# Technological aids to microbiology

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# Preface

This book is intended primarily for bacteriologists and bacteriological technicians working in routine medical diagnostic bacteriology laboratories, but it is also likely to be of interest to similar people working in other types of microbiology laboratories.

Brief expositions of many aids and the applications to which they have been put hitherto are given, but this is *not* a comprehensive review of all available devices in all categories. Such a review would be an enormous tome, since one could write a dissertation on each category of device included, and be out of date before it was published. I merely give a few examples. The suggestion is that the reader should ask himself if he has an application for any of the devices discussed, or any similar devices, in his own laboratory.

It is not appropriate to discuss the philosophy of introducing mechanization into microbiology in this book. (Care must be taken not to confuse mechanization with automation, which, at its simplest, involves far more sophisticated techniques and apparatus than any presently in regular use in diagnostic microbiology.) A number of questions, such as whether it is better to use equipment designed to perform one specific procedure or more flexible equipment and whether the sole or primary purpose of mechanization should be cost effectiveness, have to be considered. My thoughts on many such matters were published in 1973* and are unchanged. But one may well have very special reasons exclusive to one's laboratory for introducing a particular type of machine, and should not necessarily be put off by the fact that the conditions pertaining do not satisfy the so-called normally accepted criteria, about all of which there is still considerable controversy.

If the reader decides he has an application for a technological aid, it is up to him to study the literature, to look carefully at all possibilities and then to go out and acquire the apparatus he requires and develop his own procedure. Incidentally, that is the time to consider updating one's technique and reorganising one's laboratory, as opposed to merely mechanizing an existing technique.

There is no definitive section on microbiological safety, but one should always be cautious, particularly because, as I indicate in the text, some inventions seem to me to be positively dangerous. Even when that is not the case, there is often no hint that the safety of the device was considered. There appears to be a serious lack of awareness of microbiological safety by both inventor and user. No general rules can be made and certainly no regime for the safety testing of microbiological apparatus has been universally adopted. One has to devise one's own scheme, but it is very important to do so. Much more awareness of the potential hazards of devices used in microbiological laboratories is needed.

No doubt readers will be able to point out categories of devices to which no reference has been made but which might have been included. One has, of course, to make a positive decision about such matters, since neither time nor

* Trotman, R. E. (1973). The philosophy of the application of automatic methods to hospital diagnostic bacteriology. *Bio-Med. Engng* 8, 519.

space are unlimited. I have included those categories of device about which I feel competent to write and from the use of which in my view the greatest benefit can be derived: the scope of the book has been restricted to relatively simple aids, because very little work leading to the development of more complex technological systems has produced practical systems of use to more than a handful of very specialized laboratories.

It will be appreciated that one requires a great deal of assistance in producing a volume such as this. In particular, authors of published papers and books have given invaluable assistance in supplying many photographs, sketches, drawings and graphs relevant to their devices. I am most grateful to them all for so readily putting themselves to so much trouble for me and for giving permission to use the material in this way. I wish also to express my gratitude to the following: the editors of journals and publishers of books for permission to reproduce photographs and drawings that have previously appeared in their publications; the St Mary's Hospital Medical School's Audio-Visual Communications Department for producing many of the photographs (some of the tasks I presented it with, particularly in producing Fig. 1, were very difficult, but I am sure readers will agree that the effort was well worth while); Mr Kevin Byrne for producing many of the drawings; and Mrs Heather Downey and Mrs Peggy Howard for typing the manuscript.

R.E.T.

London, 1977.

# Contents

# 1
# Devices for distributing infected and/or sterile fluids

There is clearly a need for devices for distributing fluids, such as antibiotic solutions and nutrient broth, aseptically and for distributing infected fluids. We now discuss the most commonly used.

## Pipetting small volumes of fluids

### *Dropping pipettes*

In 1913 Donald described a device for producing measured small volumes of liquids, and he gave more details of its performance in 1915. The apparatus is a glass pipette with a perfectly cylindrical capillary, from which a fixed number of drops gives 1 ml of liquid. The actual number depends on the outside diameter of the capillary, and Donald described those devices giving approximately 130 drops/ml to 10 drops/ml. Wilson (1922, 1935) and Miles and Misra (1938) have exhaustively investigated various applications of the pipettes.

