INVITED ARTICLE

UPDATE OF THE RAPID DIAGNOSIS OF INFECTIOUS DISEASES I: BACTERIA, FUNGI AND PARASITES

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ABSTRACT

The application of monoclonal antibodies and DNA probes in the clinical microbiology laboratory has resulted in an array of rapid diagnostic tests. The immunofluorescent assay or enzyme-linked immunoassay is widely used in the rapid diagnosis of bacteria eg Group A streptococcus, Legionella pneumophila, Mycoplasma pneumoniae, Bordetella pertussis; parasites eg Chlamydia tachomatis, Cryptosporidium species; and fungi eg Pneumocystis carinii.

The BACTEC system was first introduced to detect bacteraemia pathogens. It has been further developed to detect Mycobacterium species in clinical specimens and this has greatly reduced turn-around time in the laboratory diagnosis of Mycobacterium species.

The discovery of the polymerase chain reaction has led to hopes of using it as a potential diagnostic tool in the microbiology laboratory.

Keywords: rapid diagnosis, infectious diseases

SINGAPORE MED J 1994; Vol 35:316-318

INTRODUCTION

Clinical microbiology has been a dynamic discipline in the past 10-15 years. Exciting developments include the recognition of several new pathogens, the re-emergence of classic pathogens and the automation of tests for identification and antimicrobial susceptibility testing as well as the exciting development of molecular diagnostic tools.

RAPID DIAGNOSIS OF BACTERIA

A. Respiratory pathogens

Group A streptococcus

Pharyngitis caused by *Group A streptococcus* is traditionally diagnosed by a throat swab culture. The commercial development of antigen detection kits stirred up great interest as it can be performed on the practice premises with the advantage that it can be interpreted before the patient leaves. Most of thesetests are based upon latex agglutination or enzyme-linked immunoassay (EIA) eg Directigen 1,2,3 Group A Strep Test (DGAST). In the latex agglutination test, latex particles coated with IgG to *Group A streptococcus* will form a lttice when exposed to the antigen. EIA uses the principle of the detection of coloured products generated by enzyme labelled antibody complexed with the *Group A streptococcus* antigen. Unfortunately, when compared with throat swab culture the sensitivity of the DGAST is often very variable (61% – 95%) although specificity may be good (88% –100%)[1-4]. Negative results with the rapid antigen assays is best followed up with culture. It is therefore not surprising that these antigen detection kits are not popularly used. In the interpretation of these test, it must be remembered that symptomless carrier rates vary from 6%–40%.

Legionella species

Legionella pneumophila causing Legionnaires disease characterised by an acute onset of pneumonia or the self-limiting Pontiac fever can be detected by the immunofluorescence (IF) test. The basic principle of this test is that fluorescein isothiocynate (FITC) irradiated with light of wavelength 490 nm will be transformed to a higher energy molecule. On reverting to its original energy state it emits fluorescence. In the IF test, antigens present in respiratory specimens eg sputum, bronchoalveolar fluid, pleural fluid will form an antigen antibody complex with the FITC labelled antisera added. This complex is then detected by the emission of fluorescence on exposure to the light of the immunofluorescent microscope. The sensitivity of the IF test is low, about 25%-50% but its specificity is high (99.9%)[5]. It is therefore useful in an epidemic setting as the predictive value of positive results may be unacceptably low when the prevalence of the disease is low. However, there is the problem of false positivity due to the cross reaction seen with other bacteria[6].

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Mycobacterium species

There is keen excitement in the clinical microbiology community over the commercially developed tests for the detection of mycobacteria in clinical specimens. Firstly, the development of the BACTEC system has greatly reduced the turn-around time for mycobacterial culture from 6 weeks to as short as 5-10 days. The system is based on the principle that growing bacteria produces CO_2 which will be detected radiometrically by a ^{14}C palmitic acid substrate. Biochemical test for identification can take up to a further 2-4 weeks. DNA probes are now available for the identification of *M. tuberculosis, M. avium, M. intracellulare* and the test is performed in about 2 hours. These probes have very high sensitivity and high specificity (97%–100%)[7]. When the BACTEC system is combined with the use of probes a better turn-around time is achieved to the benefit of the patient.

Mycoplasma pneumoniae

This is a fastidious organism and culture is available in only a few laboratories. Antigen detection by indirect IF with monoclonal antibodies and EIA, the use of DNA probes and polymerase chain reaction are now possible. Of these, the DNA probe assay is more sensitive than EIA but less sensitive than culture. Its sensitivity and specificity are both 89%[8].

Bordetella pertussis

Factors that contribute to problems in *Bordetella pertussis* culture include incorrect collection procedures, delay in transit of

specimens, overgrowth by other microorganisms, mimmunisation status of patient, time between disease onset and culture. Isolation rate of the organism is therefore highly variable especially for hospitals that do nto routinely culture the organism. An approach to its rapid diagnosis is the use of antibodies to detect the antigen in clinical specimens. Tests that have been developed include counterimmunoelectrophoresis, latex agglutination and coaggultination. The direct immunofluorescent-antibody (DFA) test is the more commonly used method. It uses polyclonal fluorescein-labelled antibodies against *Bordetella pertussis* to detect the organism in nasopharyngeal specimens. Although rapid it is limited by the problem of false positivity of 6.7%-40%[9] related to technical factors as well as cross-reactivity seen between *Legionella* species and some strains of *Bordetella pertussis*[10].

