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Molecular Diagnostics in Tuberculosis

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ABSTRACT

Molecular diagnostics in tuberculosis have enabled rapid detection of *Mycobacterium tuberculosis* complex in clinical specimens, identification of mycobacteria species, detection of drug resistance, and typing for epidemiological investigation. In laboratory diagnosis of tuberculosis, nucleic acid amplification (NAA) test is rapid, specific but not as sensitive as mycobacterial cultures. The primary determinant of successful NAA testing of tuberculosis depends on the shedding of mycobacterial DNA in secretions from caseating granuloma and its dissemination into sterile body fluids or tissue biopsies. In multibacillary diseases with high mycobacterial load, a positive Ziehl-Neelsen (ZN) smear with positive NAA is diagnostic of active tuberculosis whereas a positive ZN smear with a negative NAA in the absence of inhibitors would indicate non-tuberculous mycobacterial diseases. The role of NAA test is more important in paucibacillary diseases with low mycobacterial load. But the presence of PCR inhibitors especially in extrapulmonary specimens may produce false negative results. Although part of this inhibitor problem can be overcome by extra-extraction steps, it invariably leads to the loss of more mycobacterial DNA. To circumvent this problem, a brief culture augmentation step before NAA to enhance the mycobacterial load with concomitant dilution of inhibitors can maintain the sensitivity without excessively increases in turnaround time.

INTRODUCTION

Mycobacterium tuberculosis has infected one-third of the world's population, and is currently causing 8 million new cases as well as 2 million fatalities per year (1). In both developing and developed countries, a resurgence of tuberculosis including multidrug-resistant tuberculosis (MDR-TB) has occurred among high-risk populations such as the human immunodeficiency virus-infected patients (1). The control of tuberculosis mainly depends on rapid and accurate diagnosis, provision of effective antituberculous treatment, and thorough contact tracing. Before the introduction of molecular biology into diagnostic mycobacteriology, direct microscopy using a Ziehl-Neelsen smear of early morning sputum was the only way of making a rapid diagnosis. However, a positive smear requires the presence of about 10^4 acid-fast bacilli (AFB) per ml of sputum (2). Although the sensitivity could be improved by concentrating sputum sediment and applying auramine O fluorescent stain, direct microscopy cannot distinguish between *M. tuberculosis* and nontuberculous mycobacteria. Therefore, a positive culture of *M. tuberculosis* remains the gold standard for diagnosis of the disease. Unfortunately, growth on the most affordable solid culture medium, the Lowenstein-Jensen (LJ) medium usually takes 4-6 weeks. Moreover, most clinical specimens for culture, such as sputum and bronchoalveolar lavage require prior decontamination with N-acetyl-L-cysteine and 2% sodium hydroxide which invariably causes a substantial decrease in the number of colony-forming-units of *M. tuberculosis* (2). Sensitivity is augmented by the use of an broth culture medium such as BacT/ALERT system, Mycobacterial Growth Indicator Tube (MGIT) (containing modified Middlebrook 7H9 broth), or BACTEC 460TB system, and their automated analogues: MGIT 960 or BACTEC 9000 MB, respectively

(3,4,5,6). The mean time to detection of *M. tuberculosis* in smear-positive specimens was 13.3 days by the MGIT 960 (range 4–39) and 12.7 days by the BACTEC 9000 MB (range 7–21) respectively (7). Reports of rapid diagnosis of tuberculosis by the detection of various mycobacterial components have not lived up to expectations. The employed tools included gas chromatography-mass spectrometry assay for detection of tuberculostearic acid, and enzyme-linked immunosorbent assay (ELISA) for detection of glycolipid antigen, lipoarabinomannan antigen, and Antigen 60 of *M. tuberculosis* (8,9,10).

Molecular diagnostics in tuberculosis have enabled (i) direct detection of *M. tuberculosis* complex in clinical specimens; (ii) identification of mycobacteria, (iii) detection of drug resistance of *M. tuberculosis*, and (iv) DNA typing to address issues like reactivation of disease versus exogenous reinfection, and to track transmission and internal laboratory contamination. These technological advancements are not intended to replace the conventional tests, but would rather serve as important complementary tools in the management of tuberculosis.

