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MONITORING OF BACTERIOLOGICAL DIAGNOSTIC EFFICIENCY UNDER RNTCP - THE PUNE EXPERIENCE

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Microscopy could be used to detect two-thirds of pulmonary tuberculosis cases. However, in order to screen out the remaining one-third of sputum smear negative cases, a progressively increasing load of symptomatics had to be screened, using indirect tests.

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Diagnosis of smear-negative case of tuberculosis is more expensive than that of a sputum positive case, since diagnosis of the former involves additional costs of antibiotics and radiological examination.

* * *

For every patient of tuberculosis who can be detected using microscopy, nine have to be screened using indirect methods, due to the low sensitivity of microscopy. In addition to the expense, the resulting delay in the initiation of treatment of sputum negative cases has implications in disease control, since there is sufficient evidence of transmission from smear negative patients as well. In the background of the increasing HIV-epidemic, the detection of smear negative cases takes on significance.

I. INTRODUCTION

The cornerstone of the diagnosis of tuberculosis is the direct microscopic examination of appropriately stained sputum specimens for the identification of Acid Fast Bacilli (AFB). The technique is simple, inexpensive, and detects infectious cases who are responsible for continued transmission and maintenance of the epidemic in the community. Currently, sputum smear microscopy is widely used in Tuberculosis Control Programs worldwide for a presumptive diagnosis of tuberculosis. No other diagnostic tool offers the affordability as well as efficiency in diagnosis of tuberculosis in the public health setup, as sputum microscopy does.

Although relatively quick, easy and inexpensive, sputum microscopy has a major limitation (1,2). While the specificity of microscopy is high, ranging between 98-99%, the sensitivity is relatively poor at 50-70% or even lower in the public health setup (3). Between 5,000 and 10,000 tubercle bacilli per millilitre of sputum are required for direct microscopy to be positive. Sputum specimens from patients with cavitary disease are most often sputum smear positive.

The poor sensitivity of microscopy can be improved by examination of more than one smear from a patient. In one study, eight samples of sputa from individuals with abnormal chest X-rays were examined by microscopy (4). The results revealed that the first specimen detected 58% of the culture positive cases, and the first and second specimens together detected nearly 72% of the cases. All cases positive on culture and microscopy were detected by three specimens. Only 6% additional cases were identified by the seventh and eighth specimens together. Currently, three sputum specimens are recommended for the self reporting chest symptomatics under the Revised Tuberculosis Control Programme (RNTCP) for the diagnosis of pulmonary tuberculosis (5,6).

A negative smear result does not exclude the diagnosis of tuberculosis, as about 55% of pulmonary tuberculosis cases worldwide, harbor lower bacillary load, so that the sputum is negative upon microscopic observation. Other variables influencing the results of microscopy include the availability of saliva instead of sputum, as well as the quantity and quality of sputum (7). A large workload may also influence the sensitivity of smear microscopy. Worldwide, the ratio of smear positive to smear negative cases varies, from 1:1 in a country like the USA where there is 100% Directly Observed Treatment Short-Course (DOTS) coverage, to 1:4 in the Russian Federation, where the DOTS coverage is less than 5% (8). In 1999, India had 12,23,127 notified cases of tuberculosis, of which 11,27,553 constituted pulmonary cases. Of these, 3,49,770 were sputum smear positive, while 7,77,783 were smear negative cases, giving a ratio of 1:2 (8).

Diagnosis of tuberculosis under the RNTCP has been clearly defined (5). Chest symptomatics (especially, those having cough for three weeks or more), are offered sputum examinations. Three samples are collected: a spot specimen on the first day, and early next morning and a spot specimen on the second day. Patients with two or three positive smears are immediately started on treatment. Symptomatics with one positive smear undergo chest X-ray. Those with no positive smears are prescribed a course of antibiotic treatment. Antibiotic non-responders undergo chest X-ray and are re-evaluated. Patients are categorized into appropriate groups and are referred to the DOTS center nearest to the place of residence. The consequence of the low sensitivity of microscopy is that all chest symptomatics, who are not positive by microscopy, have to undergo the indirect screening procedure outlined above, in order to identify the smear negative cases of tuberculosis. The problems inherent in implementing this process is...
apparent from an analysis of the bacteriological diagnostic efficiency of microscopy in the RNTCP set up in the Pune Municipal Corporation (PMC) area.

RNTCP has been in operation in Pune from 1995. PMC has a population of approximately 25 lakhs. There are three Tuberculosis Units (TU), implementing NTP (National Tuberculosis Programme) as well as RNTCP. The three TU's have, under them, 101 Treatment Observation Centers and 17 Microscopy Centers. A retrospective analysis of tuberculosis data under RNTCP between July 1997 to June 2001, revealed that from 1997, the number of smear examinations increased progressively, showing a 2.3 fold increase over four years. (Fig. la). The number of tuberculosis cases being detected also showed a three fold increase from 736 cases detected in 97-98 to 2273 cases diagnosed in 2000-2001. The majority of the cases being detected were those of pulmonary tuberculosis, ranging from 74% to 83% of all cases diagnosed (Fig Ib). However, over the same period, extra-pulmonary (EP) case detection increased nearly five fold, in terms of actual number of cases, from 123 cases diagnosed in 97-98 to 582 in 2000-01. Thus, EP cases, which had constituted 16.7% of all cases in 97-98, increased to 25.6% of all cases being detected under the programme in 00-01.

During the period under review, out of those subjected to smear microscopy, the diagnosis of Pulmonary tuberculosis was made in 14.6%, 20.8%, 28.6% and 18 %, averaging about 20.6(5.9 cases annually (Table 1). In terms of actual numbers, the pulmonary tuberculosis cases being diagnosed increased from 613 cases in 97-98 to 1691 cases of pulmonary tuberculosis in 00-01, showing a 2.7 fold increase over the four years. This increase paralleled the 2.2-fold improvement in the total number of smear examinations observed (Fig la). Table-1, column iv, shows the diagnostic efficiency of microscopy, expressed as the number of sputum smear positive cases diagnosed from amongst total pulmonary tuberculosis cases. This ranged between 58% to 69% annually, averaging around 63.7 (4.57 %). Taking the average for four years, the sputum smear positive cases accounted for 64% of the cases, whilst the smear negatives accounted for 36%, giving a ratio of 2:1. (Fig.Ic).

