AN IMPROVED PIPETTE FOR MEASURING SMALL VOLUMES OF BACTERIAL SUSPENSIONS

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The glass capillary pipette, for the measurement of small volumes of bacterial suspensions or other liquids, was first used by Donald[1] and later elaborated by Wilson[2] and Withell.[3]

Glass pipettes are fragile and damage to the tips renders them inaccurate. Marston[4] described an all-metal pipette which overcame this difficulty but she found that it was a disadvantage not to be able to see the level of the liquid in the pipette. The dropping pipette described below has been designed to provide a robust tip combined with adequate observation of the liquid inside the pipette.

Description. The pipette is constructed from a 6-inch length of 7·5 mm. diameter glass tubing drawn out at one end and this end ground to fit a hypodermic needle. A stainless steel hypodermic needle of suitable diameter is fitted to the shaped end of the glass barrel, the end of the needle being accurately ground flat in order to provide a satisfactory dropping tip. The pipette is used clamped in a vertical position with a rubber teat attached to the upper end. It was found that difficulties due to the needle falling off or to leakage at the joint did not arise under the conditions of use.

Calibration. A size 20 gauge hypodermic needle was ground down and used in the initial experiments. Each experiment consisted of weighing 10 lots of 5 drops of distilled water dropped from the pipette. 3 such experiments were performed and the results gave an analysis of variance as shown in Table I.

TABLE I

Analysis of variance for single needle

Source of variance	Degrees of freedom	Sum of Squares	Mean square
Between experiments ..	2	0·258	0·129
Within experiments ..	27	4·537	0·168
Total	29	4·795	—

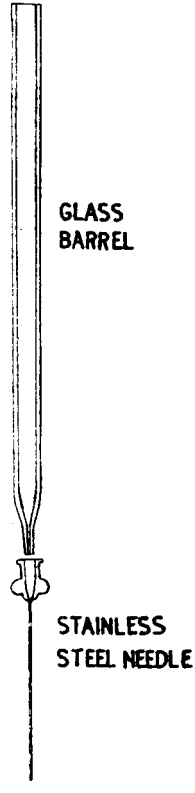

Fig. 1

As there is no significant difference between these variances, the three experiments were pooled to give:—total variance = 0·165345; standard deviation = 0·4066; standard error = 0·0742. Mean weight of 5 drops (95 per cent. fiducial limits) = 83·3 — 83·6 mg.

The greatest deviation and the mean deviation from the mean were 0·9 per cent. and 0·3 per cent. respectively, these compare favourably with 0·9 per cent. and 0·32 per cent. for 20 drops and 1·24 per cent. and 0·82 per cent. for 10 drops reported by Withell using all-glass pipettes.

Variation between Needles. The calibration experiments were repeated using eight further needles of a similar size in order to find the between-needle variance. The results gave the analysis of variance set out in Table II.

TABLE II

ANALYSIS OF VARIANCE FOR 8 NEEDLES

Source of variance	Degrees of freedom	Sum of squares	Mean square
Between needles ..	8	236·8522	29·6055
Within needles ..	261	136·4741	0·5229
Total	269	373·3263	—

Thus the between-needle variance is very significant when compared with the within-needle variance. Using the whole 270 weighings of 5 drops samples, the greatest deviation and mean deviation from the mean were 3·7 per cent. and 1·05 per cent. respectively, which figures also compare favourably with Withell's figures for glass pipettes which were 3·76 per cent. and 1·49 per cent. for 20 drops samples and 3·94 per cent. and 1·46 per cent. for 10 drops samples respectively.

Variation due to Rate of Dropping. To find the effect of different rates of formation of the drops a series of experiments was performed using different rates of dropping, and gave the results tabulated in Table III.

TABLE III

EFFECT OF RATE OF DROPPING

Rate of dropping	Mean weight of 5 drops (95 per cent. fiducial limits) mg.
2 per second ..	84·03 ± 0·33
1 " " ..	84·97 ± 0·33
2 per 3 seconds ..	85·02 ± 0·25

Adaptability. The outside diameters of several needles of different sizes were measured and the mean weights (with the corresponding 95 per cent. fiducial limits) of 5 drops of distilled water estimated from the weights of 10 lots of 5 drops for each needle. The results are set out in Table IV.