A pipette in common use today is one which gives 50 drops/ml; it is known as the 50 dropper. Donald showed that if the difference in outside diameter varies from 1.016 to 0.914 mm the number of drops/ml varies from 50 to 54, which is an error of $\pm 4\%$. This doubtless applies only if the capillary is cylindrical and if the tip of the pipette is smooth and cut at right angles to the cylinder.

There is no reason to doubt that, when carefully manufactured, 50 droppers are as accurate as this, but it is most unlikely that they are manufactured sufficiently accurately under modern routine laboratory conditions. We found that the mean volume of 12 drops dispensed from a 50 dropper manufactured in our laboratory was 0.02 ml but that the volume of individual drops varied between 0.0237 and 0.0175 ml. We found also that the mean volume of 12 drops from each of 12 different pipettes varied between 0.0172 and 0.0244 ml. These results are hardly surprising when one considers the difficulty in keeping the capillary cylindrical during manufacture, and also the difficulty in cutting the tip smoothly to within 0.1 mm in diameter, which is necessary for an accuracy of $\pm 4\%$.

One may argue that one should pay more attention to the manufacturing process, but this is a very time-consuming, and therefore costly, procedure. It is not surprising, therefore, that many people have devoted considerable efforts to devising more reliable devices for distributing small volumes of fluids.

Today a variety of dropping pipettes, both disposable and reusable, are commercially available and details of their evaluations have been published.

Unfortunately, these are usually hidden away in papers the primary purpose of which is to describe a technique in which the use of the pipettes is incidental, in such papers as those describing serial dilution techniques for antibiotic sensitivity testing.

MacLowry et al. (1970) used some 50 µl dropping pipettes marketed by Cooke Engineering Company* to dispense diluent, broth and test organisms. These pipettes are calibrated to deliver 50.0 µl of 0.9% NaCl/drop, but the size of drop varies according to the surface tension of the fluid being dispensed. The authors state, for example, that the pipettes dispensed 48.8 µl of human serum, 41.1 µl of Mueller-Hinton broth and 40.8 µl of Trypticase Soy broth, but they did not give evidence of having established these figures experimentally.

The particular pipettes used are reusable. They were placed in Amphyl for between 2 and 6 h, rinsed three to four times in distilled water, inverted and allowed to dry overnight. Each pipette was then wrapped in a paper towel sterilized with ethylene oxide and allowed to remain at room temperature for 1–2 h before reuse.

The pipettes were recalibrated every six months, or more frequently if there was evidence to suggest the results of the procedure were poor. The authors referred to a commercially available pneumatically operated dispensing device, but gave no details.

Tilton et al. (1973) used 50 µl disposable pipettes to distribute inoculum in an antibiotic sensitivity test procedure. To test the accuracy and repeatability of the pipettes they selected samples of pipettes at random, delivered 20 drops from each and determined the average drop volume for each of 0.85% saline, brain-heart infusion broth, Mueller-Hinton broth and Trypticase Soy broth. Each experiment was repeated ten times and they found the mean drop volume to be 49.2 µl, 47.0 µl, 46.0 µl and 46.2 µl respectively.

Gavan and Town (1970) found that the average size of drops dispensed by 50 µl dropper pipettes varies depending on whether or not the plastic microwell plate into which the drops are dispensed is charged with static electricity. They found that an average volume of 39.0 µl $\pm$ 10 µl was dispensed if the plate was charged and an average volume of 47.4 µl $\pm$ 3.6 µl was dispensed if it was not; these figures are for dispensing distilled water. Because of this phenomenon Tilton et al. (1973) held their dropping pipettes 2 cm above the microwell plate to keep errors due to static electricity to a minimum.

Fitzgerald et al. (1974) briefly referred to 10 µl pipette droppers. They did not report their evaluation of them but merely indicated that they required the maintenance of a constant pressure to deliver the appropriate amount of reagent without introducing air bubbles.

The above brief references to some evaluations of commercially available dropping pipettes, which are very widely used, show that they are most valuable but that, as with so many devices, a carefully controlled routine for their use and their care has to be established, and that constant checking of their performance is necessary. These factors are often neglected in such

* Cooke Engineering Company, now incorporated in Dynatech Laboratories Inc., 900 Slaters Lane, Alexandria, Virginia 22314, USA.

simple devices, because one tends to take the attitude that there is nothing to go wrong with them. The fact is that their performance very easily deteriorates and it should never be taken for granted.