The ability of microscopy to screen tuberculosis cases from amongst all chest symptomatics visiting or referred to the clinics is presented in Table 1, column v. In tuberculosis surveys, the term screening has been used to identify the population that is eligible for diagnostic tests like sputum examination or chest radiography. (9) The terminology being used here, on the other hand, expresses the ability of microscopy to diagnose tuberculosis cases from among the total number of chest symptomatics being examined annually. Between July 1997 to June 2001, a total of 24,327 symptomatics (i.e. 4173, 5097, 5512 and 9545, by the year shown in Table 2, column i) had approached the clinic and their sputum samples were examined by smear. Of these, 387, 613, 1094 and 1104 were found each year respectively, to be positive by sputum microscopy (Table 2, column ii). This indicated that the efficiency of microscopy to detect smear positive cases improved progressively from 9% to 12%, over the four years. On an average 20.5(6.35 pulmonary tuberculosis cases could be detected from amongst one hundred chest symptomatics under RNTCP, year to year. On the other hand, the sputum smear negative pulmonary tuberculosis (i.e. those cases who were not positive by microscopy), accounted for 5%, 9%, 9% and 6 % of the total chest symptomatics. (Table 2 column vi and Fig. Id). In other words, on an average, of the pulmonary tuberculosis cases diagnosed from amongst the chest symptomatics, microscopy could be utilized to identify about 13% (who were smear positive), while 7% of cases had to be identified using indirect methods, like antibiotic treatment/xray chest. Due to the low sensitivity of microscopy, identification of the latter 7% of cases, involved “screening” of the remaining 87% of symptomatics, details of which are shown in Table 2.

Table 2, shows the outcome of the differential diagnostic procedures for identification of the smear negative tuberculosis cases. The patients who were found to be negative upon smear microscopy were subsequently given broad spectrum antibiotics (Table 2, column iii). Majority of symptomatics ranging between 71 and 85% (Table 2, column iv) responded to antibiotics, indicating that they possibly did not have tuberculosis... The efficiency of microscopy and antibiotic treatment, in arriving at a conclusion on diagnosis as a firm smear positive case and as other than a tuberculosis case, under RNTCP diagnostic paradigm, ranged between 91 to 95% (Table 2 column v). The remaining 226 (5%), 450 (9%) 488 (9%) and 587 (6 %) patients (Table 2 , column vi) were started on Anti-Tuberculous Treatment (ATT) after radiography and confirmation of clinical diagnosis, i.e., more by default, than on positive evidence of active TB.

Table 2, column iii shows that over the four years of observation, the total number of symptomatics who were smear negative and required further diagnostic action, increased from 3786 to 8441 (Table 2, column iii). This was of course due to higher number of chest symptoms examined, over the years. As a result of this escalation the number of sputum negative cases had also increased 2.6-fold, i.e. from 226 cases in 1997 to 587 cases in 2000. (Table 2, column vi), with a slight increase in the proportion out of the chest symptomatics examined.
Table 1: Screening and diagnostic efficiency of microscopy

<table>
<thead>
<tr>
<th>Year</th>
<th>Total smear Examined</th>
<th>Pulmonary TB Cases</th>
<th>Number Detected by microscopy</th>
<th>Diagnostic Efficiency C/B x 100 (%)</th>
<th>Screening Efficiency C/A x 100 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 1997-98</td>
<td>4173</td>
<td>613 (14.6)</td>
<td>387</td>
<td>63</td>
<td>9</td>
</tr>
<tr>
<td>II 98-99</td>
<td>5097</td>
<td>1063 (21.2)</td>
<td>613</td>
<td>58</td>
<td>12</td>
</tr>
<tr>
<td>III 99-00</td>
<td>5512</td>
<td>1582 (28.7)</td>
<td>1094</td>
<td>69</td>
<td>20</td>
</tr>
<tr>
<td>IV 00-01</td>
<td>9645</td>
<td>1891 (17.7)</td>
<td>1104</td>
<td>65</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>24327</td>
<td>4949 (20.5%)</td>
<td>3198</td>
<td>64</td>
<td>13</td>
</tr>
</tbody>
</table>

Average for I-IV years

Table 2: Efficiency of microscopy and antibiotic treatment on differential diagnosis of smear negative cases

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of smears examined</th>
<th>No. Detected by microscopy</th>
<th>No. Undergoing antibiotic treatment</th>
<th>No. of cases eliminated i.e. responders to antibiotic treatment</th>
<th>Efficiency of Diagnostics (b+d)/a x 100 (%)</th>
<th>No. of X-ray positives E/a x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 1997-98</td>
<td>4173</td>
<td>387 (9.27)</td>
<td>3786 (90.7)</td>
<td>3560</td>
<td>94.5</td>
<td>226 (5.4)</td>
</tr>
<tr>
<td>II 98-99</td>
<td>5097</td>
<td>613 (12.0)</td>
<td>4484 (87.9)</td>
<td>4034</td>
<td>91.2</td>
<td>(8.8)</td>
</tr>
<tr>
<td>III 99-00</td>
<td>5512</td>
<td>1094 (19.8)</td>
<td>4418 (80.15)</td>
<td>3830</td>
<td>91.1</td>
<td>488 (9.0)</td>
</tr>
<tr>
<td>IV 00-01</td>
<td>9645</td>
<td>1104 (11.5)</td>
<td>8441 (88.4)</td>
<td>7854</td>
<td>93.8</td>
<td>(6.1)</td>
</tr>
</tbody>
</table>

* The figures in this column are estimates since many patients referred to the TUs undergo antibiotic treatment in the private sector.
The pertinent observations that arise from this analysis are as follows. The results show that (i) in the RNTCP of PMC, microscopy could be used to detect two-thirds of pulmonary tuberculosis cases. (Table 1 column iv) (ii) However, in order to screen out the remaining one-third of sputum smear negative cases, a progressively increasing load of symptomatics had to be screened, using indirect tests, (iii) In the PMC area the number of symptomatics being screened by sputum smear over the last four years has more than doubled (Table 1, column i). (iv) The increase in the number of sputum smear positive cases and smear negative cases has been similar (2.8 and 2.5 fold respectively), indicating that smear negative cases constitute a significant portion of the tuberculosis case load (Table 2, column ii & vi). (v) Diagnosis of smear-negative case of tuberculosis is more expensive than that of a sputum positive case, since diagnosis of the former involves additional costs of antibiotics and radiological examination. These observations raise the possibility that as attendance of symptomatics to the centres for diagnosis improves, a comparatively greater proportion of resources are likely to be diverted to screening chest symptomatics in order to identify sputum negative cases of pulmonary tuberculosis.