TABLE IV

EFFECT OF NEEDLE SIZE

Outside diameter of needle mm.	Mean weight of 5 drops (95 per cent. fiducial limits) mg.
0·70	68·06 ± 0·52
0·72	68·63 ± 0·66
0·84	72·66 ± 0·46
1·10	95·65 ± 0·79
1·28	116·87 ± 0·75
1·68	146·84 ± 1·32

Sterilisation. The sterilisation of the glass barrels presented no difficulty. Since they were interchangeable and they had no effect on drop size, sufficient clean sterile barrels for any given number of experiments were prepared and stored. The following experiments were performed to find a suitable method for sterilising the needle. A 24 hours' culture of *Bact. coli* (Lister Institute No. 5933) was used in these experiments. The culture medium was a plain peptone water. A sterile pipette was fitted up and 5 drops of the bacterial culture were dropped into 5 ml. of sterile broth. The needle was removed from the barrel and immersed in boiling water for a definite length of time. The needle was then transferred to another sterile glass barrel containing sterile culture medium and 5 drops of the sterile culture medium were transferred from this pipette into a further 5 ml. of sterile broth.

The two tubes of 5 ml. of broth were then incubated for 48 hours. No growth in the second of the tubes was taken as indicating the sterilisation process had been successful. 10 replicates were performed in each experiment, the time of immersion of the needle in the water was varied between 10 and 60 seconds. Boiling water and water at 80° C. and 60° C. were also used. The results showed that 10 or more seconds immersion in boiling water or water at 80° C. was sufficient to sterilise the needle but 60 seconds at 60° C. was insufficient.

Effect of Sterilisation of the Needle on Drop Size. A sterilisation process of 30 seconds' immersion in boiling water was chosen as giving a wide margin of safety. 10 lots of 5 drops of distilled water, from one needle were weighed; a succession of further 10 lots was then weighed, the needle being immersed for 30 seconds in boiling water between each weighing. The outside of the needle was wiped dry with sterile filter paper after immersion in the boiling water, the weighings were repeated allowing the needle 1, 2, 3 minutes respectively to cool after drying with filter paper. The results are summarised in Table V.

TABLE V

EFFECT OF STERILISATION ON DROP SIZE

Time allowed for cooling after 30 secs. in boiling water	Mean weight of 5 drops (95 per cent. fiducial limits) mg.
None (initial)	84·99 ± 0·55
1 minute	84·33 ± 0·40
2 minutes	85·16 ± 0·40
3 minutes	84·97 ± 0·21

DISCUSSION

The pipette described has one great advantage over the glass dropping pipette, namely it is more robust and at the same time possesses a slightly greater accuracy. The interchangeability of the glass barrel compensates for the disadvantage of being in two parts when compared with the single piece glass pipette. The time and temperature necessary for sterilisation, namely 30 seconds' immersion in boiling water, gives a wide margin of safety when using a vegetative culture of *Bact. coli.* Longer times may be

necessary when dealing with other cultures. This sterilisation process permits of the repeated use of the same needle and so eliminates the error due to the variation between needles. However, use of a fresh needle for each batch of drops would give an error which compares favourably with the error obtained by Withell in using a fresh glass pipette for each batch of drops.

Summary

1. A new type of dropping pipette was made consisting of a piece of glass tubing ground to fit a hypodermic needle. Various sizes of needle, with the points ground off were fitted to the glass barrel and enabled a variety of drop sizes to be measured.

2. Examination of the errors due to difference between (*a*) successive lots of drops from the same needle and (*b*) lots from different needles of the same size, has shown that those due to (*b*) were the greater.

3. It has been found possible to sterilise the needle after use with a culture of *Bact. coli* by 30 seconds' immersion in boiling water and re-use the needle on a fresh sterile barrel after 3 minutes' cooling, without loss of accuracy.

References

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4. Marstow, *Bull. Inst. Med. Lab. Tech.*, 1948, **14**, 66.