Slow progress in developing rapid methods and automation in clinical and public health microbiology


"The amazing thing in life", said a seventeenth-century philosopher, "is that all the necessary things are simple and all the complicated things are useless."

During the last ten years a lot of work has been done, and much money spent, in an effort to develop rapid methods and design equipment for automating, or at least mechanising, some of the routine procedures in clinical and public health microbiology laboratories. Progress has been slow and on the whole disappointing.

Compared with clinical biochemistry and haematology, for which machines have been in routine use for some 20 years, clinical and public health microbiology is still largely a labour-intensive cottage industry. It would therefore seem timely to try to identify and discuss some of the reasons for the present state of affairs. Is it because microbiology is inherently different from the other clinical sciences? Have we been taking too large a step at a time? Are the costs of development too great for the potential reward in relation to the work load, in spite of microbiology as presently practised being a highly labour-intensive activity? Should we pack it all up or perhaps try some new ideas? Where should we go from here?

GENERAL CONSIDERATIONS

Is there a distinction between automation and mechanisation?

'Mechanisation' usually refers to a process which imitates a sequence of manoeuvres customarily done by a human operator. Automated procedures achieve a similar result but not necessarily by imitating all or any of the manual manoeuvres involved. Highly developed automated procedures include self-regulating feedback systems controlled by a computer. Most automated procedures include elements of mechanisation. Throughout this article the term 'automation' will be used to include both concepts.

The problems

The problems in designing automated equipment for microbiology differ greatly from those in clinical chemistry and haematology.

1. Maintaining sterility of the analytical process - this is paramount in microbiology but less exacting in tests for antibiotics in serum where most progress in automation has been made.

2. Diversity of specimens - which in microbiology far exceeds that in clinical chemistry and haematology.

3. Diversity of analyses - in microbiology the diversity of organisms to be detected, identified and sometimes counted far exceeds the range of constituents determined in clinical chemistry and haematology.

4. Nature of the analytical systems - most culture media and many test reagents such as diagnostic antisera contain chemically undefined, variable and capricious biological materials.

5. Preparatory stages of analyses - to detect pathogens there is a need to 'amplify the signals'. This is achieved by:
   (i) using a microscope to search for putative pathogens in stained preparations of specimens. Staining machines are available and can work well if properly maintained.
   (ii) incubating the sample on various culture media in different atmospheres for up to 18h or longer. Here there are problems in achieving an adequate 'noise to signal ratio'.

6. Economic aspects - the technical and engineering problems of automating microbiology and devising rapid methods may not be insuperable. The main constraints are financial and the will to afford the necessary cost.

7. Personnel problems - automating microbiology could lead to a loss of job satisfaction. Automating clinical chemistry has not apparently reduced the number of chemists required but the work load ratio has increased.

DETECTING AND IDENTIFYING PATHOGENS IN CLINICAL SPECIMENS

Questions to be answered

Are there any clinically relevant pathogens in the specimen? If so, what are they and what are their antimicrobial susceptibilities? Speed and accuracy are important here, especially in emergency situations, e.g. a patient with meningitis or septicemia.

The standard routine is:

1. Microscopy - Gram-stain for most organisms - Ziehl Negelius or Auramine-pheno fluorescent stain for acid-fast organisms.
2. Machines are available, and perform well if properly maintained. Attempts to design machines to scan smears of sputum microscopically for acid-fast bacilli have met with very limited success. In addition the work load of most laboratories does not justify the cost.
3. Electrophoresis as an aid to detecting and identifying pathogens has very limited use.
4. Rapid detection and identification of anaerobes by gas-liquid chromatography has proved successful. Other forms of chromatography plus mass spectrometry have been explored but are very costly.
5. Selection of colonies of putative pathogens for tests of identification. Attempts at automation have shown that it is not likely to be cost effective.
6. Determining antimicrobial susceptibility of putative pathogens (see separate section on this topic).
7. Blood cultures present special problems. Often they are from seriously ill patients and there is an urgent need to know:
   (i) if there is bacterial growth,
   (ii) the nature and antimicrobial susceptibilities of the organism.

Measuring changes in electrical impedance or conductivity in the cultures appears promising.

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ISOLATED PATHOGENS

1. Disc diffusion techniques
   Various aids for applying discs, and machines for measuring...
zone diameters can improve accuracy but are not used in many clinical laboratories where only the presence or absence of test zones with zones of control organisms is made by eye with or without the aid of templates. The greatest present need is for a simple inexpensive device for rapidly obtaining standardised inocula of test and control organisms, which is essential for accurate work.

