The Compu-pet 100¹: A versatile dispenser-diluter for the mechanization of microbiological techniques

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SYNOPSIS The Compu-pet 100 diluter/dispenser is a versatile and accurate instrument which can be employed for a wide variety of microbiological tests and techniques. Time in performing tests is often more than halved when the diluter is used. Large numbers of tests can be performed with minimal fatigue.

Automation of diagnostic microbiological methods is not appropriate because insufficient numbers of any particular test are performed to make proper use of autoanalyzers. Full automation may be appropriate in reference laboratories but for diagnostic work mechanization which enables a technician to do a large number of tests without fatigue using versatile equipment without maintenance problems is to be preferred. The Compu-pet 100 is an example of this kind of equipment; it costs about £500.

The Diluter

Pickup and delivery is achieved by a peristaltic pump. The volume is controlled by rotation, in either direction, of a multiroller rotor assembly. Plastic tubing of uniform bore is threaded between the rollers and an adjustable 'shoe' which compresses the tubing during rotation at specific intervals advances fluid through the tubing according to the direction of rotation of the rollers. Two sets of tubing and pipettes are provided, a microdiluter which is threaded through the lower part of the rotor, deals with volumes up to 1 ml, and a macrodiluter, which is threaded through the upper part of the rotor, deals with volumes up to 10 ml. In practice it is seldom necessary to change from small to large volumes so that normally only one tube is threaded through the machine for a given procedure. The pipettes and tubing can be autoclaved when sterile fluids are to be dispensed.

The volume picked up or delivered is governed by the pressure of the 'shoe' on the tubing and the machine is calibrated by fine adjustment of the screws which alter this pressure. The distal end of

the tubing is placed in a reservoir containing diluent or reagent and the machine is primed by pressing the continuous delivery button until the whole of the tubing and pipette is completely filled. When small volumes of a costly reagent are to be dispensed slightly more than the total volume required can be picked up into empty tubing; appropriate volumes are then dispensed and waste is minimized.

Additional facilities are a delivery interval switch enabling constant volumes to be dispensed automatically at second intervals chosen from one to 10 seconds. A foot pedal is also provided which operates the switches leaving the hands free.

A separate scale of volumes operated by selection buttons is provided for each of the two pipettes. The micropipette picks up or delivers 5, 10, 20, 50, 100, 200, 500, and 1000 microlitres. When the macropipette is in use the volumes selected by the buttons are automatically tenfold higher. Delivery, but not pick up, of volumes other than these can be achieved by summation of the pick-up and delivery scales which are operated by a separate manual switch. For example, 40 microlitres can be delivered from the reservoir by pressing the 20-microlitre button on each of the pick-up and delivery scales and then pressing the pick-up + delivery switch. This facility cannot be operated automatically and when operated by the foot pedal an accessory switch must be turned to the pick-up + delivery position.

The micropipette is highly accurate when held by hand but because of the wider area of cross section of the macrotubing this pipette must be held vertically and rigidly in the goose-neck stand provided when accuracy is required.

¹Obtainable from William R. Warner & Co. Ltd., Eastleigh SO5 3ZQ, England.

The new model has been adjusted to allow pick up as well as delivery of the sum of the volumes selected on each scale.

The diluter has already been assessed for radio-immune assay by Wilde (1973) who showed that after 200 hours' pumping there was a 3.1% decrease in delivery. There was no significant change in precision after intensive pumping as determined by calculating the coefficient of variation of 20 deliveries of isotope solution at each setting. When great precision is needed the tip of the pipette must be wiped before delivery to prevent contamination from the outside of the pipette. This degree of precision is not needed in many microbiological tests. Effective wash out of isotope solutions is achieved by delivering diluent of twice the volume of reagent or serum dispensed.

Serological Tests

The precise volume of measured fluids is not often important and therefore the inflexibility of the machine in this respect does not matter, as the volumes used can be adjusted to suit the machine.

Most serological methods require a range of twofold dilutions which are normally made by dispensing a constant volume of diluent into each well or tube, mixing by sucking up and down several times, transferring the same volume to the next tube and so forth discarding one volume from the last tube. The diluter cannot be used in this way because mixing by operating the pick-up and delivery switches leads to mixing of serum with diluent in the tubing. Provided mixing is achieved when serum plus an equal volume of diluent is discharged, mechanical mixing by shaking the tubes or sucking up and down would not be necessary. Mixing will depend partly on the force with which serum plus diluent is ejected and partly on the size and shape of the receptacle. Several types are in common use. Test tubes 3 in. $\times$ $\frac{1}{2}$ in. and 2 $\frac{1}{2}$ in. $\times$ $\frac{1}{4}$ in., Dreyer's tubes 6 mm $\times$ 60 mm with tapered ends, WHO trays with wells 15 mm diameter $\times$ 10 mm depth, microtitre trays with U wells 6 mm diameter $\times$ 10 mm depth.

Twofold dilutions made by the diluter in these various receptacles were tested to discover whether mixing is satisfactory without additional mechanical aid.