NUCLEIC ACID AMPLIFICATION TESTS FOR RAPID DIAGNOSIS OF TUBERCULOSIS IN CLINICAL SPECIMENS

In order to diagnose tuberculosis rapidly, manufacturers have developed nucleic acid amplification (NAA) tests specific for *M. tuberculosis* complex for its direct detection from sputum specimens. Of the two commercially available NAA tests approved by U.S.

Food and Drug Administration (FDA): Amplified Mycobacterium tuberculosis Direct Test (MTD; Gen-Probe, San Diego, California, US) and COBAS AMPLICOR *M. tuberculosis* assay (Roche Diagnostics, Mannheim, Germany), had excellent performance when used for testing smear-positive specimens (with sensitivity > 95% and specificity = 100%). The sensitivity was lower (83% to 85%) when used for testing smear-negative specimens, though the specificity stayed high (99%) (11). Basing on these data, the FDA only recommended the use of NAA tests for smear-positive respiratory specimens from patients who had not received antituberculous drugs for seven or more days, or within the last 12 months (12). Following the initial FDA clearance, Gen-Probe enhanced the performance of MTD. A large-scale study further revealed the overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the enhanced MTD to be 94.7%, 100%, 100%, and 98.4%, respectively for respiratory specimens (13). The corresponding values were 89.4%, 100%, 100%, and 98.3%, respectively for smear-negative respiratory specimens. This enhanced version of the MTD was eventually approved by the FDA for testing respiratory specimens, regardless of the smear status. COBAS AMPLICOR *M. tuberculosis* assay maintains a reasonable sensitivity and specificity in smear positive respiratory specimens after the initial FDA clearance (14,15,16,17). However, it is limited by slow block cycler amplification process and time-consuming colorimetric detection of the amplification products, which may affect the turn around time. Recently, COBAS AMPLICOR *M. tuberculosis* assay integrates with real-time techniques using the LightCycler 2.0 instrument (Roche Applied Science, Penzberg, Germany). The procedure for template DNA extract remains the same as that of the COBAS AMPLICOR assay. With the use of LightCycler instrument to

detect the amplification products, the turnaround time can be shortening. In 146 clinical specimens being evaluated, good agreement (100% sensitivity, 98.6% specificity) were shown between the LightCycler and COBAS AMPLICOR assays (18).

The early studies on NAA tests were largely laboratory-based, emphasizing culture results as a major endpoint, and neglected integration of available clinical information into the decision making process (12). In reality, it is mandatory to consider the degree of clinical suspicion for tuberculosis in determining the clinical utility of NAA tests. There have been a number of subsequent studies addressing the use of NAA in the clinical setting. In one prospective study, the diagnostic yield of PCR in patients admitted to rule out pulmonary tuberculosis was studied (19). Among 85 patients, 27 patients had cultures positive for *M. tuberculosis*, 12 were smear-positive. A positive PCR on at least one of two specimens collected in the first 24 hours was 85 and 74% sensitive, and 88 and 93% specific for tuberculosis by the in-house and Roche techniques, respectively. Sensitivity in smear-negative patients was 73 and 53%, respectively. Thus, PCR was found to be a useful tool to evaluate patients for tuberculosis within the first hospital day. In a multicenter prospective trial, a total of 338 patients with symptoms and signs consistent with active pulmonary tuberculosis and complete clinical diagnosis were stratified by the clinical investigators to be at low (≤25%), intermediate (26 – 75%), or high (>75%) relative risk of having tuberculosis (20). Based on a comprehensive clinical diagnosis, the sensitivity of the enhanced MTD test was 83%, 75%, and 87% for low, intermediate, and high clinical suspicion of tuberculosis, respectively, and the corresponding specificity was 97%, 100%, and 100%. PPV of the enhanced MTD test

was 59% (low), 100% (intermediate), and 100% (high), compared with 36%, 30% and 94%, respectively for the AFB smear. The investigators concluded that for complex diagnostic problems like tuberculosis, clinical risk assessments can provide important information regarding predictive values more likely to be experienced in clinical practice.