2. Agar dilution techniques
Multiple inoculators allow seeding of culture plates (each containing a predetermined concentration of a particular antimicrobial agent) with as many as 50 test and control strains. The problems involved are mainly logistical. Strains of pathogens which are very fastidious or partly delicate the laboratory need to be passed to a single work station for susceptibility testing. Very often, laboratories lead to difficulties with the transcription and labelling errors and requires additional work in collating results. Loss of colonies happen to those inoculated strains have isolated a pathogen but who are deprived of completing the test because it was missed. However, the system does work well in some laboratories.

3. Optical density measurement of bacterial growth in liquid media
Spectrophotometers based on this principle perform well but the running costs are too high for most laboratories. Inadequate growth is not as easily detectable as in disc diffusion techniques. Uneven growth is not only caused by the growth of organisms but also by the liquid medium itself. The system may also cause a problem.

4. Other methods of detecting inhibition of bacterial growth
Those methods mentioned in the enumeration of living organisms section may be adapted to measure inhibition of bacterial growth in liquid media but have the disadvantages mentioned in that section.

ANTIMICROBIAL ASSAYS ON PATIENT’S SERUM
Accuracy and speed of assay (i.e., an answer within 1h) is essential for diagnostic purposes. Routine bacterial infections (especially ampicillin-sensitive) administered to patients with impaired renal function or who are being dialysed.

ENUMERATION OF LIVING BACTERIA IN URINE, WATER, MILK AND FOOD
A total undifferentiated bacterial count is usually required but a screening test for a given number of organisms is just as useful. Various approaches to automation have been made but there are problems with them all.

1. Simple aids to manual techniques
A flopped hand-set for making dilutions of bacterial suspension in agar and dispensing drops of the agar into petri dishes (droplet counter) has been most successful. Visually and electron microscopical colonies by hand and eye are slow and less useful, whilst electronic scanners for counting colonies are expensive and unreliable.

2. Electronic particle counters
There are problems in preparing the sample in order to separate the bacteria from extraneous material and thereby achieve an adequate signal to noise ratio. In addition the aperture easily becomes blocked. It should be noted that machines designed to count blood cells give poorly reproducible results with bacteria.

3. Bioluminescence
Measuring bacterial ATP as an index of bacterial content has met with limited success, and there are problems with the supply and cost of ATPase.

4. Measuring physical or chemical changes produced in liquid culture media by bacterial growth
(a) Measuring changes in pH and E of culture medium as a result of bacterial activity. Both methods are unreliable with mixed populations of unknown bacteria and are relatively insensitive.
(b) Changing optical apparatus—several machines measure optical density as an index of bacterial growth, however there are problems with uneven growth and the technique is slow. Light-scatching techniques have been tried, again with limited success.
(c) Detecting C4 in CO2 evolved by bacteria acting on substrates containing C4. This method is sensitive but the correlation with numbers of colony-forming units is poor. There are problems with handling and disposing of the radioactive material.
(d) Microcalorimetry. Measuring the change in temperature resulting from bacterial metabolism and relating this to numbers of bacteria has met with some success in dedicated hands.
(e) Measuring changes in electrical impedance of a medium may be useful in screening urine samples but is more promising for blood cultures.

DETECTING AND MEASURING SERUM ANTIBODIES
This aspect of microbiology is much more akin to clinical biochemistry which is now highly automated. The need to prevent bacterial contamination is not as great as in cultural methods but many of the reagents used are relatively unstable, being complex biological materials with batch to batch variation. Standard methods are therefore essential. For these reasons, and because they are often done in large batches, antibody tests lend themselves to automation.

Well-designed and efficient machines should do tests more cheaply and faster than skilled manual operators. Furthermore, results can be readily fed into a computer for production of routine reports and for epidemiological analyses.

To be cost-effective, tests need to be centralised, perhaps in regional or state laboratories. However this poses problems with the transportation of specimens, and with ensuring that each is accompanied by adequate clinical information. In addition there is the problem of interpreting the results at a distance and distributing reports. Attempts have been made with varying degrees of success to adapt continuous-flow analysers designed for biochemical tests to perform complement fixation tests. Three major problems were found:

(1) Slow sampling rate compatible with adequate spacing between samples.
(2) Need to replenish the machine frequently with samples by hand.
(3) Need to clean and adjust the flow-cell during use.

Despite the problems, systems for analysis seem likely to prove more suitable than continuous-flow systems for antibody tests.