## *Semi-automatic pipettes*

In addition to care, some skill is required in using dropping pipettes. In consequence, in recent years a wide variety of semi-automatic high-precision microlitre pipettes have been devised. Although they vary in detail, all consist, essentially, of a high-precision syringe with a spring-loaded plunger which has either a fixed stroke length, as in the Eppendorf* type, or a variable stroke length, as in one Pipetman† type. The plunger is drawn up and down to suck up and discharge a volume of fluid, which volume depends on the stroke length in the conventional way. A disposable presterilized tip is attached to the bottom of the barrel of the syringe, and one should not attempt to use these devices without the tip. In normal usage, the fluid to be dispensed comes into contact only with the tip, which for the highest accuracy of dispensing should be held in contact with the side of the vessel into which the fluid is discharged. In most types, the syringe plunger may be moved marginally beyond the normal end of stroke when expelling fluid to ensure that the last drop of it is ejected from the tip. A photograph showing the principle of these pipettes is shown in Fig. 1.

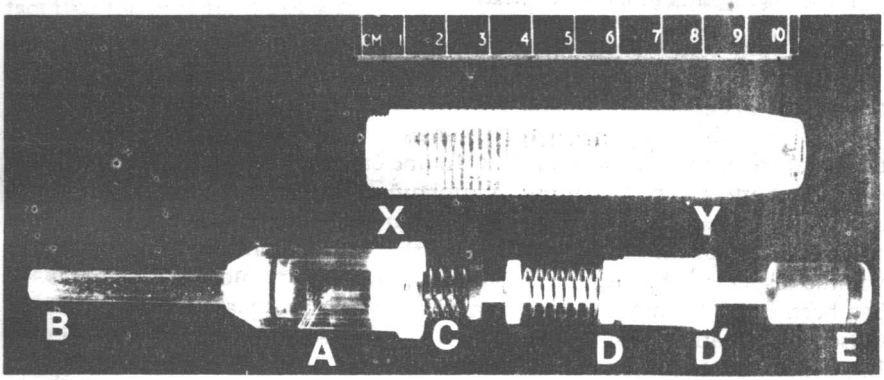


Fig. 1. Exploded view of an Eppendorf pipette. A: syringe barrel. B: rod to which the disposable tip is attached. C: spring-loaded syringe plunger. D, D': stops, the distance between which determines the stroke length of the plunger. E: actuating rod. X, Y: the threaded parts of the pipette are screwed into the corresponding threaded parts of the barrel. (Loan of exhibition model of the pipette courtesy of Anderman Co. Ltd.)

Volumes dispensed vary, but are usually a maximum of 5 ml. For example, at the time of writing, Eppendorf manufacture 25 pipettes in the range of 5 µl to 1 ml and Gilson† manufacture four Pipetman types in which the volume dispensed is continuously variable between 0 and 20 µl, 0 and 200 µl, 0 and 1 ml, and 0 and 5 ml respectively.

* Eppendorfer Gerätebau, Netheler & Hinz GmbH, 2000 Hamburg 63, Postfach 630324, West Germany.

† Gilson France, 69/72 Rue Gambetta, 95400 Villiers le Bel, France.

Joyce and Tyler (1973) evaluated the performance of five different types of semi-automatic disposable tip pipettes. The models tested and the volume dispensed are set out in Table 1.

*TABLE 1*

| *Type of pipette* | *Volume dispensed* (µl) |
|---|---|
| Biopipette | 100–200 (preset) |
| Biopipette | 100–1,000 (100 multiples) |
| New Eppendorf | 100 (preset) |
| Finnpipette | 200–1,000 (adjustable) |
| Gilson Pipetman | 0–200 (adjustable) |
| Oxford Sampler | 100 (preset) |
| Oxford Sampler | 200 (preset) |
| Marburg Eppendorf* | 100 (preset) |
| Marburg Eppendorf* | 1,000 (preset) |

*The last two pipettes are no longer marketed.