A glimpse of how extensive this problem might be on a national level is illustrated from the data on number of sputa examined in 1998-1999 (10). The data reports that 38,93,213 sputa were examined. Taking into consideration that three sputa samples were examined from each patient, the number of patients approaching the National Program with chest symptoms were possibly 12,97,738. Of these, 24.8% (3,21,920) were found to be sputum positive. Using this data, if a theoretical consideration is made that all chest symptomatics are examined using the diagnostic algorithm of RNTCP, a total of 9,75,818 symptomatics should be subjected to antibiotic treatment, followed by radiography of the non-responders, in order to diagnose the sputum smear negative cases of tuberculosis.

In a seminal study, Baily, et. al. shows that for every 100 patients visiting a general peripheral health institution, and suffering from any type of complaint, nearly 2 will have chest symptoms and require a sputum examination (11). For 10 such sputa examined, one will be sputum smear positive, and nine will require further investigations. This means that for every patient of tuberculosis who can be detected using microscopy, nine have to be screened using indirect methods, due to the low sensitivity of microscopy.

In addition to the expense, the resulting delay in the initiation of treatment of sputum negative cases has implications in disease control, since there is sufficient evidence of transmission from smear negative patients as well. For example, in a study to determine the infective potential of patients who are smear-negative, culture positive, it was found that smear-negative patients were at least 22% as likely as smear positive patients to transmit tuberculosis (12). In the background of the increasing HIV-epidemic, the detection of smear negative cases takes on significance since majority of HIV positive tuberculosis cases are smear negative.

ACKNOWLEDGEMENTS

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LEGEND OF FIGURE

Fig.1. Trends in tuberculosis case detection under RNTCP, Pune from July 1997 to June 2000. (a) shows the progressive increase in the total smears examined and the number of pulmonary and extra-pulmonary cases detected. The increasing trend of extra-pulmonary case detection is shown in (b). (c) represents the proportional distribution of sputum smear positive and smear negative cases of pulmonary tuberculosis, while the proportion of these cases to the total chest symptoms is shown in (d).
Fig. 1(a). Smears examined and tuberculosis cases RNTCP, Pune

Year

Total Smear examined
Total TB cases
Pulmonary
Extrapulmonary

Fig. 1(b) Proportion of pulmonary and extrapulmonary cases
Fig. 1(c) Proportional distribution of sputum smear positive and smear negative cases

<table>
<thead>
<tr>
<th>Year</th>
<th>Sputum positive</th>
<th>% cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>35%</td>
<td>65%</td>
</tr>
<tr>
<td>III</td>
<td>31%</td>
<td>69%</td>
</tr>
<tr>
<td>II</td>
<td>42%</td>
<td>58%</td>
</tr>
<tr>
<td>I</td>
<td>37%</td>
<td>63%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Negatives</th>
<th>% cases</th>
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<tr>
<td>IV</td>
<td>65%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>31%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>37%</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1(d) % sputum smear positive and smear negative tuberculosis cases amongst chest symptomatics

<table>
<thead>
<tr>
<th>Year</th>
<th>TB</th>
<th>Negatives</th>
<th>Sputum positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>18%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>29%</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>21%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>14%</td>
<td>9%</td>
<td></td>
</tr>
</tbody>
</table>

% cases
A world wide effort is under way to develop new tools to diagnose tuberculosis, spearheaded by the World Health Organization (WHO) under its Tuberculosis Diagnostic Initiative (TBDI) (1,2). This article discusses the advanced diagnostics being developed, and highlights the feasibility of use of these technologies in the public health facilities involved in tuberculosis control in India.

I INTRODUCTION

The global strategy for controlling tuberculosis is through a six-month treatment with anti-tuberculous drugs that interrupt disease transmission. Fundamental to this process is the correct diagnosis of patients. The problem of diagnosing tuberculosis is that no symptom or sign is typical of it, neither is the presence of bacilli indicative of disease. As such diagnosis initiates with a high clinical suspicion, and is supported through the us of various diagnostics. Worldwide, tuberculosis is still primarily diagnosed using microscopy for acid-fast bacilli (AFB). It remains the cornerstone for laboratory diagnosis of tuberculosis in clinical practice as well as the national tuberculosis control programs of the large majority of under-developed countries. Characterized by high specificity for mycobacteria, and low cost, the major limitation of AFB microscopy is its low sensitivity (Table 1). A world wide effort is underway to develop new tools to diagnose tuberculosis, spearheaded by the World Health Organization (WHO) under its Tuberculosis Diagnostic Initiative (TBDI) (1,2). This article briefly presents the advanced diagnostics being developed, and goes on to discuss the feasibility of use of these technologies in the public health facilities involved in tuberculosis control in India.

Four diagnostics are routinely used for diagnosing tuberculosis in developing countries. These are the direct visualization of bacilli by either microscopy or culture on specific media, or indirect methods such as radiography and tuberculin testing. Of these four diagnostics, the specificity of microscopy is about 99%, but its sensitivity ranges between 50-70% or less in the public health set up. Staining smears with fluorescent auramine-rhodamine dyes stain bacilli yellow green under fluorescence. Although the bacilli can be observed under low power, reducing the time required by technicians in scanning the slide, the requirement of a fluorescent microscope is an expensive limitation to the usage of this test (Table 2).