DATA PROCESSING
The end product of every laboratory is information, yet most clinical and public health microbiological laboratories are still using archaic and totally manual methods of handling the data required for producing this end product and in processing the final product itself.

There is a strong case for computerised data handling in large clinical and public health microbiology laboratories.

THOUGHTS FOR THE FUTURE
The changing epidemiology of microbial diseases may profoundly affect the planning of future developments in rapid methods and automation in microbiology, e.g. the need for rapid diagnosis of smallpox has disappeared dramatically with the worldwide eradication of the disease. Can the technology of automation keep pace with such changes? If so, what of the economics? Viewed commercially, the risks are great and the monetary rewards doubtful. Manufacturers of laboratory equipment need competent advice and guidance from medical microbiologists.

Some suggestions
1. Select and concentrate on automating basic and repetitive aspects of the technology that are not likely to change too soon or too unexpectedly, e.g. preparation and inoculation of culture media, making serial dilutions of patients' serum, adding volumes of reagents.
2. Automate those aspects of the technology which human operators perform badly, e.g. estimating titration endpoints, microscopic scanning for organisms likely to be present in small numbers, such as acid-fast bacilli.
3. Look at novel approaches to detecting and identifying pathogens in clinical and environmental specimens, e.g. various forms of chromatography.
4. Avoid trying to automate those aspects of the technology requiring a good deal of judgment and manual dexterity, e.g. scanning culture plates for colonies of pathogens and subculturing suspect colonies.
5. Automate data handling, including production and filling of routine reports, analysis of information on file for infection control, epidemiological studies and research.

First International Campylobacter Workshop
The First International Campylobacter Workshop organised by the Public Health Laboratory Service was held at the University of Reading on 24-26 March 1981. The 150 or so delegates present at the Workshop included leading medical and veterinary experts from many countries. The very full programme of lectures and poster presentations included reports of every recent finding. Considerable attempts were made in determining the special requirements of culture media and at the International Symposium on developing an efficient enrichment medium and evaluating the relative merits of various selective culture media which have been described. It is now apparent that the choice of culture medium is influenced considerably by the type of specimen.

Discussion on the special gaseous requirements indicated that much has yet to be learnt. Reports confirmed the usefulness of FBP, a mixture of ferrous sulphate, sodium metabisulphite and sodium pyruvate. This mixture increases the aero-tolerance of the organism, making it possible to grow it satisfactorily in a candle jar, thus providing Third World countries with the ability to conduct Campylobacter isolation simply and inexpensively.

In addition to many lectures and poster presentations, some of Oxford's technical staff were available to demonstrate the Company's range of products for isolating Campylobacter, including the freeze-dried antibiotic supplement developed by Professors Butcher and Drs Blaser and Wang who were all present at the Workshop. Drs Skov and Workman worked with these experts to develop versions of their formulae suitable for Oxford's equipment, and is the only company which is able to offer a complete set of Campylobacter Supplements for the isolation of all the species so far discovered.

In conclusion, the Campylobacter Workshop provided an extremely useful forum for discussion of ideas and comparison of results. Some information currently appears contradictory, but because of the degree of involvement of so many workers it is apparent that much of it will be resolved by the time of the second Workshop which has been proposed for 1983.

The following articles are based on three of the sessions at the Workshop and are a reflection of the great degree of interest currently being shown in Campylobacter.

Oxoid display at First PHL International Campylobacter Workshop. From left to right are Dr SM Skov, honorary consultant on Campylobacter to the PHLS; Dr WC Wang of the Veterans Administration Medical Centre, Denver; Dr MJ Blaser of the Centers for Disease Control, Atlanta; Professor JP Butcher of the Free University, Brussels; and David Post, Oxoid’s product development manager.
During the last few years, Campylobacter jejuni has emerged as a common cause of acute diarrhoea without prodromal symptoms. Although this organism was suspected to be a cause of acute enteritis in man as early as 1964, it was not until 1973, in Belgium, that it was first shown to be a common cause of diarrhoea. Since then other workers in the United Kingdom, the Netherlands, Sweden, the USA, South Africa and Canada, and many other countries have reported its isolation in from 5-14% of patients suffering from diarrhoea and less than 1% of people without symptoms. The few reports that have been received from the tropics suggest that the infection is particularly common and in such areas it could well prove to be a disease of much greater importance than it is in temperate zones.