They found that in most cases the volume of deionized distilled water dispensed was temperature dependent, there was a tendency for the pipettes to dispense progressively smaller volumes until the temperature reached 30 to 32 deg C, which, they state, is approximately the closed-fist hand temperature of their laboratory staff. In one case they found the temperature-dependent effect to be 'alarming'. Unfortunately, they did not state the accuracy of dispensing when the temperature has stabilized at the hand temperature, but they did say that when there was a marked change in volume dispensed this usually stabilized by the time 12 successive samples had been ejected. They also found that two continuously adjustable pipettes, Gilson Pipetman and Finnpipette,* were as precise as those (such as the Eppendorf pipettes) that deliver a fixed volume. They believed the adjustable pipettes to be generally preferable to the fixed-volume pipettes. Of the fixed-volume pipettes there were two makes in particular, the Eppendorf's and the Oxford's,† in which it was advisable to prewarm the pipette for at least 8 min before use, if highly repeatable samples were required.

Ellis (1973) evaluated the Eppendorf pipettes and the Oxford samplers and he also found that the volume delivered was temperature dependent. He indicated that the conditions have to be well controlled for the performance of the pipettes to comply with the performance quoted by the manufacturers.

He calibrated the apparatus by weighing the amount of water or solution dispensed each delivery. The mean weight and standard deviation of the mean were determined from at least 6 weighings, and the volumes were calculated using the density of the water or solution dispensed. Using an

* Labsystems OY, Pulttitie 9, 00810 Helsinki, Finland.
† Oxford Laboratories (International), 1149 Chess Drive, Foster City, California, USA.

Eppendorf 0.05 ml pipette, he found that the volume delivered at 25 deg C and at 0 deg C was 0.0501 ml ± 0.0002 ml and 0.0467 ml ± 0.0005 ml respectively. Experiments to determine the effects of prerinsing the tip, of the repetitive usage of tips and of the variation of the temperature over the whole pipette unit were also performed, but unfortunately, the evidence given was concerned mainly with the temperature variation effect. It was found that all parts of the system should be at the same temperature. For example, if the temperature of the syringe differed from that of the fluid being dispensed, the volume delivered was very dependent on the exact procedure followed.

Robinson and Johnson (1974) assessed the accuracy of the smaller volume (5–100 µl) semi-automatic pipettes. They found that the precision of dispensing decreased as the volume decreased. For example, using an adjustable Finnpipette in the range of 5–50 µl they found that when set to deliver 50 µl the mean of 10 volumes dispensed was 50.84 µl, giving an error of +1.6%, whereas when the same pipette was set to deliver 5 µl the mean of 10 volumes was 5.49 µl, which is an error of +9%. The coefficient of variation was found to be 0.4% and 2.4% respectively. They also found that with a 50–250 µl Finnpipette set to deliver 50 µl, the mean of 10 volumes dispensed was 52.06 µl, an error of +4.12%. They felt that these results suggested that the errors were related to the stroke length of the plunger of the syringe. This may well be so, although it is probably also related to the fact that the plunger is not directly in contact with the fluid being sucked up and dispensed; there is an air gap in between which is, of course, highly compressible, and the longer the stroke length the less significant errors due to this effect are likely to be.

Examples of the results obtained with fixed-volume Eppendorf pipettes are: a 5 µl pipette delivered a mean volume (of 10 volumes) of 5.32 µl, an error of +6.4%, and a 10 µl pipette delivered a mean volume of 10.1 µl, an error of +1%.

An interesting observation was that although Robinson and Johnson had been using their pipettes for a period of up to two years, they did not find any progressive deterioration in the performance over that period. Furthermore, they attributed bad performance to spurious contamination in the capillary of the pipette, to wear and tear and to lack of lubrication of the plunger mechanism. They found that on routine servicing the performance deteriorated, but this was believed to be due to contamination in the capillary which, in turn, was believed to be due to reverse pipetting, in which some tips are filled almost to capacity; this clearly increases the likelihood of contamination. In summary, Robinson and Johnson found that when dispensing volumes of 50 µl and above, the pipettes conformed to the manufacturer's claims, but that when dispensing smaller volumes there was a progressively greater discrepancy between performance and the manufacturer's claims.

Bousfield et al. (1973) used a 100 µl Eppendorf pipette to dispense cell suspension in a method for counting bacteria: 100 µl volumes of cell suspension were pipetted into 100 ml portions of diluent and mixed. Using the Eppendorf pipette, 100 µl of this dilution was transferred to a further 100 ml of diluent, thus giving a final dilution of $10^6$; 100 µl of this dilution was

then deposited as a series of 5 or 6 drops on the surface of a nutrient agar plate. The total number of colonies in the drops were counted after incubation overnight. The original suspension contained $2 \times 10^9$ *Escherichia coli*/ml in a mineral salt solution, so each drop contained approximately 200 cells. Unfortunately, they did not analyse the performance of the Eppendorf pipette separately.