Aside from the commercial NAA tests, a number of in-house studies have been developed over the years. On the whole, the commercial tests have comparable sensitivity and specificity with the in-house tests for respiratory specimens (21,22). In addition to the original intention for facilitating early diagnosis of pulmonary tuberculosis, the NAA tests have been extensively studied in patients with extrapulmonary tuberculosis by either commercial kits or in-house assay (table 1) (22-50). However, the number of publications is too small to allow a meaningful analysis, except for a few clinical entities. For tuberculous meningitis, there have been quite a number of reports with test performance somewhat at variance (34,36,37,51). In one study, the initial low sensitivity of 33% in cerebrospinal fluid could be elevated to 83% by decreasing the cutoff values for positive results of the MTD (34). In a large scale study from Sweden that analyzed 154 cerebrospinal fluid samples using the Cobas Amplicor test (Roche), the sensitivity, specificity, PPV and NPV were 55.6%, 97.2%, 55.6% and 97.2%, respectively (36). In the most recently published systematic review and meta-analysis on the diagnostic accuracy of NAA tests for tuberculous meningitis (37), it has been found that the summary estimates in 14 studies utilizing commercial NAA tests were: sensitivity 0.56 and specificity 0.98. In the 35 studies using in-house tests, the summary accuracy could not be established with confidence because of wide variability in test accuracy. Thus, on

current evidence, commercial NAA tests show a potential role in confirming the diagnosis of tuberculous meningitis, although their overall low sensitivity possibly precludes exclusion of the disease with certainty (37). For pleural effusion, one comparative study of Amplicor PCR, and conventional smear and culture methods has not shown a significant difference in the accuracy of diagnosis (29). On the other hand, some other studies using in-house PCR have yielded rather encouraging sensitivities and specificities of ≥70% and ≥90%, respectively (30,31). A recent study using real-time PCR assay in fine-needle aspirates and tissue biopsy specimens for diagnosing mycobacterial lymphadenitis revealed a sensitivity and specificity of 72% and 100%, respectively (27). The former is better than conventional staining and culture techniques. Thus, this NAA test can provide useful support for clinical decision making in children with lymphadenitis.

The more recently developed BDProbeTEC ET system (BD Biosciences, Sparks, Md) is an automated system characterized by simultaneous DNA amplification (strand displacement amplification) and real-time fluorometric detection. An early evaluation of this direct detection system has suggested its usefulness (52, 53). A recent study of 1131 clinical specimens (735 respiratory specimens and 396 nonrespiratory specimens) (125 *M. tuberculosis* culture-positive: 42 smear-positive), showed that the overall sensitivity, specificity, PPV, and NPV were respectively 90.3%, 96.9%, 78.3%, and 98.9% (54). A comparative study on the performance assessment of the enhanced-MTD test and the BDProbeTec system has also been performed (55). For the enhanced-MTD assay, the sensitivity and specificity were 88% and 99.2% respectively for respiratory specimens,

and 74.3% and 100% respectively for extrapulmonary specimens. The corresponding values for BDProbeTec were 94.5% and 99.6% for respiratory specimens, and 92.3% and 100% for extrapulmonary specimens, respectively. The difference in sensitivity between these two systems was possibly due to better detection of inhibitors by the BDProbeTec system with an internal amplification control. A recent study has further suggested the BDProbeTec ET System can be very useful for rapid detection of *M. tuberculosis* complex , especially in smear-negative respiratory specimens including pleural fluid (56). In this study, the sensitivity for smear-positive and smear-negative specimens was 100% and 81.5%, respectively. While data have been limited for nonrespiratory specimens, a recent study revealed high sensitivity (84.7%) attained in the diagnosis of tuberculous meningitis (57).

