Serological techniques

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It is rather curious that, although serum is tested for a multitude of constituents in biochemical and haematological laboratories, the term 'serology' has come to imply almost exclusively the examination of serum for antibodies, and, in particular, antibodies related to microbial diseases. So-called serological techniques tend to be regarded, therefore, as the prerogative of microbiologists.

When one considers for how long and to what extent in biochemical laboratories the automation of tests for other blood constituents has been developed, it is a little difficult to understand the delay in automating routine tests for antibodies. Although they may be imperfectly characterized substances, their physiochemical reactions have been studied for many years, and routine tests are well established. It can hardly be that the demand for antibody tests has not been enough to make automation an economical proposition. A glance at Figure 1 reveals that the number of sera examined for syphilis antibodies each year in public health laboratories alone throughout England and Wales has almost doubled over a period of 10 years, and is rapidly approaching half a million. To these figures can be added a great many more tests done in hospital laboratories and blood transfusion centres. In addition, the diagnosis of many viral diseases, including influenza, which in epidemic times can put a heavy load on diagnostic laboratories, relies to a great extent upon complement-fixation tests. If a satisfactory automated method were available, knowledge of the epidemiology of viral diseases, and especially of influenza, might be greatly extended by large-scale population studies. It is even conceivable that regular sampling of the population might enable outbreaks of influenza in particular areas to be predicted in sufficient time to allow preventive measures and useful administrative decisions to be taken.

In many parts of the world, and especially in the developing countries, there is a great shortage of trained laboratory workers; machines which could do large numbers of diagnostic antibody tests would be exceptionally useful. Quite clearly, the need exists for a reliable means of automating such tests, and complement-fixation tests in particular.

Compared with most routine biochemical tests, antibody tests, and particularly the complement-fixation test, are considerably more complicated. Moreover, the reagents used are relatively unstable, complex, and notoriously capricious biological materials with appreciable batch-to-batch variation. Strict adherence to a standardized method, therefore, is essential by whatever means the test is done; there is little doubt that an efficient machine would do tests more reliably than the most skilled manual operator.

It may be useful at this point to try to clarify the meaning of the term 'automation'. Theoretically, 'automation' and 'mechanization' imply two different concepts. The term 'mechanization' usually refers to a process which imitates more or less exactly some manoeuvre or sequence of manoeuvres customarily done by a human operator. The term 'automation' is sometimes used to describe a system which achieves an end result similar to that of the manual method but not necessarily by imitating all or any of the manoeuvres involved in the hand-operated process. Furthermore, highly developed automated procedures include self-regulating feedback systems controlled by a computer. Since fully automated procedures may develop from simple mechanization
of a part or parts of a process, and most automated procedures include elements of mechanization, there does not seem to be any useful purpose served in practice by distinguishing between them. Throughout this paper, therefore, the term 'automation' will be used to include any elements of mechanization.

In many laboratories, various simple aids have been used for many years for dispensing constant volumes of sera and reagents into tubes or into wells of plastic plates. One of these is the so-called 'automatic' pipette, which is held in the hand and enables a predetermined volume, eg, 0.1 ml, to be repeatedly and quickly pipetted into tubes or into the wells of a plastic plate (Fig. 2). Such a syringe can be fitted with two one-way valves and a side connexion to which can be fitted a piece of flexible tubing to enable the syringe to be filled from a reservoir.

One of the first successful attempts to automate antibody tests was made by Weitz (1967) at the Lister Institute, London. The apparatus developed by Weitz (Fig. 3) allowed the performance of up to 12 titrations in a single operation, with even less manipulation than that required for a single test done by a more conventional technique. The machine was designed for the measurement of volumes of liquids ranging from 0.01 ml to 1.0 ml, and could, therefore, with advantage, replace more usual apparatus such as graduated pipettes, standard droppers, burettes, and other volumetric glassware. According to Weitz, the dispenser could be used for a variety of tests, such as agglutination tests, flocculation tests (including optimal proportions), precipitin ring tests, complement-fixation tests, or any kind of inhibition test; moreover it could be used also for the simultaneous serial dilution of 12 different liquids, such as sera, in any stages desired. The apparatus was designed to achieve at least the same accuracy as that obtained by the use of grade B British Standard serological volumetric glassware.

