Technical methods

Automatic apparatus for Wassermann and Reiter complement-fixation tests utilizing the 'discrete-analysis' principle

R. E. TROTMAN *From the Wright-Fleming Institute, St Mary's Hospital Medical School, London*

In many laboratories large numbers of sera have to be tested for the diagnosis of syphilis by the Wassermann and Reiter complement-fixation tests, and it is therefore desirable to mechanize as many stages of the procedures as possible.

Pugh and Gaze (1965, 1966) have used the Auto-Analyzer for performing the Wassermann and Reiter reactions. While this method works, many difficulties have arisen, due mainly to the fact that the Auto-Analyzer utilizes a continuous-flow system and the problem of 'carry over' of serum antibody is troublesome (Taylor, Kershaw, and Heimer, 1968).

Sequeira (1964) produced hand-operated apparatus for dispensing the reagents into tubes: the apparatus is simply a mechanical aid for performing the manual 'discrete-analysis' method. This apparatus has the advantage of being flexible, and is therefore particularly suitable for performing a variety of different procedures. When, however, the number of specimens to be tested becomes large, it is preferable to have a greater degree of mechanization than Sequeira's apparatus gives, at the cost of lack of flexibility. The evidence suggests that apparatus based on the 'discrete-analysis' principle is preferable to apparatus based on the 'continuous-flow' principle.

This paper describes an apparatus that dispenses most of the reagents used in the Wassermann reaction and the Reiter complement-fixation test into the reaction tubes. The apparatus has been in routine use for over a year and has effected a substantial saving in technician time.

The complement-fixation tests comprise the following four main steps. (1) Distribution of the patient's serum into each of four tubes; (2) the addition of 1.0 MHD complement and diluent to one tube, of 1.25 MHD complement and Wassermann antigen to the second tube, of 1.25 MHD complement and Reiter antigen to the third tube, and of Reiter antigen and 1.5 MHD complement to the fourth tube: 0.2 ml of each reagent is required; (3) the addition of sensitized red blood cells to all four tubes; (4) reading the results. The apparatus described here performs the second of those steps, the first and third steps being performed manually in the conventional way; the lysis is read visually.

FIG. 1. Photograph showing racks of reaction tubes, attached to the circumference of the turntable, passing underneath the valve outlet needles: the reagent reservoirs are also shown.

THE APPARATUS

The apparatus (Fig. 1) consists of a turntable of 12 in. diameter, to the circumference of which can be attached five curved racks. Each rack holds 12 rows of four disposable AutoAnalyzer cups, and is so constructed that it can stand on the bench and fit on to the rotary table. The mechanism for rotating the table is almost identical to that described earlier (Trotman, 1967a); in this case the table has to stop in 60 positions per revolution.

THE REAGENT-DISPENSING MECHANISM For purely technical reasons, it is convenient to have four mechanisms dispensing 0.2 ml of Wassermann antigen, Reiter nantigen, Reiter antigen, and 1.5 MHD complement respectively, actuated by a single device; one mechanism, which operates twice per cycle, dispensing 0.2 ml of 1.25 MHD complement into tubes 2 and 3, and one mechanism dispensing 0.4 ml of diluted 1.0 MHD complement. The latter mechanisms must be actuated by independent devices.

Each reagent-dispensing mechanism comprises a simple, pin-type valve (Becton Dickinson & Co. Ltd.) connected to a 1 ml syringe, the piston of which is actuated by a pneumatic cylinder to fill the valve and expel the liquid. The stroke length of the cylinder piston, and hence the volume dispensed, is easily varied by a simple screw-thread device so arranged that the valve syringe unit may be removed from the apparatus and replaced without affecting the calibration of the mechanism, which repeatedly dispenses volumes of the order of 0.2 ml within a tolerance of $\pm 1\%$. When four valve/syringe units are actuated by a single pneumatic

cylinder, the volumes dispensed are not independently variable and there are significant absolute differences in them. However, the overall accuracy is $\pm 2\%$ and this is adequate; the valve/syringe units were not specially selected.

The advantage of a pneumatic cylinder over an electrically driven reciprocating mechanism was discussed in an earlier paper (Trotman, 1967a) and the same considerations apply here. Also included in that paper is a general discussion on the use of pneumatic equipment, which is particularly suitable for this apparatus, in pathology laboratories; the volume of air required in this apparatus is such that a compressor is essential.

THE COMPLETE APPARATUS The reagent-dispensing mechanisms are mounted in two groups, the four 0.2 ml volume valve/syringe units which are actuated by a common cylinder on one support and the other two mechanisms on another support. The reagent reservoirs are mounted behind the supports. The valve outlet tubes are connected to serum needles which are fixed, in appropriate positions, in a holder which is mounted in such a way that it may be moved radially: the four tubes containing one patient's serum are in a straight line along a radius.