The inoculation of concentrated bacilli from processed clinical specimens on solid media is a standard approach for confirmation of tuberculosis. Culture methods are more sensitive than microscopy for detection of bacilli, since approximately 100 mycobacteria per milliliter of sample is required for a positive result. Despite its enhanced sensitivity, culture is of little clinical use, since it takes six to eight weeks for the colonies to grow. Tuberculin test is widely used for the diagnosis of tuberculosis, especially in children, as well as for epidemiological investigations of infection. Other than pediatric cases and cases of advanced disease, tuberculin has little value in India, since wide-spread BCG immunization, and high exposure to atypical environmental mycobacteria, result in nearly all individuals reacting to the tuberculin. Radiography is an important tool in the diagnosis of pulmonary tuberculosis and is widely used for diagnosing cases under the National Tuberculosis Program (NTP) in India. Considerable subjectivity exists in the interpretation of a pulmonary opacity, since determination that the opacity represents an active tuberculous foci depends on clinical experience.

The limitations of these routinely used tests and the need to speed treatment in light of the impending HIV epidemic, have brought a new impetus to improve diagnostic tools. The focus has been to permeate the state-of-the-art technologies that have evolved from the recent breakthroughs in the field of molecular biology, to appropriate technologies for the low income countries. The objective of the search for a new diagnostic is to develop a technology that replaces or facilitates the characteristics of microscopy, i.e. has enhanced sensitivity, whilst maintaining the specificity, low cost as well as speed and ease of use of the technology in public health settings of low-income countries.

An ideal tuberculosis diagnostic should address the four focal aspects of tuberculosis control in low-income countries, i.e. it should improve case detection for both smear positive and smear negative cases, simplify and speed up detection of drug resistance, detect cases of preclinical disease or latent infection. It should be patient friendly, so that it requires minimum number of patient visits to clinics. It should be simple, such that it can be administered by a general health service technician with minimum skills, and require minimum supervision. It should be based on consumables that are stable at room temperature, and require minimum technical infrastructure. The consumables for the diagnostic should be available on a countrywide basis and on a long-term.
II. ADVANCED TECHNOLOGIES

The technologies being developed or already in the market can be categorized into four sub-categories: those based on growth properties of mycobacteria, technologies based on phage susceptibility of M. tuberculosis, nucleic acid based technologies and immunodiagnostics. Considerable impetus in the search for a new tuberculosis diagnostic has come from the complete sequence analysis of the genome of Mycobacteria tuberculosis 37Rv (3) and BCG(4).

A. Technologies based on the cultural properties of mycobacteria

The technologies based on the cultural properties of mycobacteria, attempt to circumvent the slow growth of M. tuberculosis. A number of systems have been devised that detect the metabolic products of bacilli grown on specific liquid media. A radiometric technique on highly selective medium, can detect bacilli within 2 weeks. This system (commercially sold as the BACTEC system) uses 14C labelled palmitic acid as a metabolic substrate. In the presence of viable mycobacteria, 14CO2 is released which is quantitated. Other advances in rapid culture techniques include the detection of oxygen released by bacilli using a fluorescent indicator. The principal benefit of these systems are that bacilli can be detected 7 to 12 days as compared to 6 to 8 weeks required for the appearance of visible colonies on routinely used media (5). The Table shows the prohibitive cost of one such system, which excludes its use in public health facilities.

B. Phage susceptibility testing

A more useful tool, is phage susceptibility testing, commercially available in the market. Mycobacteriophages are viruses that infect mycobacteria (6). The principle of phage susceptibility testing involves the infection of clinical samples with Mycobacteriophages, and monitoring the successful replication of the phages. The rationale behind this technique is that if the clinical specimen contains M. tuberculosis, replication of phages will take place, and this can be monitored by visualizing plaques ("holes") on M. smegmatis lawns. The procedure does not require expensive instrumentation, can be supported by a basic microbiology laboratory, (having the standard biosafety requirements that is mandatory for a tuberculosis laboratory). A very high level of skill is also not necessary for executing the test.

A useful modification of this test is genetically engineered phages, where the luciferase gene from the firefly has been introduced through recombinant DNA technology (7). In this case, phage replication can be observed using a luminol-detector. Fluorescence indicates that phages have infected Mycobacteria, suggesting that the clinical sample contains M. tuberculosis. This test which is commercially available, does not require a high technical skill, but a dark room is necessary for visualization of the fluorescence.

Phage susceptibility can also be used in testing drug susceptibility, enhancing the utility of this test (8). Unlike routine antibiotic susceptibility testing, drug susceptibility testing for mycobacteria is limited by the slow growth of the bacilli, necessitating a long delay of six to eight weeks before the cultures can be declared as drug-sensitive. Using the phage susceptibility testing system, rapid assessment of drug susceptibilities can be determined. In this case, the clinical sample is divided and incubated with a different drug or antibiotic in each aliquot, followed by addition of the luciferase reporter phage. If the tuberculosis strain is sensitive to the particular drug or antibiotic, replication of the bacilli will not take place, mycobacteriophages will not occur, and thus there will be no reporter gene expression.

C. Nucleic acid-based technologies

Nucleic acids (DNA or RNA) are the chemical constituents of genes. Nucleic acid based tests are usually more accurate and rapid than conventional tests. The first group of technologies utilize the property of DNA to complementary base-pair. That is, DNA being a double stranded molecule, one strand of DNA can specifically identify its complementary strand. This principle is used in the technique of in situ hybridization, wherein labeled nucleic acids, specific to mycobacteria, are added to the processed clinical specimen. If the specimen contains the bacilli, the labelled probe will hybridize to target nucleic acid molecules of Mycobacteria, and can be subsequently detected using various methods. A limited number of Mycobacterium species can be identified by the use of commercially available DNA probes. Although highly specific and sensitive, the requirement of sophisticated infrastructure limits the utility of these tests. Signal-amplified tests are a modification of the same methodology, wherein multiple labels are used to amplify the signal of the assay. Available commercially, the necessity of expensive and sophisticated infrastructure, and use of highly skilled technicians, eliminates the utility of this technology in public health facilities.