Tests of Mixing in Twofold Dilutions

Titration of complement in preserved guinea-pig serum was used as an indicator system in trays.

WHO TRAYS

The manual method was carried out with a Pasteur pipette held in an automatic syringe which picks up

and delivers constant volumes; mixing is achieved by sucking up and delivering repeatedly in each well. Eight titrations were made in parallel for each method.

MICROTITRE TRAYS

The manual method was dilution of 25 microlitre volumes using Takatsy loops. Since the diluter will not pick up 25 microlitres in one operation¹ 25 microlitre volumes were diluted, the other reagents being adjusted accordingly. Eight parallel titrations by each method were performed on two occasions.

In each case the endpoints were easier to read in the diluter titrations because the number of residual cells in the tubes with partial lysis was more nearly equivalent in each row. In the WHO trays the endpoint was identical in all the diluter titrations. It was twofold lower than the manual method in all but two rows where the manual method showed a trace of intact cells and the reading would therefore have been the same as the diluter titration. In the microtitre trays all rows in all the diluter titrations were identical as were those in the manual method. When performed by two different people using the same reagents the titre was twofold lower in the diluter titrations but on the second occasion when both methods were performed by the same person the endpoints were identical. The degree of lysis in the first row showing intact cells was, as before, more regular in the diluter series.

DREYER'S TUBES

Salmonella paratyphi H serum was titrated against homologous antiserum by the diluter and manually using the automatic syringe pipette with the 200 microlitre standard volume, each method in triplicate. The titre, 320 in the fourth tube, was identical in each case.

3 IN. $\times$ $\frac{1}{2}$ IN. TEST TUBES

The sensitivity of the Oxford Staphylococcus penicillin was titrated in parallel with the diluter (micro and macropipettes) and manually using a graduated pipette. The volume in all titrations done in triplicate for each method, was 500 microlitres. The series was purposely longer than would normally be considered desirable extending from 5 μ g per ml to 0.01 μ g/ml. Results after overnight incubation were as follows:

Manual MIC	= 0.04 μ g/ml (8th tube)
Micropipette MIC	= 0.08 μ g/ml (7th tube)
Macropipette MIC	= 0.04 μ g/ml (8th tube)
(foot pedal and stand)	

¹The new model has been adjusted to allow pick up as well as delivery of the sum of the volumes selected on each scale.

Radioisotope Method

These preliminary tests indicated that the diluter could be used satisfactorily for a number of microbiological tests but there were minor differences in titre, the diluter tending to give lower titres. This might be due to less carry-over from the micropipette. The sensitivity test in which the macropipette and manual pipette gave identical readings in a long series of dilutions while with the micropipette the titre was one tube different made this explanation seem likely since the ends of the two large pipettes were similar in size. The much smaller end of the micropipette would carry over less.

A radioactive serum was therefore titrated so that the accuracy of dilution in each step could be studied. Sodium 22 labelled serum with saline buffer as diluent was titrated as follows, each titration in duplicate, the automatic syringe being employed manually; the standard volume was 200 μ l in each case.

- A In $2\frac{1}{2}$ in. $\times$ $\frac{1}{4}$ in. tubes without additional mixing
- B In tubes of the same size with vortex mixing of each tube after each dilution
- C Dreyer's tubes without additional mixing.

No attempt was made to calibrate the manual pipette and diluter to deliver identical volumes because each dilution step, not the exact amount, was of interest and this would not be affected. Trays cannot be tested by this method because the contents of each well cannot be individually counted.

Counts showed that each of the duplicates in each test was acceptable, the mean was therefore calculated. Appropriate adjustments were made to allow for the minor difference in volume actually delivered by the diluter and the manual pipette and figures were calculated for each dilution so that the expected figure was 2 for a perfect result; less than 2 indicates

less dilution, ie, carry over, more than 2 indicates loss of serum during dilution (table).

The best series was in the $2\frac{1}{2}$ in. $\times$ $\frac{1}{4}$ in. tubes with Vortex mixing, as would be expected, but the diluter performed better in the Dreyer's tubes without mixing than the manual pipette with the usual manual mixing.

In-use Tests

These are shown being done in the figure.

Fig The Compu-pet 100 in use.

VENEREAL DISEASE SEROLOGY

After testing more than 600 sera in parallel the diluter was employed routinely for Reiter protein complement-fixation tests. The ability to dilute each serum 1:5 without dispensing diluent and to measure automatically more than halved the time taken to do these tests and made them much less laborious. Recently the *Trep. pallidum* haemagglutination test (TPHA) replaced the RPCFT as a screening test and it has been satisfactorily performed using the diluter for the last six months, ie, for about 7000 tests of which about 5% were positive and confirmed by other tests.

