Technical method

The Micro-Compu-Pet: a multi-channel diluter and dispenser

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The Micro-Compu-Pet is a development of the Compu-Pet 100 which is aimed at serological tests performed in microtitre trays. The disadvantages of the Compu-Pet 100 are that the volumes which can be selected are severely limited and that only one volume can be dispensed at a time. This new machine enables two, four, five, 10 or 20-fold dilutions to be made of any preselected volume from 5 μ l, in 5- μ l steps, up to 9995 μ l, and eight aliquots of the chosen volume can be dispensed simultaneously. Larger volumes cannot be dispensed.

The diluter

The peristaltic pump which was tested for accuracy in the previous model (Cremer *et al.*, 1975) has been modified. It is smaller in circumference to increase torque, and the rollers are longer in order to accommodate eight narrow tubes, one for each of the micropipettes which are fixed to a handset for easy handling. The handset comprises a one-piece acrylic holder into which each of the Teflon micropipettes fits in order that they may be easily removed if all eight are not required. The fit is strong enough to prevent the pipettes from lifting when placed in the wells of a microtitre tray but only prolonged use will show whether they loosen. The whole assembly is easy to handle, allowing a certain amount of fingertip control, while being robust enough not to feel fragile. The double row of buttons on the Compu-pet 100 has been replaced by a much more versatile single volume selector in the shape of a window showing figures in 5- μ l steps which can be altered by a simple dial system using a thumbwheel edge switch.

The machine can be used as a simple dispenser by selecting the volume required and pressing the fascia button or the foot switch. One knob on the console converts the machine to a diluter, and another knob selects the dilution factor. A two-

position foot switch is employed for making and dispensing dilutions. In one position the chosen volume of fluid to be diluted is picked up, and in the other this volume is automatically diluted, by the factor selected, and dispensed. A second knob on the fascia selects the dilution factor and also has a slow position. When diluting, the delivery rate is 100 μ l in 0.8 second and, in the slow position, 100 μ l in 3 seconds. A third knob on the machine selects the facility to dispense a set amount at any rate between 1 and 10 seconds per volume.

Tests

Complement was titrated twice to test that the end-point was the same for each of the eight diluters. The complement titration end-points were identical in all 16 titrations. Complement was selected to test reproducibility because complement titration is a very sensitive indicator of dilution errors. Six hundred and five serum samples were tested for rubella antibody by the haemagglutination inhibition test using the Micro-Compu-Pet in parallel with the Compu-Pet 100. Although these tests occasionally showed slight differences in the degree of haemagglutination, this never amounted to a two-fold difference in titre. Aligning eight pipette tips into each appropriate well slightly increases delivery time but nevertheless the tests may be performed up to five times faster with the Micro-Compu-Pet than with the Compu-Pet 100.

All these comparative tests were carried out using 1:2 dilutions. It was found possible, with the speed of the rotor at its slow setting, to layer successfully sucrose gradients of the type used to differentiate rubella specific IgG and IgM antibodies. Five concentrations of sucrose are used—37.5, 31, 25, 18.75, and 12.5%; these require five of the tubes and the remaining three are left blank. Each tube is primed with a different concentration of sucrose.

The required amount of the strongest concentration is dispensed into a cellulose nitrate tube; this is followed by successive weaker concentrations, each being layered carefully. The completed gradient is then ready for addition of the serum before ultracentrifugation. Using the Micro-Compu-Pet, the tedium and technical expertise involved in hand-layering a gradient are considerably reduced. While the Micro-Compu-Pet is not recommended as an alternative to any of the commercial gradient layers available, nevertheless it performed the job in an

Figure The Micro-Compu-Pet with multiple tubing

adequate manner without the need to purchase a special item of equipment.

Problems

The machine performed well during its period of testing without causing problems or varying significantly in calibration. No attempt was made to calibrate the machine under test, but the rotor shoe adjustment system comprising 16 screws may be too complex and could lead to difficulty, in tube volumes becoming grossly different from others after calibration. (Replacement with a pair each side, giving a total of four screws, would improve the system.)

This would not, however, affect titration results because the total volume in each well, not the dilution factor, would be irregular. The multiple tubes were in a flexible conduit which kept them together while still allowing reasonably free movement. Each tube was marked by a coloured number at both ends to aid identification. However, it was not always easy to ensure that the tube ends were below the level of diluting fluid so a hollow glass weight was attached via several Auto-Analyzer joints to give a single pick-up point. Changing the tubing set was a time-consuming operation, and care had to be taken to route the tubes the correct way on either side of the rotor or else four sucked and four blew. All tests were performed with one set of tubing which performed satisfactorily.

Until the operator is familiar with the machine care must be exercised during use as it is easy to miss a well near the edge of the microtitre plate with the multiple pipette handset.

Advantages

The chief advantage of the machine is its ability to undertake up to eight multiple dilutions at a time in a microtitre plate with the minimum of effort and operator time while retaining the required accuracy and precision.

The highly variable system of volume and dilution selection allows the instrument to be adapted to most tests that require large numbers of dilutions and deliveries to be made.

The Micro-Compu-Pet would appear to have a useful role to play in the diagnostic and reference microbiology laboratory, especially as a companion instrument to the Compu-Pet 100. It cannot replace the Compu-Pet 100 because it is cumbersome to use for single titrations, eg, of antigens, and it cannot be used to dispense large volumes of fluid, eg, for feeding tissue culture tubes.

Reference

Cremer, A. W., Mellars, B., and Stokes, E. J. (1975). The Compu-pet 100: a versatile dispenser-diluter for the mechanisation of microbiological techniques. *Journal of Clinical Pathology*, **28**, 37-41.