Data concerning the impact of the *M. tuberculosis* complex NAA tests on patient outcome have so far been gathered from uncontrolled studies or observations only, and are not based on well-designed clinical trials. The impact of the NAA tests on patient outcome mainly depends on the status of the AFB smear. In smear-positive patients, hospital infection control and public health resources are largely affected, regarding drug therapy and isolation of hospitalized patients for air-borne infections, as well as contact investigations. Appropriate use of isolation beds is especially important in high prevalence areas, regional outbreaks, or where such beds are scarce. In smear-negative patients, the NAA test has a greater potential in influencing the outcome of patients regarding treatment, and avoiding the need for costly and/or risky diagnostic investigations/procedures. Thus, molecular diagnostics of *M. tuberculosis* complex has a

potential to improve clinical care through a substantial reduction in the time required for mycobacterial detection, and may provide material savings in the overall cost of care of a patient. A cost-effectiveness analysis of the MTD (Gen-probe) direct test as used routinely on smear-positive respiratory specimens has been published recently (58). The authors considered that while routine MTD testing of smear-positive specimens would not be expected to have cost saving for most individual hospitals, centralized reference laboratories might be able to implement MTD in a cost-effective manner across a wide range of settings. Prospective studies are required concerning the cost-effectiveness of NAA tests in patients suspected to have tuberculosis. While awaiting more clues for evidence-based practice, one pragmatic approach at present is still to concentrate using NAA tests for smear-positive patients with intermediate or low probability of tuberculosis, and smear-negative patients with high or intermediate clinical suspicion of the disease.

Although the NAA tests provide a rapid diagnosis for pulmonary and extrapulmonary tuberculosis, the sensitivity is still far from ideal. The limitation of the utility of NAA tests is largely attributed to the lack of shedding from patients with tuberculosis and the susceptibility of the amplification reaction to the inhibitor. Secretion of antigens from the bacterial cell of *M. tuberculosis* invariably excites a granulomatous inflammation in immunocompetent hosts which produces a walling off effect by the epithelioid cells and fibroblast at the site of infection. No bacteria will be shed and excreted via the luminal passages such as the airway into the exterior or potential body cavities such as peritoneum, pericardium, pleura, synovium, and arachnoids. Shedding only starts to

occur when the granulomatous inflammation is so severe that caseous necrosis has occurred with erosion into the mucosal lumen or coelomic cavity. In the case of the airway, the necrotic material carrying the AFB is carried by the mucociliary blanket and coughed into the environment. It is usually at this stage that a diagnosis can be made in an immunocompetent host using either culture or NAA test. It is also important to note that extrapulmonary specimens such as whole blood and various body fluids could be more useful clinical specimens in immunosuppressed hosts where mycobacterial dissemination readily occurs without the walling off effect of granulomatous inflammation. As expected, studies have shown a reasonable sensitivity of NAA test using peripheral blood in AIDS patients with tuberculosis (45).

Although the advantage of NAA over culture is that the sensitivity is not decreased by the prior decontamination step, this advantage is offset by inhibitors of the polymerase enzyme. The incidence of such inhibitors varies from 4% in pulmonary to 18.6% in extrapulmonary specimens (59). Part of this inhibitor problem can be overcome by refining the nucleic acid extraction step. But these extra-extraction steps invariably lead to the loss of more mycobacterial DNA. This is a serious problem in extrapulmonary tissue specimens where inhibitors are especially abundant (59). This may be circumvented by a short culture augmentation step with concomitant dilution of inhibitors. A brief culture of 2 to 3 days on LJ medium significantly increased the sensitivity of NAA tests in tissue samples exerting PCR inhibition from 63% to 92% (60). False positive results from NAA tests could be minimized by automated testing, good laboratory practice, and the use of uracil-N-glycosylase (UNG) and dUTP-UNG

(2,61). However, biological false positive has also been reported as a result of the shedding of dead mycobacteria after adequate antituberculous treatment (62). Except for the turnaround time, the initial expectations that these NAA tests are at least as rapid as the ZN smear, more sensitive and specific than the smear, more sensitive than the culture, especially those smear negative specimen from extrapulmonary sites where the diagnosis is always difficult due to the paucibacillary nature of the disease, have not been fulfilled (63).