The main parts of the machine consisted of the pumping units, the pipette-clamping device, the carrier lift, and several accessory racks for the glass test tubes and pipettes. In operation, a rack containing a row of 12 special pipettes was mounted on the rack carrier lift, which was then raised by a lever until the open tops of the pipettes fitted into a series of holes in the clamping device into which the pipettes were locked by means of another lever. The empty rack was then lowered and the pipettes remained in position in the clamping mechanism ready for use. In Fig. 3 can be seen the two levers on the right-hand side of the machine, as well as a row of pipettes clamped in position. A series of 12 test tubes in a rack could then be lifted so that the open lower ends of the row of pipettes just dipped into the liquids contained in the test tubes. By moving the main volume control lever, which acted on the pumping units, a measured volume was aspirated into each of the 12 pipettes. The whole, or measured portions, of the liquid thus contained in the pipettes could then be expelled into another series of tubes simply by reversing the movement on the volume control lever. These motions could be performed in any sequence, with consequently great flexibility of performance. The rapidity and efficiency of this machine can be judged from the fact that it enabled a single technician to carry out up to five thousand individual precipitin ring tests in a morning, results being read the same afternoon.
A hand-operated machine for dispensing measured volumes of liquid into rows of wells in plastic plates, and for enabling serial dilutions to be made simultaneously in the wells of plastic plates, has been developed by Sequeira (1964), and marketed by the Shandon Scientific Company Limited (Fig. 4). In certain respects, this machine is similar to the one devised by Weitz but was designed primarily with the object of assisting the performance of complement-fixation tests in plastic plates.

Trotman (1967a, 1967b, 1969) has described an apparatus which dispenses complement, antigens, and diluent used in the Wassermann reaction and the Reiter complement-fixation test. The distribution of patients' sera and the addition of sensitized red blood cells are done manually. Results of the tests are read visually. The apparatus (Fig. 5) 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 either stand on the bench or fit on to the rotary table which stops in 60 positions per revolution. 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 syringe and expel the liquid. The four cups containing one patient's serum are in a straight line along a radius. There is a control unit which governs the mechanism required for carrying out a predetermined sequence of events. As each rack is completely filled with reagents, it may be removed and placed in the waterbath; a freshly prepared rack of sera may then be put in place of the completed rack, and the procedure repeated. The apparatus takes 20 seconds to complete one cycle, which is equivalent to 20 minutes for 60 sets of reaction tubes. It may be allowed to run continuously for at least three hours without any detectable deterioration of the reagents. In that time, 540 specimens can be processed.

A machine used in many hospitals for routine biochemical tests is the AutoAnalyzer. The continuous-flow principle, on which it is based, has many attractions. A measured volume of serum is taken up at one end of a continuous system of plastic and glass tubing through which it is propelled by a peristaltic proportioning pump. At appropriate time intervals during its passage through the system, the sample meets and mixes with predetermined amounts of reagents which are flowing continuously. The reaction temperature is usually controlled by allowing the mixture of serum sample and reagents to pass through delay coils in a special temperature-controlled oil bath.
but a series of coils in a temperature-controlled water bath offers more flexibility for experimental purposes. The reaction mixture finally passes through a colorimeter which is connected to a pen recorder so that the result of each test is expressed as a peak on a sheet of continuously moving chart paper.

Efforts have been made, with variable degrees of success, by many workers, to use the AutoAnalyzer for doing routine antibody tests (Badin, Martin, and Schmitt, 1960; Sobota and Gillem, 1965; Vargues, 1965a, 1965c; Vargues, Studievic, Moraud, and Gonthier, 1965; Vargues, Studievic, and Ripault, 1965; Cohen, 1966; Gailllon, Ripault, Studievic, and Daussset, 1966; Pugh and Gaze, 1966a, 1966b; Trinquier and Morel, 1966; Vargues, Studievic, and Audurier, 1966; Morris and Bywater, 1967; Valette and Joubert, 1967; McGrew, DuCros, Stout and Falcone, 1968; Taylor, Kershaw, and Heimer, 1968). The machine has been used also to study the kinetics of complement activity (Osler and Hill, 1955; Vargues and Ayeva, 1963, 1964; Studievic, 1965; Vargues, 1965b, 1966; Vargues and Audran, 1966; Vargues, Gonthier, and Moraud, 1967). Microbial antibody tests, which have been done by means of an AutoAnalyzer, include complement-fixation tests for treponemal, gonococcal and viral antibodies, as well as tests for streptolysin antibodies. The machine also has been used for measuring thyroid and gastric complement-fixing antibodies (Irvine, 1966).