Bousfield and his colleagues compared this method with the pour plate and the spread plate methods. They found it slightly less precise than the pour plate method but more precise than the spread plate method. However, it involves less preparative work and is easier and quicker to perform than the pour plate method.

From the above discussion on semi-automatic disposable tip pipettes one can understand why they have begun to supersede the old dropping pipette. Nevertheless, all is not plan sailing and I can do no better than quote Joyce and Tyler (1973). 'However, they [the disposable tip pipettes] can differ considerably in ease of use, price, accuracy and precision.' So, like the dropping pipette, their performance cannot be taken for granted. Furthermore, they have one disadvantage in that they will dispense 1 drop of fluid per filling whereas a dropping pipette will dispense many drops per filling. We return to that matter later.

Recent additions to the pipettes that are commercially available are the multichannel Labpipettes.* These are 4– or 8–channel pipettes very similar in principle and in operation to the single-channel Finnpipette. There are 22 pipettes, in the range 5–200 µl, available in both the 4– and 8–channel systems. The 'average accuracy' is claimed to be $\pm 1.5\%$. The tips are suitable for both single– and multichannel versions.

## Transferring small volumes of fluids

### *Diluting loops*

In 1955 Takatsy devised a simple device for titrating small measured volumes of liquid, which has had considerable impact in a variety of pathological laboratories. This device, known as the microtitrator loop, consisted of a lightly wound wire spiral closed at each end (Fig. 2). One of his loops titrated

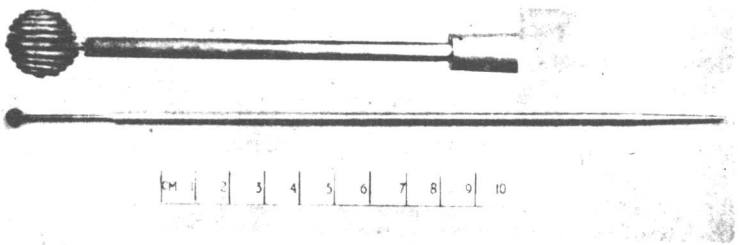


Fig. 2. A modern wire spiral-type microtitrator loop.

*Labsystems OY, Pulttitie 9, 00810 Helsinki, Finland.

0.025 ml of liquid and another titrated 0.05 ml. One simply dipped the loop into the liquid, rotated the loop a few times, removed it from the liquid and the appropriate volume was held in the wire spiral by capillary action. The liquid so held can be transferred and mixed with another liquid.

The microtitrator loop was first used (for serological titrations) by Sever (1962), but he found many difficulties. The wire, which was not made of stainless steel, tended to rust and it was easily distorted, thus becoming very inaccurate. Furthermore, in order to ensure that it transferred the correct volume, the loop had to be prewet and all particles and grease had to be removed before use. Those factors indicate that the original loops were very delicate and became inaccurate very easily.

In recent years, the quality of the microtitrator (now more commonly called the microdilutor) loops, many of which are no longer lightly wound wire spirals but are made from solid metal (Fig. 3), has improved considerably and many are commercially available.

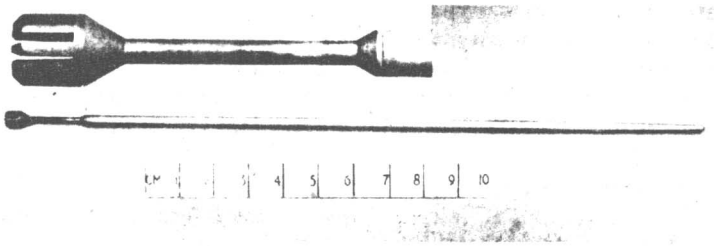


Fig. 3. The type of microtitrator loop which is fabricated from solid metal.

A detailed comparison of the performance of both types of loop was carried out by Ashcroft et al. (1971). This work was concerned primarily with the performance of the loops in the haemagglutination inhibition test, so we will leave detailed discussion of this paper until later (see p. 59). Suffice it to say here that Ashcroft and his co-workers found no really significant difference in the performance of the two types of loop. They found the loops transferred, on average, 90% of the nominal volume of fluid. They found also that the performance did not deteriorate significantly over a 'period of use'. Unfortunately, they did not say how long this period was.