A modification of this method is that of peptide nucleic acid (PNA) probes, which consist of standard or modified nucleobases with a protein-like, polyamide backbone. The structure of PNA's provide more favorable conditions for targeting DNA. A PNA probe designed for specific detection of mycobacteria of the tuberculosis complex was shown to be able to penetrate the mycobacterial cell wall and subsequently hybridize in situ to complementary rRNA. M tuberculosis strains were detected by FISH (fluorescent in situ hybridization) using specific fluorescein-labelled PNA probes directly in smear positive sputum samples. Thus, PNA probes allow for rapid diagnosis of tuberculosis in smear-positive cases, but are limited by the need for sophistication in
terms of infrastructure as well as technical skill required to perform the test (9).

Of all the nucleic acid based technologies available, Nucleic Acid Amplification (NAA) assays have drawn the maximum attention, due to the enormous potential of this technology in overcoming the limitations of microscopy and culture in terms of sensitivity and specificity. The principle behind this test is the Polymerase Chain Reaction (PCR), a method by which minimal quantities of nucleic acid can be amplified (10). The technique uses primers (short complementary DNA sequences) that are specific to mycobacteria of the tuberculosis complex, and the renewed amplification of the targeted region using a thermostable DNA polymerase and repeated round of amplification. PCR based kits are in use in Europe and in the USA for increasing diagnostic certainty of tuberculosis in samples with lower bacilli loads (11).

Due to its significantly higher analytical sensitivity, a large number of studies have explored the role of PCR as a tuberculosis diagnostic in developing countries (12-19). A theoretical study on the cost effectiveness of using PCR versus smear examination for the diagnosis of tuberculosis in a government hospital in Kenya showed that the PCR method can potentially be a cost effective screening procedure for tuberculosis provided that the largest contributing cost component, the costs of the PCR-kit, can be reduced substantially (16). A number of studies however, have shown the extreme technical care necessary for PCR. In a study to determine the reproducibility of PCR, results obtained from six laboratories in six different Latin American countries showed large differences in sensitivity and specificity. The study concluded that PCR could not be used as a single diagnostic tool for tuberculosis (14). A major drawback of PCR in the Indian context is its high false positivity (17,18), although a recent article identified that PCR for tuberculosis was related to experience and accuracy of the personnel conducting the assay (19).

D. Immunodiagnosis

Immunodiagnosis of tuberculosis faces two major challenges. Unlike infection with other pathogens, tuberculosis infection is characterized by the slow generation of antibodies. Secondly, the immunodiagnostics of tuberculosis are limited by lack of both sensitivity and specificity, since many antigens do not discriminate between M. tuberculosis and atypical mycobacteria. Some of the diagnostics available are as follows: the lymphocyte proliferation assay which detects the cellular reactivity to tuberculin PPD, the gamma interferon assay, also based on cellular immunity, while the ELISA (enzyme-linked immunosorbent assay) is based on humoral responses.

Of all these immunodiagnostics, a commercial kit of promise, quantitates the gamma -interferon levels in sensitised lymphocytes after a 18 hour incubation period with specific antigen (PPD-tuberculin). The assay takes advantage of the fact that individuals primed in, vivo with exogenous or endogenous antigen have lymphocytes in their blood that maintain an immunological memory for the priming antigen. Stimulation of whole blood with a test antigen followed by the quantitative measurement of IFN-gamma in plasma can be used to measure an individual's cellular immune response. The commercially available kit uses four antigens, which include two controls - a mitogen (PHA) which stimulates T-cells and an avian antigen which detects infection with atypical mycobacteria. The test has a sensitivity and specificity of 90% and 98% respectively, and provides a dramatic improvement over the routinely used tuberculin skin test. A recent study however, showed that the test did not efficiently distinguish BCG-vaccinated individuals, from individuals with disease due to M. tuberculosis. The test is being modified in order to distinguish between TB infection, atypical mycobacterial reactivity and reactivity due to BCG vaccination (20).

III. CONCLUSION

The accompanying (Table 2) summarizes the key features of the diagnostics being developed. The Table shows the large number of parameters that come into play when laboratory research, completes its trajectory and becomes an application. From the point of view of India and other low income countries, an immunological test is of maximum advantage, since infrastructural requirements are minimum for such tests. However, the biggest limitation of immuno-diagnostics for tuberculosis is the lack of specificity. Furthermore, recurrent consumable costs such as the need to have disposable syringes, ensuring proper disposal of these syringes, and the fact that patients need to return for reading the results somewhat limit the use of these tests. The second contender in the challenge is PCR, which remains a useful diagnostic in clinical practice. However, large-scale epidemiologic studies with this tool are lacking, and therefore the cost-benefit aspect of this diagnostic in the public health setup remains yet to be investigated in India. Phage-based kits also hold promise, but here again, large scale cost-benefit studies are yet to be undertaken, that would determine the actual utility of the diagnostic.

India has had a tuberculosis control program for over forty years. With the impending HIV epidemic, the prospect of tuberculosis control becomes a more daunting task than ever. Tuberculosis control in India, will not only depend on resources, drugs and managerial aspects of the control program, but also on a simple, inexpensive test that will lead to the rapid detection and treatment of cases.
REFERENCES


11. Nucleic Acid Amplification for Tests for Tuberculosis MMWR July 07.20007 49(26) 593


### Table-1: SPUTUM MICROSCOPY

**EQUIPMENT REQUIREMENT**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Cost</th>
<th>Skill Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope</td>
<td></td>
<td>from 30,000/-</td>
<td>Minimum</td>
</tr>
<tr>
<td><strong>Indigenously</strong></td>
<td>manufactured</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CONSUMABLES</strong></td>
<td>Stains, slides etc locally available, inexpensive</td>
<td>Approximately 10/- per test</td>
<td></td>
</tr>
<tr>
<td>TIME REQUIRED</td>
<td></td>
<td>Less than one hour</td>
<td></td>
</tr>
<tr>
<td>TECHNICIANS SKILL LEVEL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of Admission</td>
<td>Length of Stay</td>
<td>Diagnosis</td>
<td>Treatment</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>01/01/2023</td>
<td>14 Days</td>
<td>Hip Fracture</td>
<td>Cast</td>
</tr>
<tr>
<td>02/02/2023</td>
<td>21 Days</td>
<td>Knee Injury</td>
<td>Surgery</td>
</tr>
</tbody>
</table>