VIROLOGY

Six hundred and fifty Rubella haemagglutination inhibition tests (HAI) were satisfactorily titrated in parallel with the manual Takatsy loop method. The diluter is now used routinely for this test and also for viral complement-fixation tests. It was shown by parallel tests that reducing the unit volume from 25 to 20 μ l to suit the diluter does not affect the endpoint. It might be supposed that the loop method in which eight rows can be diluted simultaneously

Radioactivity of Twofold Serum Na^{22} Dilutions

	A		B		C	
	Manual	Diluter	Manual	Diluter	Manual	Diluter
	1.65	1.98 ¹	1.92	1.93 ¹	1.76	2.00 ¹
	1.93	1.93	1.87	2.14	1.94	1.94
	1.90	1.97	2.10	1.91	2.01	1.96
	1.98	2.01	1.91	2.00	1.88	2.00
	1.87	1.96	2.04	2.00	2.00	1.76
	1.92	2.00	1.84	2.03	1.89	2.16
	1.94	2.00	1.83	2.00	2.00	—
Mean	1.89	1.98	1.93	2.00	1.93	1.97

Table Comparison of accuracy of two-fold dilutions made manually and by the diluter

Two is the expected result in each case (see text)

- A In $2\frac{1}{2}$ in. $\times$ $\frac{1}{4}$ in. tubes without additional mixing
- B In tubes of same size with vortex mixing of each tube after each dilution
- C Dreyer's tubes without additional mixing.

might be less laborious than the diluter which can only dilute one row at a time. This is not so, however, because diluent must be predispensed for the loops whereas the diluter dilutes and dispenses simultaneously. Moreover less skill is required to operate the diluter than to dilute with the loops.

The macropipette is used routinely to feed tissue culture tubes with medium and to dispense kaolin absorbent for Rubella HAI tests.

Other Tests

BACTERIAL COUNT

A bacterial culture was diluted with the micropipette to see if it could be used for counting viable bacteria. As might be expected the bacteria could not be washed out of the pipette sufficiently to make this possible.

ANTIBIOTIC SENSITIVITY TESTS

The minimal inhibitory concentration (MIC) of antibiotic was estimated by the plate dilution method. The macropipette was compared with a manual method. Master dilutions were made and then 1 ml was dispensed into Petri dishes for incorporation into the medium. The pipette was used without a stand to test whether minor inaccuracy with this large volume would matter. Results were as follows:

1 The MICs of gentamicin for *Ps. aeruginosa* and *E. coli*, by the manual method and diluter were identical.

2 The MIC of fusidic acid (known to bind to cellophane) for *Staph. aureus* was identical by all methods.

3 To find the MIC of gentamicin for *Staph. aureus*, titration was performed with the pipette in sextuplicate. There was a twofold difference in results between the first and last row, the first row corresponding with the manual method.

4 The MIC of fusidic acid for *Staph. aureus* (3) was repeated. On this occasion all titrations gave identical results.

The makers' recommendation to use the stand and foot pedal should be followed. An experienced worker would not save time or attain greater accuracy by using the diluter for this method. One experienced in using the diluter and inexperienced in the manual technique would probably prefer to use the diluter.

Washout Test

Washout of antibiotic was tested by picking up 500 μ l penicillin solution 10^5 μ g/ml with the micropipette; the solution was then discarded and the tip of the pipette was wiped. Then 500 μ l of broth from the reservoir was dispensed into each of a series of

tubes which were inoculated with the Oxford Staphylococcus. Growth was seen in the sixth or seventh tubes in four wash-out tests, the quantity of penicillin dispensed by the pipette having been reduced from 10^5 μ g/ml to about 10^{-2} μ g/ml despite lack of repeated wiping of the pipette between washouts.

Shortcomings

The range of volumes that the diluter will handle is limited. It is not suitable for diluting cultures. It cannot dilute more than one row at a time. The summation of volumes facility cannot be operated with the automatic delivery interval switch. The largest volumes handled by the micropipette are delivered slowly because of the narrow bore of the pipette, speed is increased by using the macropipette but a stand is then necessary which is not always convenient. When a technique demands delivery of the last of a series of aliquots air must first be sucked into the tubing to prevent mixing of this aliquot with diluent.

The pump is probably rather low powered for the macrotubing. One machine had to be returned to the makers because of staggering of the rotor and finally failure to deliver the larger volumes. This may have been a single faulty machine because for the last six months another machine has delivered tissue culture fluid and kaolin suspension through the macropipette twice weekly without trouble.

Advantages

The diluting facility cuts out tedious repeated pick-up and delivery from reservoir to tubes and predispensing of diluent. It is more accurate than manual methods and mixing of twofold dilutions is unnecessary. Aliquots are dispensed accurately and automatically.

The machine can be used for a great variety of tests including those which need a sterile technique. In changing from one technique to another no adjustment is necessary other than changing tubing and pipettes when larger or smaller volumes are to be handled, or, when a sterile set is required, this can be done in about one minute. The machine is portable and can be stored in a cupboard or on a shelf when not in use. No special skill is required to use it; a trained technician can learn to handle it for all purposes within one day or less. It is robust and has withstood repeated use and changes from one technique to another for more than one year. Calibration is seldom necessary for microbiological techniques. The machine is economical in the use of reagents.

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Reference

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