Microtitre techniques are becoming increasingly widely used, primarily because of the saving in reagents that may be effected by using them; earlier techniques use much larger volumes of reagents.

A very common application of the microdilutor loops is in serial dilution antibiotic sensitivity testing. Marymont and Wentz (1966) used a 50 µl loop for this purpose. They used 50 µl volumes of diluent and, with an initial antibiotic concentration of 200 µg/ml, serially diluted the antibiotic 12 times, using the now familiar multiwell reusable or disposable plastic plates. The minimum concentrations of up to three antibiotics required to inhibit growth of up to 60 bacteria were determined by this method and also by the standard tube dilution method; a total of 111 minimum inhibitory concentrations (MICs) were measured by each method. In 70 cases the tube dilution method

gave a MIC which was either 1 or 2 dilutions higher than that given by the microtitre method, and in 4 cases the tube dilution method gave a minimum inhibitory concentration which was 3 or more dilutions higher than that given by the microtitre method.

Harwick et al. (1968) also used the microtitrator system to determine the MIC of antibiotics, and, in addition, to determine the minimum bactericidal concentration (MBC). Seventy-six organisms isolated from blood cultures were tested against up to 16 antibiotics (penicillin G, kanamycin, ampicillin, methicillin, tetracycline, chloramphenicol, lincomycin, 5-cloro-7-deoxylincomycin, carbenicillin, streptomycin, polymyxin B, colistin sulphate, rifampicin, cephalothin, erythromycin glucoheptate and vancomycin) by both the standard tube dilution method and by the micro method.

The standard tube dilution method was as follows. Suspensions of antibiotics in distilled water, at a concentration of 1,000 µl/ml, were prepared and serially diluted 9 times; the dilutions were twofold; 0.1 ml of each dilution of antibiotic solution was added to a test tube containing 0.8 ml of nutrient broth and the tubes were frozen at −16 deg C and stored. As required, tubes were thawed and 0.1 ml of culture containing $10^5$ organisms/ml was added. Cultures were incubated for 18 h at 37 deg C and the first dilution with no apparent growth was considered to be the MIC. This culture and all cultures in subsequent dilution tubes were subcultivated on to blood agar and incubated for a further 24 h. The original cultures were also incubated for a further 24 h. Colonies growing on each subculture were studied and the lowest dilution from which one or no colonies grew was regarded as the MBC (a 99.9% kill).

In the microtitre method, cultures were prepared as before but antibiotic solutions of a concentration of 800 µg/ml were used. The technique was as follows: 50 µl of nutrient broth was added to each well (U bottom) of an 8 × 12 well plate; 50 µl of antibiotic stock solution was added to each well in column 2 and the fluid in the wells mixed by rotating the microdilutors for approximately 5 s. They were then transferred to the wells in column 3 and rotated to mix the broth and antibiotic. This process was continued so that serial dilutions of the original antibiotic solution were made from column 3 through to column 7. Then 50 µl of nutrient broth was added to each well in column 8 and 0.1 ml of nutrient broth was added to each well in the plate. The plates were stored at −16 deg C. To perform the test, 50 µl of the culture was added to each well in columns 1–7, the final titre being approximately $10^5$ organisms/ml. Column 8 was a media control. The plates were incubated for 18 h. The minimum inhibitory concentrations and the minimum bactericidal concentrations were determined as before.

Minimum inhibitory concentrations determined by the macro and micro methods were compared in 922 determinations and 853 (92.5%) agreed to within 2 dilutions. Minimum bactericidal concentrations were compared in 903 determinations and 807 (87.4%) agreed to within 2 dilutions.

The precise combinations of organism/antibiotic tested were not given, but the way in which each of the 16 antibiotics performed was given. The results ranged between 100% agreement (45 tests) with carbenicillin and 75%

agreement (18 out of 24 tests) with lincomycin for the MICs, and between 100% agreement (24 tests) with methicillin and 81% agreement (57 out of 70 tests) with polymyxin B for the MBCs.

The authors found that the microtitration techniques produced a good saving in the labour costs of performing the determinations, and that a technician can perform three times as many determinations with the micro method as he can perform in the same time with the standard tube dilution method. They estimated the total cost to be about one-third of the standard method.