**Table 2: Profile of Diagnostic Tests for Toddler**
A COMPARATIVE STUDY OF THE DIAGNOSIS OF PULMONARY TUBERCULOSIS USING CONVENTIONAL TOOLS AND POLYMERASE CHAIN REACTION*

Kavita Modi – Parekh 1, Vikas Inamdar 2, Anagha Jog 3 and Anita Kar 4

Summary
Background: The sensitivity of Polymerase Chain Reaction (PCR) makes it a potential diagnostic test for detection of M. tuberculosis in samples with low bacillary load.
Aim: To assess the efficiency of PCR as compared to routine diagnostics in detection of M. tuberculosis from sputum samples of suspects referred to a tuberculosis clinic and those identified during a morbidity survey.
Methods: Respiratory samples (sputum with or without saliva) from 144 individuals were examined by PCR, using MPB64 primers, culture and microscopy. 97 samples were from suspects referred to a tuberculosis clinic, 26 were from suspects identified during a morbidity survey and 21 were from patients with diseases other than tuberculosis. Study was conducted blind.
Results: Total cases considered to be positive for tuberculosis by all criteria was 71. PCR detected 98% of 'culture positive', 97% of 'smear positive, culture positive', and 100% of 'smear negative' culture positive samples. PCR was also positive for 86% of smear negative samples, from tuberculosis suspects diagnosed on the basis of other routine diagnostics and supporting clinical evidence. Seventeen samples were positive only by PCR but based on clinical parameters only 7 were considered as true positives.

The sensitivity of PCR was 91.5% compared to 51% for smear microscopy and 68% for sputum culture. This was due to the fact that PCR could pick up bacterial DNA even from saliva mixed sputum specimens, which are generally not considered appropriate for microbiology. The specificity of PCR (88%) was found to be lower than other diagnostic tests mainly due to lack of a suitable gold standard to assess its efficiency. This is an important limitation in evaluation of the test.

Conclusions: PCR using MPB64 primers has potential and can be a useful adjunct to diagnose clinical tuberculosis, particularly in smear negative paucibacillary cases. However, the major limitation of PCR results from the absence of a suitable gold standard by which to evaluate the results. [Indian J Tuberc 2006; 53:69-76]

Key words: Tuberculosis, Polymerase Chain Reaction, MPB64 primers

INTRODUCTION

Diagnostic process of tuberculosis initiates with a high clinical suspicion, and is supported through the use of various diagnostics 1,2. The only rapid test for presumptive diagnosis of tuberculosis is smear examination of the patient’s specimen for acid-fast bacilli (AFB). Culture remains the final confirmatory laboratory diagnostic for tuberculosis 3. The need for more sensitive and specific techniques thus become obvious. Nucleic acid amplification using the principle of polymerase chain reaction (PCR) has the potential for the diagnosis of tuberculosis in a few hours with a high degree of sensitivity and specificity 4. The potential of PCR as a diagnostic test for tuberculosis has been investigated in a large number of studies 4,14. While sensitivity of microscopy is 60-70% in culture positive respiratory material, the sensitivity of PCR is 90-100% and 60-70% on smear positive culture positive and smear negative culture positive respiratory samples respectively 11. The limitations of PCR have also been discussed 12. The overall reported sensitivity of PCR ranges from 58% to 100%. Sensitivity is reported to be higher in smear-positive samples (95% to 100%) than in smear-negative samples (46 to 63%) 6. In many studies, problems with false-positive PCR results, at rates ranging from
0.8% to 30% have been reported. Specificity of PCR results varies between laboratories due to procedural differences, differences in cross-contamination rates and the choice of primers.\(^3\)

The purpose of this study was to determine the efficiency of PCR as compared to other routine diagnostics like smear microscopy and culture, amongst sputum/saliva samples from a pool of highly probable tuberculosis suspects referred to a tuberculosis clinic and from symptomatics who were identified during a morbidity survey in a slum.

**METHODOLOGY**

**Research design**

A total of 144 respiratory samples (sputum with or without saliva) from as many individuals were tested. Of these, 123 samples were from individuals suspected of having pulmonary tuberculosis, and 21 samples were from hospital patients having a disease other than tuberculosis. The latter samples were controls for all the investigations carried out on the test samples. Of the 123 samples, 97 samples were taken from 97 highly probable tuberculosis suspects who were referred to or who presented at a tuberculosis clinic. Diagnostic and treatment decisions were made by site physicians according to the Revised National Tuberculosis Control Program (RNTCP) guidelines. Single overnight sample was used for culture and PCR examination, after it had been examined for smear microscopy at the tuberculosis clinic.

Twenty six samples were collected from 26 chest symptomatics identified during a morbidity survey carried out in a slum. These individuals reported a productive cough with or without sputum for over three weeks along with one or more cardinal signs of tuberculosis like low grade fever in the evening, weight loss and chest pain. Samples from these individuals were collected by holding health camps (n=12), by referring symptomatics to a nearby municipal clinic (n=1), or through the collection of samples by health workers (n=13).

All the 144 samples, whether overnight or spot collections, were examined by routine smear microscopy, culture and PCR. The data were compared with available clinical information. Radiological data was available from 61 subjects. The study was conducted blind.

**Quality of samples submitted**

Of the 144 sputum samples, 45 were mixed with saliva. All the samples were processed for bacteriological investigations, namely smear, culture and PCR.

