POLYMERASE CHAIN REACTION IN THE DIAGNOSIS OF TUBERCULOSIS

Manjula Sritharan1 and Venkataraman Sritharan2

1Department of Animal Sciences, School of Life Sciences, University of Hyderabad, Hyderabad-46.
2 Biological E Ltd., Azamabad, Hyderabad-20

ABSTRACT

A rapid, sensitive, specific and yet economical method for the diagnosis of M. tuberculosis and other mycobacteria in clinical specimen is a desperate and urgent requirement of the day in the laboratory diagnosis and hence management of tuberculosis. This need is further accentuated by emerging diseases like multi drug resistant tuberculosis, tuberculosis in AIDS patients and opportunistic mycobacterial infections, which do not respond to conventional anti TB therapy. Molecular methods, particularly PCR based detection of M. tuberculosis, has come a long way since it was first described about fifteen years ago. Several probes have been developed and some of them, particularly the IS6110 and TB400 have been validated on several clinical samples. The latter has been validated on a variety of clinical specimens along with a simple sample processing method. Polymerase chain reaction based diagnosis of M. tuberculosis has been introduced as one of the routine/confirmatory tests in clinical microbiology laboratory in some countries like Canada, the United States and the United Kingdom several years ago. The possibility of introducing PCR based direct diagnosis of drug resistance is being explored in some laboratories, particularly for drugs like rifampicin. The evolution and application of PCR for diagnosis of M. tuberculosis is being analysed and discussed in this review.

KEY WORDS: Polymerase chain reaction, tuberculosis, DNA probes.

Tuberculosis and its impact on public health

Tuberculosis (TB) is an important public health problem worldwide. About a third of the world's population (about 1.7 billion) is infected with M. tuberculosis. The great majority of the world's population and thus the majority of the infected people reside in developing countries. As 75% of infected people are less than 50 years of age (the most productive period in life), TB has the most devastating effect in the developing world. According to WHO, TB causes 25% of avoidable adult deaths in the developing world and worldwide about 2.5 million people died of TB in 1992 alone of which 4,50,000 deaths are from children less than 15 years old from developing countries.

The magnitude of the problem is enormous in view of the epidemic of HIV infection that has radically changed the epidemiology of TB (1). Eradication of TB in most developed countries will not be substantially influenced by HIV infection because the percentage of individuals in the 20-50 years age group with M. tuberculosis infection is low. The close connection between HIV infection and TB was shown in the USA (2) and particularly in 1992 in Africa (3) where 15-70% of patients presenting with TB are HIV infected. Further, TB as a concurring problem in HIV infection is thought to be due to endogenous reactivation of the tubercle bacilli, as a result of the loss of immune control

Author for correspondence:
Dr. V. Sritharan, General Manager,
Biological E Ltd., 18/1&3, Azamabad,
Hyderabad-500 020
e-mail: vsrita@yahoo.com
mechanisms in HIV infected individuals. This, coupled to outbreaks of multiple drug resistant TB warrants stringent measures to combat this disease which is increasing at alarming rates.

Control of the disease should not only mean to treat the clinically proven cases of TB but more important to identify early those individuals who are in the early stages or at risk of developing the disease. An important key to successful control of TB is, thus, timely diagnosis. Additionally, because of the differences in the drug sensitivity pattern, it has become necessary to identify \textit{M. tuberculosis} from other species, as a number of non-tuberculous mycobacteria such as \textit{M. avium} and \textit{M. intracellulare} have become opportunistic pathogens in AIDS patients. The timely identification of persons infected with \textit{M. tuberculosis} and the rapid laboratory confirmation of tuberculosis are two key ingredients of effective public health measures to combat the resurgence of tuberculosis and the outbreaks of nosocomially transmitted tuberculosis (4).

The timely identification of infected persons is important because only about 10% of infected immuno competent persons will develop active tuberculosis during their lifetime and the overt disease symptoms appear fairly late in the infection. Appropriate preventive chemotherapy for persons whose infection is progressing toward overt disease can dramatically reduce the development of infectious tuberculosis (5). In fact, preventive therapy for these infected persons may be the most cost-effective way to reduce the general public health impact of tuberculosis in populations in which the incidence is low. On the other hand, rapid diagnosis and treatment/monitoring of infected individuals would help to reduce the development of active disease but also help to reduce the spread of the disease in an endemic population.

\textbf{Conventional methods of laboratory diagnosis of tuberculosis}

Current procedures for identifying infected persons are limited by a lack of sensitivity or specificity or both and the laboratory confirmation of an infection may require several weeks. Indeed, a workshop of tuberculosis experts conducted a few years ago concluded that all "current techniques for the diagnosis of \textit{M. tuberculosis} are beset by serious limitations" and that "the rapid and specific diagnosis of \textit{M. tuberculosis} is one of the most pressing needs in efforts to eradicate the disease (6).

The usefulness of the historic tuberculin test is limited by its lack of specificity for tuberculosis and by its inability to distinguish between active disease, prior sensitisation by contact with \textit{M. tuberculosis}, BCG vaccination and cross sensitization by other \textit{Mycobacterium} species (7, 8). The tuberculin skin test also fails to detect a substantial proportion of persons co-infected with HIV and \textit{M. tuberculosis} and of persons with advanced tuberculosis. Despite these limitations, the tuberculin skin test is still a very useful tool, especially when conversion to a positive skin test is used to identify recently infected persons for preventive therapy or to help confirm a physicians' suspicion of tuberculosis.

Confirmatory diagnosis of TB requires the identification of \textit{M. tuberculosis} in the patients' samples. Conventionally laboratory diagnosis starts with microscopic examination of smears for the presence of acid fast bacilli followed by culture isolation and biochemical testing of the isolated mycobacterium for the species identification. The entire process often requires 4-6 weeks from specimen collection to species identification, primarily because of the slow growth rate of mycobacteria. Determination of drug susceptibility of an culture isolate can add 2-4 weeks to this already long process.

Acid fast bacilli smear examination is time tested and remains extremely valuable in testing sputum for \textit{M. tuberculosis} while other specimen like bronchial lavage, blood and other aspirates may also be used. The diagnostic yield of this technique is limited primarily because the number of organisms required for a positive smear is between 5000 and 10,000 per ml for reliable detection. The immediate diagnosis of tuberculosis by direct sputum examination ranges from 40 to 75%. However, today with the widespread nature of TB