RAPID IDENTIFICATION OF MYCOBACTERIA BY MOLECULAR TECHNIQUES

Identification of mycobacteria by growth characteristics and conventional biochemical tests takes many weeks. The CDC has recommended use of more rapid identification methods, such as nucleic acid probes, the NAP (p-nitro-α-acetylamino-β-hydroxypropiophenone) test, and high-performance liquid chromatography (64).

Several systems exist for the rapid identification of mycobacterial species from cultured isolates (64). The MicroSeq 500 system (Applied Biosystems, Foster City, California, US) is an assay based on sequencing a portion of the 16S rDNA gene (65,66,67). It is time-consuming to perform and requires sophisticated instrumentation for data analysis. The AccuProbe assay (Gen-Probe, San Diego, California, US), a chemiluminescent DNA probe, involving hybridization with species-specific probes, is highly specific. But the test is only able to identify a limited number of species (68). Like MicroSeq 500 system, it cannot differentiate *M. tuberculosis* from the other species in the complex. DNA strip

assays based on the reverse hybridization of PCR products to oligonucleotide probes bound on a membrane strip can be applied to identify myccobacterial species. Two commercial preparations of DNA strip assay are available for laboratory diagnosis (68). Inno-LiPA Mycobacteria (Innogenetics NV, Ghent, Belgium) identifies mycobacteria by the 16 to 23S rDNA spacer region of *Mycobacterium* species (69) and GenoType Mycobacteria (Hain Lifescience, Nehren, Germany) identifies mycobacteria by 23S rDNA of *Mycobacterium* species (70). Both can also identify *M. tuberculosis* from clinical isolates (70,71) or broth culture systems (72), but does not allow differentiation among members of the *M. tuberculosis* complex. A single test can identify a range of species (73). Inno-LiPA assay has been recently improved (Inno-LiPA Mycobacteria v2) by increasing the number of identifiable mycobacterial species to 16 (74). GenoType assay can identify 13 clinically important mycobacterial species. In a prospective evaluation of 178 clinical isolates, GenoType assay produced an overall agreement of 89.3% with AccuProbe assay and 16s rDNA sequencing (70). Other rapid identification method included PCR amplification-restriction analysis of the rpoB DNA which can detect pathogenic mycobacteria including *M. tuberculosis* complex in clinical specimens (75).

Furthermore, an extension of the principle of solid phase detection of nucleic acids has been developed. Using high-density DNA probe arrays on a microchip, the system can provide rapid strain identification, as well as, assessment of drug resistance in cultured isolates (76,77). While these microarrays are technologically advanced, the associated cost can be great. One simpler way is to use the peptide nucleic acids probes as an in situ

hybridization assay by a fluorescent stain format. Peptide nucleic acids are DNA-like structures in which the sugar-phosphate backbones are replaced with peptide-like structures. The binding of peptide nucleic acid to DNA is sequence specific, and the interaction is stronger than that of a DNA-DNA interaction. This technology has been found to work well for cultured isolates, and formalin-fixed histological samples (78,79).

RAPID DRUG SUSCEPTIBILITY TESTING USING MOLECULAR TECHNIQUES

Giving an effective antituberculous drug combination is as important as making a rapid and accurate diagnosis of tuberculosis. Without a rapid drug-susceptibillity testing method, most clinicians are prescribing a standard regimen empirically for patients with no known risk factors for drug-resistant disease. Traditionally, drug-resistance testing requires the growth of sufficient bacterial colonies in order to allow standardization of inoculums used in the agar proportion method (2), which usually takes another 2 weeks. The turnaround time is shortened to only 4 days with the BACTEC 460TB methods, which incorporates standard dilution of antituberculous drugs (isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin) in the broth medium and monitor the growth curve after inoculation (2).