Clinical features
Firstly, not all Campylobacter infections produce symptoms. Symptomless excreters commonly occur among the close contacts of infected patients, although their incidence in the total population is less than 1%. For symptomatic patients the incubation period averages from two to five days, but occasionally it extends to ten days. Usually, the disease starts with fever coupled with a general feeling of weakness, headache and confusion. This prodromal state lasts for up to two days and is followed by nausea and abdominal cramps. These symptoms are rapidly followed by diarrhoea, which may be profuse, watery or bloody, with or without fever. The clinical features vary from those of a brief insignificant gastroenteritis to an enterocolitis with abdominal pain and bloody diarrhoea which may last for several weeks. Patients presenting with signs of acute colitis have been mistakenly thought to have ulcerative colitis. The distinction is important because treatment with steroids can have serious consequences. The help of the laboratory is therefore required in order to establish the correct diagnosis.

Complications - the "acute abdomen"
In a few patients the abdominal pain is so severe that they are admitted to hospital as cases of acute appendicitis, cholecystitis, or peritonitis. Some undergo emergency surgery and occasionally they do indeed have peritonitis from an acute appendicitis, but most of those who undergo laparotomy have inflammation of some part of the ileum and jejunum coupled with mesenteric adhesions which have more than once been mistaken for the lesions of typhoid fever. Most of the patients who end up in hospital are young adults and teenagers. Another potential complication is gastric perforation which may occur in infants. Diarrhoea is very common and at this age yet blood may appear in the stools and mislead the clinician into thinking there is an intestinal ulceration. Six instances are known where babies underwent laparotomy on suspicion of this condition. Some patients develop reactive arthritis and ulcers in the stomach which have subsequently happened in patients with Campylobacter enteritis and colitis.

Persistence of organisms in stools
In the absence of chemotherapy, the faeces of patients remain positive for about two to seven weeks after the illness. However, a mild case may excrete the organism for only a few days and there is always the odd patient who excretes for a much longer period.

Prognosis and therapy
In general, campylobacter enteritis has a very good prognosis and the isolation of campylobacter from the stools does not necessarily warrant chemotherapy. Indeed, by the time a bacteriological diagnosis is made, it is common to find that the patient is already recovering.

Figure 1. Electron micrograph of C. jejuni showing a spiral form with a flagellum at each end of the rod (x 25,000). Electron microscopy studies from the Department of Prof. deKeijzer, Pasteur Institute, Brussels.

BC Selective Agar with Supplement
Bacillus cereus is now recognized as a potential food poisoning organism. It is important, therefore, to quickly identify its presence among the many harmless Bacillus species found in foodstuffs. The distinctive turquoise to peacock-blue colonies and the other described characteristics of B. cereus, make this possible.

Antibiotics as selective agents for brucella have advantages over dyes, which can be very inhibitory. Brucella Selective Supplement is formulated on the proven mixture of antibiotics described by Farrell & Robinson (1972). It can be used to isolate dye-sensitive strains of Brucella abortus, as well as other species of the Brucella genus.

The full list of references have been omitted due to lack of space, but are available on request from the publishers, Oxoid Limited.
Campylobacter jejuni: the serological approach

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A session of the International Workshop on Campylobacter Infections was devoted to the problem of serotyping strains of Campylobacter jejuni and to techniques for the serological diagnosis of infection. The following account is an attempt to summarize the current "state of the art."

Serotyping

There is clearly a need for a definitive serotyping scheme for C. jejuni, which would be comparable to the Kauffmann-White scheme for salmonellae. Without such a scheme, it is impossible to compare results obtained by different laboratories. Furthermore, the very high rate of virulence of C. jejuni means that any vaccine must be serologically specific and capable of inducing immune responses to a wide range of strains. This has been approached in two ways: either by selecting a limited number of strains to be used as prototypes or by determining the antigenic relationships between all the strains available.

The current situation is that many workers have been trying to type C. jejuni strains using a variety of techniques, and there is considerable variation in the results obtained.

The advantage of using a limited number of reference strains is that it is possible to compare results obtained by different laboratories. However, the disadvantage is that it is impossible to be sure that all the strains involved are included, and that the prototype strains are representative of the strains encountered in the community.

On the other hand, using a reference scheme to examine sera for evidence of past or current infection is a further area of serology which has been neglected. There has been a tendency to use C. jejuni strains as an antigen in order to look for significant changes in antibody titre in sera collected early in an illness and in convalescence. An alternative is to use the strain isolated from an outbreak and to examine sera by a serological test specific for the strain. This approach is useful for the identification of specific strains and for the determination of the antigenic relationships between strains. However, it is not possible to be sure that the prototype strains are representative of the strains encountered in the community.

The following account is an attempt to summarize the current "state of the art."