Results of syphilis screening tests done by means of an AutoAnalyzer in the Diagnostic Reference Laboratory at Colindale, London, compared satisfactorily with those of tests performed manually in the Venereal Diseases Reference Laboratory, Whitechapel, London (Taylor, Kershaw, and Heimer, 1968). It was concluded, however, from this work that the design of the AutoAnalyzer contained three major shortcomings if it were to be used for doing large numbers of complement-fixation tests. First and foremost was the relatively slow sampling rate compatible with adequate discrimination between samples. Second was the necessity for frequently replenishing the machine with samples. Third was the need for adjusting or cleaning the flow cell during use. In order to compare favourably with existing manual methods, an automated system for doing complement-fixation tests needs to operate with a sampling rate of at least a hundred samples per hour. As far as complement-fixation tests and other serological tests are concerned, it seems doubtful whether the problems inherent in any continuous-flow system can be easily overcome. For the time being, at any rate, the best prospects for automating serological tests, and complement-fixation tests in particular, would seem to lie in the development of discrete analysis systems. Such a project is in hand at the present time.

What are the future prospects for automating antibody tests? The complement-fixation test should lend itself exceptionally well to automation because the end result can be measured either as a colour change or as a change in opacity of a red cell suspension, both of which can be readily estimated automatically. Furthermore, by changing only one reagent, namely the antigen, a whole range of antibody estimations can be carried out with the same apparatus. On the basis of present experience, it would seem that efforts could most usefully be directed towards designing suitable equipment based on the principle of discrete analysis. It is just conceivable that ultimately the techniques of continuous-flow analysis and those of discrete analysis may be in some way combined in one machine to include the best of both systems. At present, it is difficult to envisage such a combination but it may be important to bear this prospect in mind lest, in the event of a sudden swing of the pendulum from continuous-flow to discrete analysis systems, some of the advantages of the former may be disregarded.

There would be a considerable advantage if automated equipment for antibody tests had a refrigerated compartment to hold reagents and samples, and from which reagents and samples could be fed automatically into the analytical section. It is also desirable that results of tests should appear in the form of a printout on a laboratory report form as well as being suitable for data processing by a computer. For reasons of economy, the equipment should be capable of running continuously without attention for about 20 out of 24 hours.

Processing specimens by machine at the rate of several thousand per day would inevitably require some degree of centralization of work, in which case it would be vital to ensure well organized services for the transport of specimens and for the distribution of laboratory reports. These features, as well as the need for trained staff to operate and service the machines, are common to all forms of laboratory automation. It may well prove desirable, therefore, that all automated aspects of pathology, including serology, should be located together in relatively few large laboratories. This may result in the present division of pathology into separate specialties disappearing, at any rate as far as the routine examination of specimens is concerned.

**SUMMARY**

The need for automation of antibody tests, and the complement-fixation test in particular, is discussed.
Examples of various attempts to mechanize and partly automate antibody tests are described. The limitations inherent in a continuous flow system are emphasized. At the present time, the best prospects for automating antibody tests would seem to lie in the development of discrete analysis systems. The possibility of combining certain aspects of continuous flow with a discrete analysis system should be kept in mind. Ideally, automated equipment for antibody tests should have a refrigerated store for reagents and samples. Results of tests should appear as a printout and be suitable for data processing by computer. For reasons of economy, equipment should be capable of running continuously for long periods without attention.

Automation will inevitably lead to some degree of centralization of work, which will require well organized services for the transport of specimens and for the distribution of laboratory reports. It may prove desirable for all automated aspects of hospital pathological services to be located together in relatively few large laboratories.

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REFERENCES