**Sample processing, culture and PCR**

Samples were processed using either Petroff's method or N-acetyl-L-cysteine-sodium hydroxide method.\(^5\) A small amount of the processed pellet was used for culture on Lowenstein Jensen (LJ) medium. For PCR, DNA was extracted, by incubating the remaining pellet in extraction buffer (1mg/ml proteinase K in 10mM Tris-HCl pH8.0, 1mM EDTA, 10% SDS). Proteinase K was inactivated by heating at 100°C for ten minutes. PCR was done using MPB64 primers (Sigma Aldrich/ Bangalore Genei) which are specific for *Mycobacteria* of the tuberculosis complex.\(^6\)

Amplification reaction was typically performed in a 50µl reaction mix containing 0.25nmoles of forward and reverse primers, 2mM final concentration of dNTP, 1.5 U of Taq polymerase in 1X buffer and proteinase K digested sample. The sequence of the forward and reverse primers used were 5'-TCCGCTGCCAGTCGTCTrCC-3' and 5'-GTACTCGCTCCTTAGGGCA-3'. Forty cycles of amplification were performed using an initial denaturation step of 95°C for five minutes, followed by denaturation at 95°C for one minute, annealing at 55°C for one minute and extension of 72°C for one minute. A final extension was carried out at 72°C for seven minutes. The 0.2Kb amplified fragment was detected on a 2% agarose gel through ethidium bromide staining. DNA from *M. tuberculosis* strain H37Rv was routinely used as a positive control. Appropriate negative controls were set up for each sample. Culture results were monitored at one, two and four weeks and reported positive if growth was found after five to six weeks. Positive cultures were confirmed by microscopy for AFB. Cultures were declared negative if there was no growth by twelve
weeks. Characterization of Mycobacteria was done at the Corporation laboratory by primary differential tests for atypical Mycobacteria.

Statistical analysis

As no single gold standard was available for comparison of the performance of the individual tests, an analysis of results was done using a variety of standards. Efficiency of microscopy, culture and PCR in terms of sensitivity, specificity, positive predictive value and negative predictive value was done using the gold standards of culture for the culture positive samples and smear microscopy, combined microbiological data, response to ATT, chest radiographic findings and clinical follow up data for culture negative samples.

RESULTS

A total of 144 samples, one from each subject (97 from a tuberculosis clinic, 26 identified during a morbidity survey in a slum community and 21 from cases having chest diseases other than tuberculosis) were examined by smear microscopy, culture on LJ slants, and PCR using primers specific for MPB64. Results for sputum smear microscopy and PCR required less than 48 hours whereas results for culture were available 4-8 weeks later.

a. PCR results amongst samples positive by culture and smear microscopy

Thirty five percent (50/144) samples tested positive by culture. Two culture positive samples were Mycobacteria Other Than Tuberculosis (MOTT), identified as M. scrofulaceum and M. intracellulare. PCR and microscopy were negative for both samples identified as MOTT and positive for 98% (47/48) of the remaining samples (Table 1A, serial-a).

There were 36 samples that were positive by Ziehl Neelsen staining. PCR was positive for 94% (34/36) of these samples. Thirty two samples were positive by microscopy and culture (S+C+). PCR was positive for 97% (31/32) of these samples (Table 1A, serial-a).

Table 1A: Efficiency of PCR amongst samples positive by other diagnostic tests

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Sample Description</th>
<th>Number (ii)</th>
<th>PCR positivity (iii)</th>
<th>PCR Efficiency* (%) (iii/ii x 100) (iv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>Culture positive M. tuberculosis MOTT</td>
<td>50</td>
<td>47</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Culture positive M. tuberculosis MOTT</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smear positive samples</td>
<td>36</td>
<td>34</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Smear positive culture positive</td>
<td>32</td>
<td>31</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Smear positive culture negative</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>b.</td>
<td>Smear negative samples From persons with radiological positivity</td>
<td>28</td>
<td>24</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td>Smear negative culture positive</td>
<td>16</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Smear negative culture negative</td>
<td>12**</td>
<td>8</td>
<td>66.6</td>
</tr>
<tr>
<td>c.</td>
<td>Persons (control) with respiratory disease other than tuberculosis (sputum and X-ray negative)</td>
<td>21</td>
<td>0</td>
<td>@</td>
</tr>
</tbody>
</table>

* Efficiency expressed as proportion of PCR positive samples amongst samples positive by other routine diagnostics.
** Out of 13 initially interpreted as X-ray active tuberculosis, one was later confirmed as having non tuberculous lesion
@ 0% positivity of PCR is the expectation in this group

Indian Journal of Tuberculosis
b. PCR data of smear negative samples from tuberculosis cases diagnosed on basis of radiological examination and other routine diagnostics

Twenty-nine individuals whose samples were negative by smear microscopy were diagnosed as having tuberculosis by other routine diagnostics. Of these one did not respond to anti-tuberculosis treatment and was excluded from the data presented in Table 1A. PCR was positive for 86% (24/28) of these samples. Fifty-seven percent (16/28) of these smear negative samples were positive by culture (S-C+). PCR was positive for 100% of these samples (Table 1A, serial-b).

c. PCR data of smear negative samples from individuals diagnosed as not having tuberculosis

In addition, PCR was positive for 17

Table 1B: Status of persons negative by smear and culture but positive by PCR

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
<th>Considered true positives (clinically)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Started ATT subsequently</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Not started ATT, but highly probable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family contact</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Deceased*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Highly probable†</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Lost to follow up</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>7</td>
</tr>
</tbody>
</table>

* Immunocompromised with past history of tuberculosis
† with symptom complex

PCR, culture examination and smear microscopy were performed on 21 individuals with individuals who were declared not to have tuberculosis using the diagnostic paradigm of the RNTCP. Thirteen such individuals could either be traced subsequently, within a period of one year or clinical information could be obtained within a year of first presenting to the clinic. Seven individuals took anti-tuberculosis treatment (ATT) subsequently, one HIV positive individual with recent history of extra-pulmonary tuberculosis was dead. Three individuals were immediate family contacts of tuberculosis cases, two of whom were on anti-tuberculosis treatment (Table 1B). The remaining two individuals were highly probable cases of which one had prominent cervical lymph nodes suggestive of tuberculosis and the other had previous history of tuberculosis. On follow up, one submitted a sample for PCR. Figure 1 shows that within a period of six months after being diagnosed as not having tuberculosis, the PCR profile of the individual had changed markedly, suggesting an increase in the bacterial load in the sputum. Only seven of the 17 cases i.e. those who took ATT subsequently were considered as true cases of tuberculosis, even though the other six could be rated as highly probable cases of tuberculosis (Table 1B). Table 2 summarizes the final diagnosis of tuberculosis made in 71 cases following a diagnostic review consisting of response to ATT/clinical follow up/information from X-ray/sputum investigations.

