BRITISH MEDICAL JOURNAL

LONDON, SATURDAY 1 MARCH 1986

Bedside and rapid bacteriology

A move is afoot from clinicians for reviving side room diagnostic tests. The past 30 years has seen much centralisation of pathology; in some regions many diagnostic procedures are now carried out in large laboratories distant from the patient's ward or general practitioner. The advantages, mainly of accuracy, have to be weighed against the disadvantages of clinical convenience and cost effectiveness.

In the past rapid and straightforward investigations such as direct microscopical examination and a Gram stain of urine, sputum, and cerebrospinal fluid were performed by clinicians as a matter of course and provided them with important diagnostic information in the ward side room or the general practitioner's surgery. Few younger clinicians nowadays would claim to be technically adept at performing such investigations.

Nevertheless, simpler methods are now becoming available, a good example being dipsticks to detect white blood cells in body fluids. These new tests are simple; and their good correlation with direct microscopy makes them suitable for detecting white cells in both urine[1] and peritoneal fluid[2] in the ward. At present, however, these tests are not sufficiently sensitive to detect polymorphs in other body fluids such as cerebrospinal fluid. C Reactive protein is a sensitive indicator of inflammation and has been used to monitor progress during treatment; and in many hospitals it is measured with enzyme immunoassays or radial haemolysis, investigations which take one and 24 hours respectively. A new latex agglutination kit can detect 6 mg C reactive protein per litre in a matter of minutes (CRP-Wellcotest, Wellcome Diagnostics, Dartford, England). Widespread use of such a kit is probably precluded by its cost and the need for further laboratory investigations, but the method might prove valuable in high dependency areas such as special care baby units, where junior medical staff could be trained to perform such tests and follow up strongly positive reactions by specific investigations for possible infection. In its present form the kit cannot measure concentrations of C reactive protein and so would be of less use in monitoring progress.

In recent years various rapid immunological techniques have also been developed. These include counterimmunoelectrophoresis, coagglutination, latex agglutination, enzyme immunoassay, and radioimmunoassay. Only two of these methods, coagglutination and latex agglutination, are suitable for use at the bedside. Commercial latex agglutination kits are available to detect *Haemophilus influenzae* type b, *Neisseria meningitidis* (serogroups A, C, Y, W135), pneumococcal antigens and group B streptococci in body fluids, group A streptococci in swabs and body fluids, rotaviruses in the stools, and rubella antibodies in serum. Although not available commercially, latex agglutination has been used to detect cryptococcal and histoplasma antigen in cerebrospinal fluid and serum,[3] and coagglutination has been used to detect salmonella and shigella in stool samples (A A El Marsafy *et al*, paper to 2nd European Congress of Clinical Microbiology, Brighton, 1985). These produce results in 10-15 minutes, are easy to perform, require little equipment, and would reduce tedious laboratory work.

There are, however, drawbacks to their use. False positive reactions have occurred with *H influenzae* type B antibody coated particles,[4] and false negative reactions have been reported with particles coated with pneumococcal omniserum.[5] Reading the test is subjective—strong agglutination reactions are easy to read while weak reactions are difficult to evaluate. Experience in interpretation is required. Nonspecific reactions have been reported when the kits have been used to detect antigen in serum.[6] These reactions were not removed by heating the serum at 56°C for 30 minutes as recommended by the manufacturers. Polyclonal antibodies used in *N meningitidis* kits have a high specificity for serogroups A and Y but do not detect serogroup B, the commonest cause of meningitis in Britain. One kit which detects Lancefield group A streptococcal antigen seems suitable for use in a general practitioner's surgery, but it gives a negative result in patients whose throat cultures yield scanty growth of the pathogen (fewer than 10 colonies of group A streptococci per culture plate[7]). Further clinical studies and possible refinements are, therefore, required before the kits can be substituted for throat cultures. The sensitivity of the latex agglutination rotavirus kit is slightly less in comparison with other methods in tropical areas (M Mounier *et al*, paper to 2nd European Congress of Clinical Microbiology, Brighton, 1985). Handling potentially infectious samples may be hazardous if the tests are performed by untrained staff. Finally, the kits are expensive and may increase further in cost as current techniques are being replaced by the more specific tests employing monoclonal antibodies.[8]

