4
78	0·406	112·9	21·9
77	0·457	101·0	21·74
75	0·533	87·0	21·6
72	0·633	73·5	21·4
68	0·787	58·5	21·4
66	0·838	56·5	21·1
62	0·965	51·6	20·1
60	1·016	49·8	19·9
58	1·067	48·3	19·53
57	1·092	47·9	19·1
54	1·397	38·9	18·4
52	1·612	34·0	18·25
43	2·261	25·5	17·4
33	2·870	20·3	17·2
28	3·569	16·7	16·8

At different rates of dropping the drop-count differs as shown by the following observations:—

	Diameter.	Sec. per drop.	No. of drops obtained.
	mm.		
Dropping-tube (throttled on separator cylinder with Mariotte's tube)	14·4	0·5	41·0 from 10 c.c.
		0·6	41·5
		1·0	42·0
		2·73	46·0
Dropping-tube similarly fitted	8·4	0·5	76·0
		2·5	84·0
Mercury tube pressure, Morse 33.........	2·87	0·5	22·2 from 1·125 c.c.
		1·0	23·0
		2·7	23·2
		3·0	23·9
		4·3	24·3
		12·0	24·6
,, ,, Morse 56.........	1·181	0·27	45·0
		0·44	46·0
		1·0	50·0
		1·6	51·3
		3·0	51·5
,, ,, Morse 80.........	0·34	0·5	68·0 from 0·5 c.c.
		0·75	67·0
		1·0	66·0
		2·0	67·0
,, ,, D.P. VIII	0·29	0·32	95·0
		0·6	90·0
		1·0	86·4
		1·33	86·1
		1·63	86·2
		3·0	86·4
Hand-teat pressure, D.P. X	0·25	0·43	116·0
		0·7	106·0
		1·0	99·0

From the above observations it may be seen that just in the cases where fine accuracy is most desirable—namely, in measuring by hand one or two drops—these small drops have in their favour the peculiarity (pointed out by Ollivier) of being practically constant in weight at all expulsion-rates slower than one drop per second. Such rates can be easily and reliably secured by the use of a hand mercury-plunger tube—a miniature form of the mercury tube described above.

The narrow limits of drop-rate required for large drops, *e.g.* of 1/5 to 1/4 c.c. in the Wassermann test, may be secured by the use of the arrangement shown in fig. 2.

A separate cylinder of convenient size has a small Mariotte's tube fitted by a rubber cork through the upper aperture. Various dropping-tubes, smaller to telescope inside, or larger to telescope outside the stopcocked tube, may be fitted on by a ferrule of washed rubber tubing or of good cork. A dropping-point to give watery drops of ¼ c.c. may be formed of tubing 14½ mm. external diameter. The dropper is held in a stand at a height convenient to allow a rack of test-tubes to be slid under the dropping-point.

The drop-rate may be regulated as desired by fitting inside the dropping-tube, and within forceps-reach of the orifice, a throttle made of suitably drawn out capillary tube.

The author has found the apparatus efficient in the following applications:—

(1) Comparison of surface-tension of liquids, in quantity as little as ½ c.c., *e.g.* in testing the value of the meiostagmin reaction in syphilis.

(2) As the drop yielded by one pipette differs by not more than a fraction of 1 per cent. from the drop similarly yielded by another pipette similarly gauged in the same hole, the method may be used, with successive fresh pipettes, for rapidly and accurately measuring off small quantities of any liquid, from 0·25 c.c. to, say, 0·004 c.c. of watery liquids of high surface tension, or even smaller quantities of liquids of low surface tension, for instance, in:—

(*a*) Measurements of various small quantities, in *e.g.* Wassermann reaction, micro-Wassermann reaction, and other complement-fixation tests; Widal reaction; alkalimetry, acidimetry, and other volumetric tests; testing cultures and disinfectants; and in pharmacy.

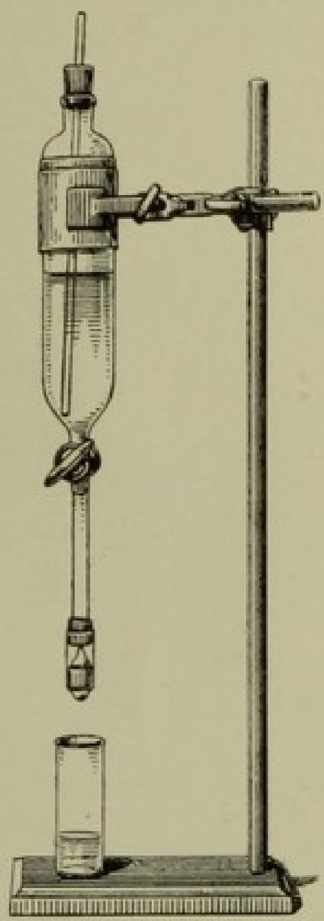

Fig. 2.

(*b*) Direct estimation of cells, numerically, and roughly qualitatively, in small drops of cerebro-spinal fluid, dried on a slide.

(*c*) Blood-count, red cells and white cells, from drops of diluted blood, similarly dried.

(*d*) Direct counting of bacteria in small dried drops of diluted vaccines, distilled water, domestic water, sewage effluent, diluted milk.

(*b*), (*c*), and (*d*) yield permanent preparations.

Harrison and Sons, Printers in Ordinary to His Majesty, St. Martin's Lane.