Automation in diagnostic bacteriology

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Diagnostic bacteriological laboratories in hospitals suffer the same problems as the other diagnostic services: an ever-increasing load of work not matched by an increase in the number of people available to do it. The laboratories have managed to increase their 'productivity' in terms of reports per technician partly by a greater use of technical short cuts and reliance on simpler tests, and partly, presumably, simply by working faster. Hitherto practically nothing has been done to improve the productivity of the staff by providing them with mechanical aids. The reasons for this neglect become apparent when one analyses the work done in a hospital bacteriological laboratory. Four main categories may be distinguished.

ANALYSIS OF WORK OF BACTERIOLOGICAL LABORATORIES

1 SERUM ANTIBODY TITRATIONS In these the reagents are sterile, and the reaction vessels need to be clean but not sterile. Infected material is not usually added during the test, which can ordinarily be completed sufficiently quickly for chance contamination from the laboratory to be unimportant. Serological tests bear many resemblances to the chemical tests that have been so fruitfully adapted to automation, and they represent the one area of the bacteriological laboratory's field in which a substantial amount of work on automation has been carried out. This forms the topic of the paper by Dr C. E. D. Taylor in this Symposium.

2 SERUM ANTIBIOTIC TITRATIONS AND TITRATIONS OF ANTIBIOTIC SENSITIVITY OF BACTERIA Although formally similar to serum antibody titrations, these demand the addition of living bacteria and involve a period of incubation; it is therefore necessary to use truly sterile equipment, to prevent contamination with bacteria, the growth of which would invalidate the result, and to protect the operator and instrument from contamination by the test microbe.

3 ENUMERATION OF LIVING BACTERIA, FOR EXAMPLE,

IN URINE OR IN MILK In such tests the requirement is to determine the total number of bacteria but it is often (at least in screening tests) unnecessary to identify or subculture the bacteria counted. There is again a need for fully sterile handling and for safe disposal of the used equipment.

4 IDENTIFICATION OF PATHOGENIC MICROBES The specimens for such examinations may come from areas of the body that are normally sterile, or from those that harbour many commensal and, for the purposes of the test, irrelevant bacteria. The relevant bacteria may be any one of a great variety of species and may be present in large or small numbers. It is usually necessary to subculture them for further tests. These requirements provide additional and formidable restrictions on the development of mechanical or automated equipment.

We may review some of the work that has been done on the development of automatic equipment for performing tests in the last three of the categories mentioned.

AUTOMATIC ANTIBIOTIC TITRATIONS

Three elements can be recognized in these tests: (1) the sterile preparation of the dilutions of the patients' serum or the antibiotic solution; (2) either the addition of a suspension of the test bacteria to tubes containing the antibiotic, or the addition of the antibiotic dilutions to culture plates spread with the test organism; and (3) the determination of the highest dilution of antibiotics that inhibits, or (after further subculture) can be shown to kill the bacteria.

In making the dilutions of antibiotic it is generally considered satisfactory to use a single pipette for the successive dilutions and the various hand-operated syringe-type pipettes (Aimer Products Ltd.) are much faster in use than calibrated pipettes with rubber teats. They are also far more accurate than the dropping pipettes as ordinarily made. If any numbers of dilution-series have to be made, however, the use of mechanical equipment is fully justified. We have devised and used an instrument,

that can prepare, simultaneously, four series of two-fold falling dilutions (Trotman, 1967), and this produces a great saving of time and probably an increase in accuracy. The advantage of such equipment lies not in the actual speed with which it makes the dilutions, but in the fact that, while the machine is diluting one set of reagents, the technician can be preparing another set.

Mechanization of these tests has not yet reached beyond the stage of making the dilutions, although some consideration has been given to ways of recording bacterial growth. These are more conveniently referred to in the next section.

AUTOMATIC BACTERIAL COUNTING

The methods available for estimating numbers of bacteria include: (1) a direct cell count, as for example, provided by the Coulter electronic cell counter; (2) indirectly, by measuring some physical or chemical change produced in a medium by bacterial growth; (3) observation of the degree of turbidity developing after incubation in a liquid medium; and (4) a count of the colonies developing on or in a solid medium after incubation.