Yet despite their limitations latex agglutination kits offer

clinicians the possibility of making a preliminary diagnosis of the cause of purulent meningitis, pharyngitis, and diarrhoea in infants when there is no access to a laboratory. In normal circumstances the laboratory would still be needed to confirm the diagnosis and check the appropriateness of treatment. In developing countries, where these kits may be potentially most useful, the cost and the relative instability of latex during transport in tropical areas might limit their use. In Britain and other developed countries the diagnostic kits would be of most value when a bacteriological diagnosis has to be made outside routine laboratory hours. Not only will this reduce the costs of the "on call" services but it may also produce more immediate results. If junior medical staff are trained to perform and interpret the results of the tests close liaison will be essential between the main laboratory and the user in order to ensure a reasonable standard of accuracy, precision, and reliability. In practice, however, this type of approach is unlikely to replace conventional methods.

Certain other methods of antigen detection such as enzyme immunoassay and radioimmunoassay are most suited to automation. Instruments have been developed for use in laboratories, but they are not suitable for use in side rooms, as the whole procedure may take up to six hours for enzyme immunoassay and 40 hours for radioimmunoassay, the latter method carrying the added risk of handling radioactive isotopes. Before the use of solid phase immunoassay instruments can be envisaged in side rooms methods must be fully automated and non-radioactive labelling systems introduced. Quicker procedures, possibly linked to a computer system able to give clear instructions and results to the user, would be the ideal—but somewhat unrealistic.

A rapid microbiological diagnosis is needed most in the diagnosis of septicaemia, where information about the causative organisms and possibly their antibiotic susceptibility allows treatment with the appropriate antimicrobials to be given as soon as possible. Considerable interest in the rapid identification of bacteria has led to the development of several new techniques. Though still in the early stages of development, these methods might be adapted for use directly on clinical samples[9] and have a great potential for automation, rapid identification,[10] and possible use at the bedside. Circular intensity differential scattering is a technique in which light which is alternately right and left polarised is shone on a sample. The sample differentially scatters the polarised light, and the pattern (known as the circular intensity differential scattering spectrum) varies with each species (and strain) and may be measured using a spectrometer. Preliminary data show that strains of bacteria which even have a similar guanine-cytosine content, such as *Morganella morganii* and *Escherichia coli* K12, have different circular intensity differential scattering spectra and may be differentiated in 15 minutes. Hepatitis B virus has also been detected by this method directly from clinical samples.[9]

Fourier transform infrared spectroscopy uses more sensitive and faster recording spectrometers to identify organisms by their infrared absorption bands.[10] Infrared spectra of intact bacteria are composed of many absorption bands. Characteristic bands may be identified for each of the different constituents of bacteria (for example, cell walls, proteins, and membranes), indicating that such spectra might serve as a spectral fingerprint. Comparison of unknown spectra with those stored on a reference database allows rapid identification of bacteria. Further studies of this technique on clinical samples would be of great value.

More recently conventional microbiological practices have been challenged by a totally different approach based on deoxyribonucleic acid (DNA) matching using hybridisation or DNA probes. DNA probes have been used to identify specific gene sequences of infecting agents directly from clinical samples.[11] They may also be used to distinguish pathogenic and non-pathogenic strains. Several methods are available to perform nucleic acid hybridisation, but they are all complex and time consuming, and some require radioactive isotopes; thus nucleic acid hybridisation remains a research tool. Attempts are being made to simplify the procedures and to use non-radioactive labelled probes such as biotin. Indeed, non-radioactive labels are the main hope for the future, as they would avoid the problems of decontamination and storage. Furthermore, if only one labelled sequence is used (as in the "sandwich hybridisaton" technique) the mass production of probes would be facilitated and costs reduced. The sensitivity of the tests using non-radioactive labels is currently being compared with that of tests using radioactive labels.[12] Nucleic acid hybridisation may revolutionise conventional microbiological practices making the investigation of infection a primary rather than a confirmatory diagnostic service. It may only be a matter of time before we see prototype probe kits or automated machines containing DNA probes used to detect infective agents directly from a clinical sample in laboratories or side rooms.