With unraveling of the genetic basis of antituberculous drug resistance through identification of the main genes in question (80), namely rpoB (rifampicin), katG (isoniazid), inhA (isoniazid and ethionamide), ahpC (isoniazid), pncA (pyrazinamide), embB (ethambutol), rrs (streptomycin), and rpsL (streptomycin) and gyrA

(fluoroquinolone), various molecular techniques for detection of drug resistance (largely against rifampicin, isoniazid, pyrazinamide and streptomycin) in *M. tuberculosis* have been evaluated. These principally include direct DNA sequencing (81), heteroduplex (82), and restriction fragment length polymorphism (83), as well as oligonucleotide arrays (84). Two commercial forms of the molecular assays, namely INNO-LiPA Rif.TB (Innogenetics, Belgium) and MisMatch Detect II (Ambion, U.S.A.) have performed well by correlating with standard methods of detection of *M. tuberculosis* and rifampin susceptibility testing in 94.7% and 100% smear-positive respiratory specimens, respectively (85). For the cultured isolates, the correlations reached 100%. The former technique is a line probe assay based on the reverse hybridization principle. The latter represents a RNA/RNA duplex, base pair-mismatch assay. Direct DNA sequencing and DNA arrays also appear very promising for future use due to high sensitivity and specificity. In recent years, there have been additional reports on direct detection of rifampicin and isoniazid resistance rapidly in *M. tuberculosis* strains present in clinical specimens. Most of the techniques embrace the use of improved PCR amplification, such as real-time PCR (86) or allele-specific on-chip PCR (87). Another new development in recent years has involved the molecular beacons. These are molecules that emit light following upon a chemical reaction involving a colored fluorophore (88). Such molecular beacon assays based on binding of DNA primers with specific targets in PCR amplicons can provide a rapid and sensitive method of diagnosing drug resistance (89). However, application of DNA arrays and molecular beacons is associated with high cost. A recent study has described the potential utility of the peptide nucleic acid probes in

detecting mutated KatG and rpoB genes in *M. tuberculosis* (90). This study utilizes the PCR-ELISA format and is likely to be more economical.

UTILITY OF MOLECUAR FINGERPRINTING IN TUBERCULOSIS

Tuberculosis often has a long incubation period which makes outbreak investigations more difficult than for other acute respiratory infections. Discriminatory typing methods would have an important place in the confirmation of clusters in outbreak investigations especially in areas where the disease is highly endemic. Strain typing has been used in community or institutional outbreaks involving family households, prisons, laboratory cross-contamination and outbreaks due to drug-resistant strains (91,92,93). These typing techniques are also important in the differentiation of reactivation or exogenous reinfection. At the national level such tests could also be used for the evaluation of regional control programs and will allow the design of more rational control measures. In the last 10 years, the most widely used technique is restriction fragment length polymorphism (RFLP) analysis of chromosomal DNA using IS*6110*, an insertion sequence found throughout the *M. tuberculosis* complex, typically in 5 to 20 copies (94). While general principles regarding interpretation of RFLP patterns are present, there are no universally accepted criteria. Besides, the analysis is labor-intensive and cannot be applied to strains with five or fewer copies of IS*6110*, which are not uncommon in some communities rendering this approach less useful (95). Other fingerprinting techniques that have been developed to complement RFLP analysis include blotting for polymorphic guanine-cytosine-rich sequences (96), substituting IS*1081* (97) or other repeat sequences such as the variable-number tandem repeats (98), and spoligotyping (99).

OTHER POTENTIAL UTILITY OF MOLECULAR DIAGNOSTICS IN TUBERCULOSIS

Molecular detection of mycobacterial DNA has also been investigated regarding its potential to predict treatment success, failure or relapse. Initially monthly qualitative PCR on sputum specimens were performed. The majority of the treated patients either did not produce sputum or have a negative PCR test by 6 months (62). Around 30% had persistent positive test but most of these patients had extensive pulmonary disease, underlying medical problems (62). A small number of these persisters actually had known MDR-TB which accounted for the failure (62). Most of these persisters could be explained by the persistent shedding of dead AFB. Since microbial load is the result of the dynamic interaction between the microbe, the host defense and the drug treatment, a serial quantitative monitoring of the microbial load throughout the course of treatment may be important for both prognostic predictions and individualization of the regimen and duration in the long run. This is possible with the advent of the real time PCR using the LightCycler (100). However studies comparing the quantitation by AFB smear, colony counts and genome copies by quantitative PCR showed that the initial bacillary load are only well correlated before treatment. But the rate of disappearance of the AFB and the quantity of DNA did not correlate with the rate of decline of the viable colony count and therefore not useful for monitoring of the efficacy of drug treatment. This was not unexpected since positive smears are well known to persist for years in patients with severe cavitary disease leading to extensive lung destruction. The hard cell wall of mycobacteria may well have protected the microbial DNA from host enzymatic

degradation. There were attempts to use relatively shorter lived mRNA encoding 85kDa protein for monitoring the progress (101). The clinical usefulness of such approach would await validation by quantitative PCR in larger clinical studies.