Harwick and his colleagues also performed reproducibility tests on the microtitration system. Isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus* and *Streptococcus faecalis* were each tested 11 times against all 16 antibiotics. Their results indicated that a 95–98% probability existed that the MIC end-point observed would fall within 1 dilution of the mode, and that a 93–97% probability existed that the MBC end-point would fall within 1 dilution of the mode.

## *Semi-automatic diluting apparatus*

Although it is not specifically stated, one is left to conclude that in the methods described above, the loops were held and manipulated manually. Quite clearly, when one is trying to hold and manipulate a number of loops simultaneously, a considerable amount of manual dexterity is required. In consequence, a variety of semi-automatic devices, in which the loops are supported and rotated automatically, have been devised and one of the earliest was by MacLowry and Marsh (1968).

In their device (Fig. 4), which was devised specifically for serial dilution antibiotic sensitivity testing, 12 microdilutors, of either 25 or 50 µl capacity, are attached in a straight line to a vertically movable head; all dilutors may be rotated simultaneously, a preset number of times, by means of an electric motor. The microtitre (U) plates in which the tests are carried out, and the reservoirs of reagents, are placed on a holder, which moves horizontally under the microdilutor loops: the spacing between the loops is identical to the spacing between the wells in the plates. The horizontal movement of the holder and the vertical movement of the head in which the loops are supported are controlled manually by means of levers. Actuation of the motor controlling rotation of the loops is performed automatically by the head as it is lowered.

They gave little detail of their experiments, but they measured minimum inhibitory concentrations of a variety of combinations of organisms and antibiotics by their semi-automatic microtitre apparatus and compared the results with the results obtained by the standard tube dilution method. Eighty-eight MICs were performed by both methods: 68% produced the same end-point, 19% produced end-points which varied by 1 dilution, 7% by 2 dilutions and the remaining 6% by 3 dilutions. The differences did not appear to be related to any particular antibiotic or organism. They also carried out reproducibility studies on their equipment, and of 336 repetitive assays, 86% gave identical results, 13% differed by 1 dilution and 1% by 2 dilutions.