To look even further into the future, biosensors seem likely to make increasing contributions to clinical practice.[13] The most exciting prospect for biosensors in relation to clinical microbiology is their use in continuous in vivo monitoring of antibiotic concentrations, with feedback control of delivery rate for agents with a narrow therapeutic range such as aminoglycosides. Optical sensors have already been used to detect antigen-antibody interactions in vitro[14]; might it be possible to monitor antibody responses to an infective agent using optical sensors in vivo? No doubt we shall hear more about biosensors and these other methods in the coming years.

NEETA MANEK
Senior registrar

RICHARD WISE
Reader and consultant

Department of Medical Microbiology,
Dudley Road Hospital,
Birmingham B18 7QH

1 Kusumi RK, Grover PJ, Kunin CM. Rapid detection of pyuria by leukocyte esterase activity. *JAMA* 1981;**245**:1653-5.
2 Chan LK, Oliver DO. Simple methods for early detection of peritonitis in patients on continuous ambulatory peritoneal dialysis. *Lancet* 1979;ii:1336-7.
3 Dee HD. Mycoserology: its status as a diagnostic adjunct. In: Rytel MW, ed. *Rapid diagnoses in infectious disease*. Florida: CRC Press, 1979:157-97.
4 Leinonen M, Merva E. The latex agglutination test for the diagnosis of meningococcal and Haemophilus influenza meningitis. *Scand J Infect Dis* 1977;**9**:187-91.
5 Coonrod JD, Rylko-Bauer B. Latex agglutination in the diagnosis of pneumococcal infection. *J Clin Microbiol* 1976;**4**:168-74.
6 Intram DL, Pearson AW, Occhiuti AR. Detection of bacterial antigens in body fluids with the Wellcogen Haemophilus influenzae b, Streptococcus pneumoniae and Neisseria meningitidis (ACYW135) latex agglutination tests. *J Clin Microbiol* 1983;**18**:1119-21.
7 Gerba MA. Detection of group A streptococcal antigen by coagglutination and latex agglutination. In: Habermehl K-O, ed. *Rapid methods and automation in microbiology and immunology*. Berlin: Springer-Verlag, 1985:357-66.
8 Dick HM. Monoclonal antibodies in clinical medicine. *Br Med J* 1985;**291**:762-4.
9 Gregg CT, McGregor DM, Grace WK, Salzman GC. Rapid microbial identification by circular intensity differential scattering. In: Habermehl K-O, ed. *Rapid methods and automation in microbiology and immunology*. Berlin: Springer-Verlag, 1985:184-7.
10 Giesbrecht P, Nawmann D, Lablschirski H, Branickel G. A new method for the rapid identification and differentiation of pathogenic microorganisms using Fourier transform infrared spectroscopy. In: Habermehl K-O, ed. *Rapid methods and automation in microbiology and immunology*. Berlin: Springer-Verlag, 1985:198-206.
11 Falkow S. The use of DNA hybridization for the identification of pathogenic bacteria. In: Habermehl K-O, ed. *Rapid methods and automation in microbiology and immunology*. Berlin: Springer-Verlag, 1985:30-3.
12 Sethabut RO, Hanchalay S, Echeverria P, Taylor DN, Leksomboon U. A non-radioactive DNA probe to identify shigella and enteroinvasive Escherichia coli in stools of children with diarrhoea. *Lancet* 1985;ii:1095-7.
13 Bickup JC. Biosensors: a clinical perspective. *Lancet* 1985;ii:817-20.
14 Sutherland RM, Dahne C, Place JF, Ringrose AS. Optical detection of antibody-antigen reactions at a glass-liquid interface. *Clin Chem* 1984;**30**:1533-8.