RAPID METHODS FOR THE MOLECULAR DIAGNOSIS OF INFECTIOUS DISEASES: CURRENT TRENDS AND APPLICATIONS

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Key words: Diagnostic microbiology - Immunoassay - Nucleic acid hybridization - Nucleic acid probes.

Methods for the rapid diagnosis of infectious diseases have become increasingly common in the last decade. The impetus for the development of such techniques has stemmed from the need to provide clinically relevant information without the length of time and complexity inherent to traditional cultivation methods. This is particularly important in high-risk populations for which more effective antibiotics and antiviral compounds are now available.

Assay systems for the immunological detection of microbial agents occupy a central role in the molecular diagnosis of infectious diseases and are rapidly appearing on the market. Novel methods of organism detection by nucleic acid hybridization, long considered usable only in highly specialized laboratories, have the potential for use in routine microbiological laboratories and some systems are now commercially available.

As more efficient and rapid diagnostics systems are being developed, the selection of the optimal method will depend on the environment in which the system is to be used. Rapid methods for the detection of infectious agents might markedly improve health care in a variety of clinical, laboratory and epidemiologic situations.

INTRODUCTION

Sensitive, specific and rapid diagnostic techniques for the detection of microbial agents in body fluids are important for the prevention, control and management of infectious diseases in clinical medicine as well as for the large scale study of epidemiology of infectious diseases.

Traditionally the diagnosis of infectious diseases has been accomplished by direct microscopic examination of clinical specimens or by the isolation of the infecting organism in culture. However direct microscopy suffers from limitations of low sensitivity and lack of specificity.

Cultivation is sensitive, specific, and capable of detecting agents whose presence is not suspected. Although it is considered the standard to which other methods should be compared, cultivation is often insufficiently rapid to provide information of practical use in clinical management or in the identification of outbreaks in close settings. The diagnosis by culture can also be limited by practical constraints in the case of viral agents for which the availability of numerous cell lines and sterile areas is required. Such requirements have so far limited the identification of viral agents to central laboratories. Furthermore cultivation of certain fastidious viruses such as rota-
virus, Norwalk virus, hepatitis A and B virus and Epstein-Barr virus is problematic in generally available cell lines while important bacterial pathogens such as *Treponema pallidum* and Mycobacteria cannot be cultivated in artificial media. In the common clinical situation where antibiotics have been administered, cultivation may be inadequate in identifying a pathogen in antibiotic containing body fluids.

Finally, virulent, toxigenic strains such as *E. coli* and *Staphylococcus aureus* cannot always be distinguished in culture from avirulent strains.

In recent years a number of newly recognized viral, bacterial, fungal and parasitic agents have been associated with a wide range of human disease. In order to be clinically relevant such associations have to be tested in several different populations. It is important to ascertain the geographic and environmental range of potential pathogens and to identify populations at high risk for infections. The limitations of standard cultivation techniques have led to the development of more practical and more rapid methods for the diagnosis of human infections. In addition, in case of viral infections, the recent development of many antiviral compounds has provided the incentive for a more timely diagnosis of such infections in high-risk individuals like cancer chemotherapy and organ transplant recipients.

Advantages of rapid diagnosis in such settings include:

1. Aggressive pharmacologic management of potentially lethal infections.
2. Establishment of measures of isolation and patient cohorting, which are essential for the containment of communicable diseases.
3. Reduction of unnecessary use of antibiotics.

Most of the assays applied to the rapid detection of infectious organisms are based on the fact that such organisms express specific antigens and that antigen-antibody reactions can be completed and measured in a relatively short period of time. In addition, over the last few years other alternative techniques have been developed which do not rely on immunological interactions to detect microbial agents.

Nucleic acid hybridization, for example, has many features which make it a potentially efficient and practical system for the detection of microorganisms in a wide variety of health care settings.

**SELECTION OF DIAGNOSTIC SYSTEMS**

Although assay systems have been developed for the detection of a large number of infectious agents relevant in human pathology, most are utilized as research tools and relatively few of them have become commercially available. This reflects the fact that high level of test reproducibility is necessary before such assays can be applied in diverse situation and compare favorably with more cumbersome but highly reproducible standard microbiologic techniques. The efficiency of assay systems for microbiological diagnosis must also be clearly established when they are to be used extensively for screening purposes in high-density urban areas or under field conditions.

It is apparent that the degree of efficiency which is required from an assay system i.e. sensitivity, specificity, predictive value as well as speed of performance and practically varies widely upon clinical, laboratory and epidemiologic environments. Thus, as more rapid diagnostic systems are being developed and marketed, the choice of the appropriate method to achieve an optimal yield in the diagnosis of infectious diseases will depend on the individual characteristics of such environments.

**IMMUNOASSAY SYSTEMS**

Assay systems which make use of the specific interactions of labeled antibodies with microbial antigens have followed the successful application of radioimmunoassay, fluorescent immunoassay, enzyme immunoassay for the detection of drugs and hormones in body fluids (10, 32, 39, 45).

Many of the principles involved in the detection of microbial organisms are essentially the same as for the detection of these compounds and involve the binding of an antigen molecule to an antibody molecule and the detection of this reaction through another labelled ligand. In most applications a solid phase surface, generally of plastic material, is used to separate bound from unbound immune reagents. However, there are differences in the molecular composition and steric presentation of the antigen which may account for lower degrees of efficiency at times present in the detection of infectious agents. The relative large size (often greater than 200,000 MW) and polymeric nature of microbial antigens as well as the variable form in which the antigen is present in body fluids make it difficult to apply the same separation and detection techniques as for smaller and chemically defined molecules. Fortunately, antigen-antibody reactions generally occur under a wide range of condition of temperature, ionic strength and pH, thus making it possible to detect microbial antigens in media as different as blood, cerebrospinal fluid, urine, gastrointestinal and respiratory secretions.

To increase the efficiency of antigen detection in such media it is at times necessary to use antibodies which react with a large number of different antigenic determinants to insure reaction with the form of the antigen which is expressed during the course of the infection. This is particularly problematic when monoclonal antibodies