The Coulter counter¹ was first used for counting bacteria in suspension by Kubitschek (1958), and was further investigated by Curby, Swanton, and Lind (1963) who found that the count depended on the magnitude of the electric field applied across the orifice of the counter, and that the relationship between count and electric field varied with different bacteria. This has been confirmed by Dr P. N. Hobson (personal communication, 1968). Moreover the electronic counter cannot distinguish live from dead bacteria, or bacteria from other particles that may be in the suspension, nor can it distinguish single bacteria from aggregates. The effects of all these factors must vary considerably from specimen to specimen and it is likely to prove difficult to formulate general criteria for relating the electronic count to the total viable count for all bacteria commonly occurring in diagnostic bacteriology. It might well be possible, however, to use cell counters for detecting the growth of an organism in suspension and so for measuring antibiotic sensitivities.

Growth of organisms in suspension has been detected by measuring the changes in pH (Faine and Knight, 1968) or the Eh (Hewitt, 1950) of the culture, but the difficulties of developing a technique, based on these principles, for measuring growth in cultures for routine diagnostic purposes are considerable.

Photometric methods have been widely used in

experimental bacteriology for many years, at least since 1933 when Alper and Sterne measured growth curves of *Salmonella gallinarum*. The application of photometric methods to bacteriology has been reviewed by Norris (1959), Kavanagh (1963), and Meynell and Meynell (1965). Many devices, such as the Vitatron Universal photometer/densitometer (Vitatron Scientific Instruments) and the Unicam spectrophotometers (Pye Unicam Ltd), are commercially available and new techniques are still being devised (Cobb, Crawley, Crowshaw, Hale, Healey, Pay, Spicer, and Spooner, 1969; Norris, 1969). Photometry is more likely to become a diagnostic technique for measuring the concentration of bacteria in suspension than cell-counting techniques, although its sensitivity is rather poor, and the optimum operating conditions differ for different organisms, as in electronic cell-counting techniques.

Bowman, Blume, and Vurek (1967) have developed a much more sensitive method for counting viable bacteria in which the bacterial suspension is mixed with agar and run into a capillary tube which can be scanned by a linear tungsten filament lamp focused on the centre of the capillary. A microscope objective collects the light scattered within the capillary and feeds a photomultiplier. The instrument counts light pulses scattered from growing colonies and from other points, such as the junction between the agar and the capillary. When the tube is scanned at intervals during incubation the light pulses due to colonies increase whereas those due to contaminants do not, so a colony count can be derived. The minimum number of organisms that formed a recognizable micro-colony was of the order of 20. The method has considerable potential, because growth can be detected in a few hours and the method may, therefore, offer a means of producing some bacteriological test results on the day the specimen is sent to the laboratory, but much further development is required. Micro-colony techniques were also investigated in a preliminary way by Mahony and Chadwick (1966).

Apparatus for automatic counting of colonies on solid agar plates was described by Alexander and Glick (1958) and was evaluated by Malligo (1965); a cathode-ray tube flying spot was focused on to the culture and variations of light transmitted during a scan were measured on a photomultiplier. The chief source of error in scanning techniques is coincidence (Le Bouffant and Soule, 1954) and it appears that, whilst Malligo achieved reasonably accurate results, a much more detailed investigation of the errors in this method is necessary before one can decide whether the method is likely to become a useful diagnostic procedure. Apparatus, using

W. H. Coulter (1953). U.S. patent 2,656,508.

similar principles, for measuring sensitivity-disk zone diameters was demonstrated by Lightbown, Isaacson, Tattam, and Wright (1969).

POSSIBLE AUTOMATED METHODS FOR IDENTIFICATION OF PATHOGENIC MICROBES

Current bacteriological technology relies heavily on the expert inspection of plate cultures for the recognition of the presence of pathogenic microbes in a specimen, their distinction from the commensal or contaminating bacteria that may also be present, and an indication of the nature of the confirmatory tests required. The visual inspection might be replaced by some automated method if it were possible to develop selective culture media so efficient that the only colonies developing on them would be those of the pathogen sought, or if one had a method for recognizing some chemical change in the growth medium that was quite specific for each pathogen.