The control unit (Fig. 2) produces the following sequence of events. The three pneumatic cylinders are actuated to dispense 4×0.2 ml volumes (Wassermann antigen, Reiter antigen, Reiter antigen, and 1.5 MHD complement), 1×0.2 ml volume (1.25 MHD complement) and 1×0.4 ml volume (diluted 1.0 MHD complement) into the appropriate reaction tubes. The needle holder is then moved radially, by means of a fourth pneumatic cylinder, so that the output needle of the mechanism dispensing 0.2 ml of 1.25 MHD complement is over tube 3, and the three dispensing cylinders return to their original positions thus refilling the valves; 0.2 ml of 1.25 MHD complement is dispensed into tube 3, the needle holder moves back to its original position, the

valve is refilled, the table rotates until the next set of tubes is located under the needles and the process is repeated a preselected number of times. Further details of the control unit are given elsewhere (Trotman, 1967b).

As each rack is completely filled with reagents it may be removed and placed in the water bath: a freshly prepared rack of sera may be put in place of the completed rack and the procedure repeated as necessary.

COMPARISON OF MANUAL AND AUTOMATIC METHODS

Two hundred and forty-eight specimens of sera were taken, and the complete diagnostic procedure was carried out on each by the routine manual method. Samples of the same 248 sera were taken and the procedure was carried out on the machine. Complete agreement was obtained even in a few cases when the Wassermann reaction was positive and the Reiter reaction negative. We had, therefore, every reason to assume that the apparatus performs its function well.

EVALUATION OF AUTOMATIC METHOD

The apparatus takes 20 seconds to complete one cycle, which is equivalent to 20 minutes for 60 sets of reaction tubes. It takes approximately 10 minutes to prime and prepare the valve mechanisms and, since it takes approximately 10 minutes to dispense all the reagents into 12 sets of tubes manually, it is quicker to use the apparatus even if one has as few as 24 tests to perform, but considerable saving of time is effected if 100 specimens have to be processed. The priming volume of a valve mechanism is 6 ml and this volume of reagent is wasted. Consequently the waste of reagents is more significant when small numbers of sera are processed than when large numbers of sera are processed.

The maximum number of specimens that can be processed depends mainly on how long the diluted complement and the antigens may be left standing at room

FIG. 2. Photograph of the control unit.

temperature without deteriorating. We therefore performed some simple experiments to assess this.

Twelve sera were taken, six known to be positive and six known to be negative to both antigens. The reagents were made up and distributed on the machine and the test was completed in the usual way. The apparatus was left standing for two hours and the procedure repeated using fresh samples of the same 12 sera. The results were identical even in two cases in which only a few cells remained. The procedure was repeated after a further two hours with identical results. The apparatus may thus be run continuously for at least three hours without any deterioration in the reagents, and there is a considerable safety margin. In that time, 540 specimens could be processed.

One could have devised automatic apparatus for distributing the patients' sera, adding the sensitized red blood cells and for reading the results. However, in view of the complication that would be introduced and the fact that the time taken to perform each of these stages manually is very small compared with the time taken to dispense the reagents, we decided to continue to perform these stages manually.

FINAL COMMENTS

We have demonstrated that the machine is a satisfactory aid in performing the serological diagnosis of syphilis. The apparatus is moderately cheap, many of the components are commercially available, and it does not suffer from the disadvantages of the AutoAnalyzer method. It is suitable for carrying out the procedures on up to at least 500 to 600 specimens per day, and in smaller laboratories, for which it is particularly suitable, pathologists might well be encouraged to store samples, thus effecting a considerable saving of time.

The basic principles of the apparatus could be used for a method of performing any complement-fixation technique. If the apparatus were modified to accommodate more reaction tubes, either by increasing the number of racks that could be attached to the turntable, which number is limited only by the size of turntable required, or by using a 'continuous-flow' mechanism operated by a chain, the apparatus could be used for very large batches of sera: in this case, more attention would have to be paid to the deterioration of reagents, and more exhaustive experiments than those reported here would have to be performed before the design was finalized.

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Use of microplates for monolayer tissue cultures

I. F. C. MCKENZIE¹ AND P. J. MORRIS *From the University of Melbourne Departments of Medicine and Surgery, The Royal Melbourne Hospital, Victoria 3050, Australia*

Monolayer cultures of cells are used extensively in research laboratories and for routine detection of viruses. One disadvantage of the technique is that large numbers of cells are required, and for quantitative experiments the use of sterile flasks becomes expensive. We describe the use of microplates for monolayer cultures, a technique enabling a large number of tests to be done with a small number of cells.

METHODS

THE PLATE The microplates are available commercially (Falcon Plastics) and consist of plastic trays fitted with a lid (Fig. 1). There are 60 shallow wells in the plate, each

FIG. 1. The microplate used for the tissue culture studies. (Falcon Plastics.)

fitted with shelving sides, and capable of holding 15 μ l of culture medium. Monolayers are viewed using an inverted phase contrast microscope. These plates are continually used in tissue typing laboratories and were developed for the lymphocytotoxicity microtest by Terasaki (Terasaki, Vredevoe, and Mickey, 1967).

CULTURE OF KIDNEY CELLS A primary culture of human kidney cells was set up in a milk dilution bottle using a standard technique (Paul, 1960), in a growth medium of

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