d. PCR data on controls with diseases other than tuberculosis

PCR, culture examination and smear microscopy were performed on 21 individuals with

Table 2: Summary: Final diagnosis of tuberculosis patients

<table>
<thead>
<tr>
<th>Tuberculosis patients</th>
<th>Diagnostic review</th>
<th>Final diagnosis of TB patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Number</td>
<td>Culture positive &amp; response to ATT</td>
</tr>
<tr>
<td>Smear positive</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>Smear negative*</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>PCR positive</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>48 (67.6%)</td>
</tr>
</tbody>
</table>

*1 smear negative case was false positive which was later determined on the basis of response to ATT and radiological profile after 3 months of treatment initiation and hence not considered in the final tally.
respiratory diseases other than tuberculosis (Table 1A serial-c). These cases were as follows, two each of asthma, pneumonia and cancer lung, one each of post CABG, COPD and lung abscess and 12 with cough and cold. All samples from these individuals had tested negative for tuberculosis by all three diagnostics. No PCR positivity being the expectation for this group, the PCR results could be interpreted as 100% efficient (Table 1A serial-c).

**e. Comparative efficiency of PCR to routine diagnostics like microscopy and culture**

Table 3 presents the comparative efficiency of PCR to diagnostics like microscopy and culture. The sensitivity, specificity, positive and negative predictive values for each of the diagnostics was compared using the gold standards of smear microscopy, culture, and combined microbiological data along with chest radiographic findings and information on clinical follow up. Of the 144 samples, 48 were confirmed on the basis of culture and response to ATT, while 23 culture negative samples were confirmed on the basis of response to ATT, microbiological data and on clinical follow up.

PCR was the most sensitive diagnostic with a sensitivity of 91.5% as against that of culture (68%) and microscopy (51%). However, its specificity was only 86% when compared to sputum microscopy (100%) and culture (97%) (Table 3). Comparison of PCR to conventional methods using McNemars test ($\chi^2=5.26$, df=1, $P<0.05$) showed a significant difference.

**Figure 1:** PCR profile of respiratory sample from suspect at time of first reporting to the microscopy centre (Lane 2) and after six months (Lane 3). MW represents DNA molecular weight marker.

**Table 3:** Comparative sensitivity, specificity, predictive value and efficiency of PCR to routine diagnostics.

<table>
<thead>
<tr>
<th>Diagnostic</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>51 (36/71)</td>
<td>100 (73/73)</td>
<td>100 (36/36)</td>
<td>67.5 (73/108)</td>
<td>76 (109/144)</td>
</tr>
<tr>
<td>Culture</td>
<td>68 (48/71)</td>
<td>97 (71/73)</td>
<td>96 (48/50)</td>
<td>75.5 (71/94)</td>
<td>83 (119/144)</td>
</tr>
<tr>
<td>PCR</td>
<td>91.5 (65/71)</td>
<td>86 (63/73)</td>
<td>87 (65/75)</td>
<td>91 (63/69)</td>
<td>89 (128/144)</td>
</tr>
</tbody>
</table>

Saliva mixed sputum specimens constituted 31% (45/144) of all respiratory samples. Thirty-one of these were negative by all diagnostics while seven of them were positive by routine diagnostics. Of the latter, only one was positive by microscopy while the remaining 6 belonged to individuals diagnosed on the basis of radiological examination. Four of these seven samples were also positive by culture and five
by PCR. In addition, seven saliva samples were positive by PCR only (Table 1B). Thus microscopy, culture, radiological examination and PCR could diagnose one, four, six and eight cases respectively of those individuals whose respiratory samples were considered as inappropriate for microbiological processing.

DISCUSSION

The need for an efficient tuberculosis diagnostic becomes evident from the fact that for every patient of tuberculosis who can be detected using microscopy, nine have to be screened using indirect methods due to the low sensitivity of microscopy. This is the primary impetus for a worldwide effort for developing new tools to diagnose tuberculosis. The use of a molecular technique like PCR for the laboratory detection of Mycobacteria in respiratory and other tissue samples from tuberculosis suspects has thus attracted enormous attention. The present study demonstrates the utility and limitations of PCR.

Among the total of 144 specimens studied, the sensitivity of smear, culture and PCR was 51%, 68% and 91.5% respectively. Smear microscopy was positive in only 67% of the culture positive samples. In comparison, PCR was positive in 98% and it could detect 83% of the smear negative cases that were only radiologically positive. This aspect has great potential in the laboratory diagnosis of tuberculosis, particularly in paucibacillary cases. However, its overall specificity was only 86% when compared to smear and culture. PCR was negative in all negative controls and did not show any cross reactivity with the two MOTT isolates, which indicate good specificity of the primers used.

This study has also indicated that PCR can be a useful tool in those who are not able to expectorate a proper sputum sample. Out of 45 such samples, PCR was able to detect 12 positives while the routine diagnostic tests were positive in only seven. Three of the additional seven cases detected by PCR were considered as true positives by the clinicians. In one of the subjects who had persistent chest symptoms and whose sample was available for PCR at the time of first presentation and on follow up after 6 months, a dramatic increase in bacillary load could be detected by PCR.

The primary limitation of PCR arises from the absence of a suitable gold standard to assess its efficiency. When culture is used as a gold standard in comparison studies, samples containing non-viable Mycobacteria may lead to a false positive PCR, thereby misleading clinicians. The primers MPB64 used in this study proved to be specific and should hold promise for the future. However, studies with larger numbers need to be taken up in order to validate these results. In this context, a recent study examined the cost-effectiveness of polymerase chain reaction versus Ziehl-Neelsen smear microscopy for diagnosis of tuberculosis in a high-burden, resource-starved environment. The study demonstrated that costs per correctly diagnosed case were US dollar 41 and dollar 67 for smear microscopy and PCR, respectively. When treatment costs were included, including treatment of culture-negative cases, PCR was found to be most cost-effective at dollar 382 versus dollar 412.

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