B. Urinary tract infection pathogens

Rapid bacteruria screens include microscopic, enzymatic, filtration and automated procedures. The Bac-T-Screen is a simple 2 minute urine screen based on a colorimetric filtration technique. It detects bacteria present in urine rather than bacterial growth. One millilitre of well mixed urine is suctioned through a piece of filter paper attached to a test card. Safranin dye is then added followed by a final rinse with a decoloriser. The major advantage of this test is its ability to obtain results within minutes. Evaluation by various centres gave an average sensitivity of 93.9% with a high false positive rate (>25%)[11,12].

C. Bacteraemia pathogens

A wide range of automated and semi-automated blood culture systems are now available. An example is the Becton Dickinson Bactec system (similar to that used for mycobacterial culture) which gives a faster detection time when compared with the conventional system of blind subculturing of culture bottles. It also has the advantages of being labour-saving and reducing contamination rate since it eliminates the step of blind periodic subculture.

RAPID DIAGNOSIS OF PARASITES

Chlamydia trachomatis

Cell culture has been the primary laboratory method for diagnosing chlamydial infections. The problems associated with this are cost, labour-intensive method, and turn-around time for results. Nonculture methods have been developed with the potential for use in the offices of obstetricians, gynaecologists and paediatricians. These tests detect the various chlamydial antigens directly from clinical specimens eg DFA, EIA and nucleic acid probes. They have both high sensitivity and specificity of 97%-99% and are excellent for screening high risk patient population. Their limitation lies in the fact that they cannot be used as a test for cure because the antigen persists in the host for long periods of time, even after appropriate antimicrobial therapy.

Crpytosporidium species

These are small coccidian parasites infecting the microvillus of epithelial cells lining the digestive and respiratory tract. They cause profuse and watery diarrhoea in both immunocompetent and immunocompromised hosts. In the most severely immunocompromised host such as persons with AIDS, the infection becomes progressively worse and may be a major factor leading to death. The diagnosis of cryptosporidiosis is made by the detection of acid fast oocysts in faeces which require considerable experience with the concentration and staining methods. The development of indirect fluorescent test with *Cryptospordium* specific polyclonal or monoclonal antibodies overcomes this problem[13,14]. Although a rapid and sensitive test, it is unfortunately expensive. One commercial kit (Meridian Diagnostics) combines monoclonal antibodies for the detection of *Giardia* and *Cryptospordium* oocysts and is particularly useful in water testing.

RAPID DIAGNOSIS OF FUNGI

Pneumocystis carinii

Pneumocystis carinii gained importance after the advent of AIDS. It is also a major respiratory pathogen in many non-AIDS immunocompromised patients eg those with organ transplants and carcinoma. It is diagnosed by the demonstration of oocysts and trophozoites in stained preparations of respiratory specimens. The gold standard, Gomori's methenamine silver stain, has now been replaced by newer methods of which the immunofluorescence is one of them. Although expensive, it is more sensitive (80%) than the other non-immunofluorescence methods (72%-75%)[15]. It has the added advantages of short processing time, rapid screening time (<5 mins) especially for smears with low count of organisms.

NEWER MOLECULAR BIOLOGY TECHNIQUES

Molecular biology techiques include recombinant deoxyribonucleic acid (DNA) technology, protein biochemistry, monoclonal antibody production and other tests with which microorganisms can be studied at the molecular level. Recently, the application of polymerase reaction (PCR) has creatd an impact on the rapid diagnosis of pathogens especially those that are fastidious or non-cultivable. Each PCR cycle consists of 3 steps: denaturation step (2 stands of DNA separated by high heat of about 94°C), annealing step and an extension step when DNA synthesis occurs at about 72°C. During each cycle, there is doubling of DNA material. Repeating the cycle 20-50 times would therefore lead to an exponential increase of DNA sequences in the region of millions in a few hours. The whole process is now automated and there are many ongoing studies exploring its potential use in the routine diagnostic microbiology laboratory. However, there are problems that the laboratory has to content with - cost and false positive reactions due to contamination. Hence, although an attractive tool for a rapid and sensitive diagnosis of pathogens, the consequence of a false positive result has to be considered carefully in terms of therapy or possible litigation.

CONCLUSION

Many of these rapid diagnostic techniques are being used in the microbiology laboratory to help in the early diagnosis of infectious diseases especially in the immunocompromised host. The benefit is clearly seen in the diagnosis of fastidious pathogens eg *Legionella pneumophila, Pneumocystic carinii, Bordetella pertussis* and *Mycobacterium* species. The turn-around time for the detection of these organisms in clinical specimens is greatly reduced in contrast to the culture technique. Appropriate therapy can then be instituted early to the patient. This is a far cary from the days when management of most infectious diseases was a matter of empiric therapy.

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