MOLECULAR DIAGNOSTICS FOR HOST SUSCEPTIBILITY

The future of the molecular diagnostic test for tuberculosis will no longer be confined to the bacterial genome. The unfolding of the human genome has led to the discovery of more susceptibility or resistance genes associated with tuberculosis (102). These genes are related to effective killing of intracellular mycobacteria or granuloma formation. The effector mechanisms include the iron scavenging function of transport proteins of macrophages which competes with the siderophores of mycobacterium, the activation of macrophage function by Vitamin D, the antigen presentation, and even the cytokines, cytokine receptors and intracellular signaling molecules which are part of the immunological pathway of activation for a T-cell helper-1 response (103). Important examples are the natural resistance associated macrophage protein (NRAMP-1), Vitamin D receptor, HLA-DR2 and HLA-DQB1 loci, on chromosome 15 and X (104,105,106). Importance of the mutations involving the receptors IFN R1, IFN R2, STAT1, IL12R β1 associated with interferon-γ mediated immunity is uncertain in tuberculosis though they have been found to be linked to disseminated diseases due to atypical mycobacteriosis and other intracellular pathogens (107,108). The use of microarray for a host genome survey of tuberculosis susceptibility would not be too far from reality.

CONCLUSION

In the past decade, there has been an exuberant progress in the discovery and evaluation of new techniques in molecular diagnosis for tuberculosis. Many of these are likely to have major complementary roles to the conventional tests. Some technologies have opened up potentially novel approaches in the fight against this important infectious disease worldwide. As for new drugs, the use of these new diagnostics is also fraught with budgetary considerations. This is especially relevant in the developing world with heavy disease burdens and severely compromised resources. Thus, continuing efforts must be made to address the clinical applicability and cost-effectiveness of these novel tools in the strategic management of tuberculosis globally.

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Table 1. Overview of sensitivity and specificity of polymerase chain reaction using either in-house or commercial methods in detection of *Mycobacterium tuberculosis* from direct clinical specimens

	Overall sensitivity (%)[!]	Overall specificity (%)	Ref
Anatomical site			
Pulmonary			
Respiratory specimens*	77.1 - 100	99.3 - 100	22,23
Gastric aspirates	44 - 60	93.7 - 98	24
PTNA[@]	65	100	25
Extrapulmonary			
Lymph node (fresh tissue)	71.6 - 87.5	NM	26,27[$]
Pleural fluid	27.3 - 81	90 - 100	28-32
Pleural biopsy	90	100	33
Cerebrospinal fluid	31.4 - 56	98	34 - 37
CAPD fluid	CR	CR	
Ascite fluid	CR	CR	
Liver biopsy tissue (paraffin-embedded)	58 - 88	96 - 100	38, 39
Urine	55.6 - 95.6	98.1 - 98.9	40, 41
Skin	60 - 80	100	42, 43
Bone & synovial tissue	CR	CR	
Peripheral blood	30.4 - 100 [#]	NM	44 - 46
Marrow blood	42 - 73.2	NM	47, 48
Paraffin-embedded tissues	60 - 68	NM	49, 50

Note. [!] *Mycobacterium tuberculosis* culture as gold standard for respiratory specimens whereas clinical diagnosis with or without radiological and histological findings as gold standard for other specimens; * sputum, bronchoalveolar lavage fluid, endotracheal aspirates; [@] Percutaneous transthoracic needle aspiration [#] 82% in HIV-positive; [$] Real-time PCR is performed; CR, case report only; NM, not mentioned; PCR, polymerase chain reaction; Ref, references