Although selective culture media have been in use for many years, practically none of them is completely specific in allowing the growth only of the required pathogen, and, in general, the more selective the medium the more it tends to be somewhat inhibitory even for the bacteria selected. The selective culture media available make the visual recognition of important colonies on a plate immensely easier, but they are not yet at a stage when the eye could be reliably replaced by an automatic recognition device.

The specific identification of particular bacteria is now often possible by the use of staining methods involving labelled antibody; the most used label is fluorescein, and bacteria stained by such reagents can be readily recognized by ultraviolet microscopy. Radioactive labels have also been employed. Either of these methods can undoubtedly be employed to recognize specific bacteria automatically, although there will certainly be considerable problems in ensuring the specificity of the reagents.

It has often been said that the methods of infrared spectroscopy ought to be adaptable to provide a truly automated identification system. At present this seems some distance from realization, partly because of the large quantity of bacterial growth required and partly because insufficient work has been done on the variations in the spectrogram to be expected among the members of one clinically significant bacterial species, and of the variations attributable to variations in cultural conditions. In any case it is difficult to conceive that it would be possible by infrared spectroscopy to detect the pathogen among a mixture of non-pathogenic bacteria, so that the use of the method would

imply the sorting of pure cultures from the mixed primary cultures. If this is necessary it may well be better to automate conventional identification methods rather than to demand extensive exploration of an entirely new method. Similar considerations apply to the use of chromatography.

At this point it is convenient to distinguish what may be called the 'public health' approach from the 'hospital diagnostic' approach. In investigating an outbreak of *Salmonella* infection, for example, one may need to examine large numbers of specimens for the presence of the particular salmonella type known to be implicated; one is simply asking the question, Is *Salmonella x* present or not? This provides the ideal field for the use of labelled antibody recognition methods, or for the use of a specific selective medium.

The hospital diagnostic laboratory is generally asked a different question, in the form, What, if any, bacteria are present in the specimen; are they present in numbers likely to be significant, and what is their antibiotic sensitivity? To answer the first part of this question with the use of labelled antisera or specific selective media would require the use of a considerable battery of reagents; to answer the third part requires the isolation of the specific bacteria in pure culture, which is not greatly facilitated by labelled antibody methods.

It seems to us, therefore, that we are a long way

FIG. 1. Automatic culture-spreading apparatus.

FIG. 2. Swab culture spread with automatic apparatus.

off the situation where we can envisage a fully automated system for examining the specimens sent to the hospital diagnostic laboratory, but there are nevertheless ways in which mechanization ought to improve the output of the laboratory.

It should be possible to mechanize the inoculation of specimens on to culture plates and their transfer to the incubator, and similarly, when the bacteriologist has picked the colony of interest and selected its identification programme, it should be possible to transfer it to the various identification media, or set up cultures for antibiotic sensitivity determination mechanically without undue difficulty.

We have so far constructed one part of the equipment for such a system, namely, a machine for spreading the culture over a Petri dish to give a display of discrete colonies. The machine has a wire loop, which can be sterilized by passing a current through it (Trotman and Drasar, 1968), that is drawn radially across the culture plate while the latter is rotated, thus tracing a spiral (Figs. 1 and 2). A similar method has been examined by Williams and Bambury (1968). Preliminary investi-

gations have shown that this is as good as the conventional spreading method for revealing the different colonies in a mixed culture (Trotman, 1969).

The passage of an electric current is a convenient method of sterilizing a wire loop, but is not suitable for tubes or pipettes that will be needed to transfer cultures into various diagnostic reagents. As a preliminary to designing equipment for this purpose we have explored the use of radio-frequency (rf) induction heating. In this method a radio-frequency current in a hollow copper coil surrounding the workpiece produces a radio-frequency magnetic field in the workpiece and this field leads to losses of eddy current in a non-magnetic conductor or hysteresis and losses of eddy current in a magnetic conductor, thus heating the workpiece. A few experiments utilizing this method to sterilize a stainless steel pipette are illustrated in Table I.