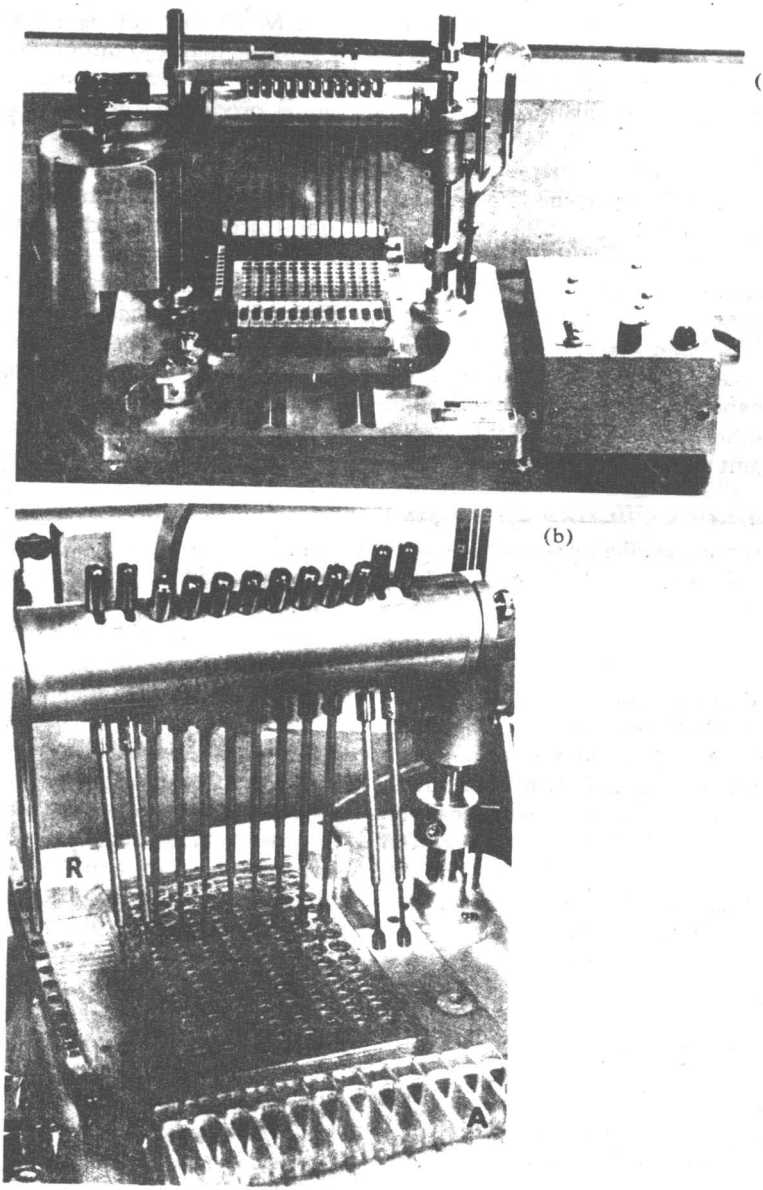


Fig. 4. The machine devised by MacLowry and Marsh for serial dilution antibiotic sensitivity testing by the microtitre method. (a) The complete apparatus. The reservoirs in the foreground, immediately in front of the microtitre tray, contain the antibiotic solutions. The loops are shown situated over a reservoir containing a solution in which they are rinsed at the end of the cycle. (b) Close-up view of the microdilutors, so arranged to utilize only 8 of the 12 loops. A: reservoirs containing antibiotic solutions. R: reservoir of solution in which the loops are rinsed. (From MacLowry, James D. and Marsh, Harry H. (1968). Semiautomatic microtechnique for serial dilution antibiotic sensitivity testing in the clinical laboratory. *J. Lab. clin. Med.* **72**, 685–687.) (Photographs courtesy of Dr James D. MacLowry.)

MacLowry and his colleagues (1970) later published a most valuable paper in which considerable detail of their methodology was given. They discussed the equipment, the calibration and sterilization procedures, the media, the preparation of the antimicrobial agents, and the quality control procedures they introduced. In addition, some most useful tips on reading the plates were also given.

They evaluated the 50 µl microdilutors independently, which were found to deliver a mean volume of 50.4 µl (standard deviation 0.65) of 0.9% NaCl. After 8 months' use the mean volume was found to be 51.6 µl (standard deviation 0.64). Mean volumes of 49.4 and 49.9 µl (with standard deviations 0.36 and 0.71 respectively) of Trypticase Soy broth (and also of serum) were delivered immediately and after 8 months' use respectively.

The preparation of the microdilutors was as follows. They were flamed to incandescence, quenched in distilled water, touched with blotting paper to remove most of the water and then flamed briefly to dryness. This procedure was adopted every morning.

In the experiments described below a commercial version (American Instrument Company*) of the semi-automatic instrument referred to in their earlier paper was used. No details of the machine were given other than a statement to the effect that the instrument holds twelve 50 µl microdilutors.

The accuracy of the twofold dilutions was determined by diluting a standard solution of sodium chloride and measuring the chloride content in each well. The eighth dilution was found to be $\pm 5\%$ of the expected value. All previous dilutions were found to be more accurate than that. The number of experiments used to determine these values was not given. Of course, the error in the serial dilution process is due to the errors in pipetting the diluent and other solutions (discussed on p. 3), as well as due to the serial diluting process. It is not at all clear if the expected values of sodium chloride concentration quoted took that into account or not.

Their apparatus was normally kept in an 'ultraviolet (uv) hood', except when dilutions were being made; the uv light was turned on if the equipment was left unused for more than 15 minutes. The apparatus had been operated for periods of up to 6 h duration without the uv light, but under the hood. The authors stated there had been no contamination problem.

The plates were prepared by dispensing 50 µl of broth diluent into all wells and 50 µl of the initial concentration of each antibiotic into the first well of each row. As the dilutions were made the operator noted the height of the diluent in each of the wells to ensure that it did not rise above the top of the microdilutor 'chamber'; it is not clear if this means the well or the head of the dilutor. The height to which the diluent rises in the chamber should be constant. After use the loops were rotated in sterile distilled water, blotted on filter paper and flamed briefly to dryness. Then 50 µl of a 1/1,000 dilution of a suspension of the organism, prepared by mixing 6–10 isolated colonies in 2 ml of broth, was added to each well. In the case of *Proteus* organisms, a 1/10,000 dilution was used, since the end-points were said to be much sharper and much more reproducible with the smaller inoculum.

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