DATA PROCESSING AND DISPLAY OF RESULTS

As will be seen our philosophy has been to devise methods for the safe and sterile handling of the material used in diagnostic bacteriology rather than to devise methods for reading the results. This approach was forced on us by the technical considerations already mentioned, but in any case it would be necessary to devise methods for handling cultures before a completely automated system could be contemplated.

It does not seem that there should be serious difficulty in devising methods for printout or other display of the results from tests in which bacteria are to be counted, so that an automated system for antibiotic titrations, antibiotic sensitivities, or for quantitative urine cultures ought to be possible. The interpretation of diagnostic cultures will be considerably more difficult. In theory it is a simple matter to feed the results of a set of diagnostic tests into a computer previously provided with a memory store relating various patterns of test results to bacteriological diagnoses. Some of the difficulties involved in using such methods are illustrated in a recent paper by Dybowski and Franklin (1968) but Dr S. P. Lapage (personal communication, 1969) has obtained very encouraging results with cultures tested by standard methods at the National Collection of Type Cultures.

The use of computers and other data-handling equipment for the laboratory records and for transmitting reports from laboratory to wards is no different in principle in bacteriology from other branches of pathology though more complex coding systems will be needed (Flynn *et al*, 1968). This aspect is discussed elsewhere in the Symposium.

TABLE I

STERILIZATION OF STAINLESS STEEL PIPETTE BY RADIO-FREQUENCY INDUCTION HEATING

Rf Oscillator Valve Current (milliamperes)	Time for which Power Applied (sec)	Number of Failures in 20 Tests with Pipette Contaminated with		
		<i>E. coli</i>	<i>Staph. aureus</i>	<i>B. stearothermophilus</i>
300	4	9	19	20
	8	4	1	8
	12	2	0	0
	14	0	0	0
325	4	4	17	20
	8	0	0	4
	12	0	0	0
	14	0	0	0
350	4	6	14	5
	8	0	0	0
	12	0	0	0
	14	0	0	0

The hospital bacteriologist has epidemiological as well as diagnostic responsibilities and a useful byproduct of improved data transmission should be the prompt display to the bacteriologist of the diagnostic results in a form that could provide a basis for preventive action.

THE PLACE OF MECHANIZATION AND AUTOMATION IN DIAGNOSTIC BACTERIOLOGY

In the foreseeable future we see more place for the development of mechanical aids to provide technical assistance to the bacteriologist than for automated systems that will turn the bacteriologist into a machine-minding technician. It ought to be possible to achieve some real saving in technician time with the employment of mechanical equipment not too complicated or expensive to be justified in a laboratory handling 50 to 100,000 requests per year. But diagnostic bacteriology rarely has the urgency of some chemical or haematological tests and a concentration of the bacteriological bench work in central laboratories equipped with extensive mechanical aids may well be justifiable before long.

At the same time there is a need to develop more rapid diagnostic techniques; it should not require more than a few hours to determine whether or not a bacterium is inhibited by antibiotics. One great advantage of mechanization should be that tests can be started by the equipment on some cultures while the bacteriologist is still working on others, and the methods that are needed for the automatic recognition of growth, or of some consequent change in the growth medium, ought to permit the determination of significant changes in growth at an earlier stage than is possible by conventional methods.

CONCLUSION

Our studies lead us to think that there is a real place for automation in diagnostic bacteriology, but it does not seem probable that machinery will usurp the bacteriologist's skill in searching culture plates for potentially important colonies and in deciding on the pathway for their further investigation. Mechanization ought to make it possible, within reasonable resources, to offer a better service to the clinician: to give answers more quickly, to monitor antibiotic therapy more precisely, to make more extensive investigations into the sources of infecting bacteria, and to allow the bacteriologist time to be, if he wishes, an active member of the clinical as well as of the laboratory team.

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