Adjunctive Tests for Diagnosis of Tuberculosis: Serology, ELISPOT for Site-Specific Lymphocytes, Urinary Lipoarabinomannan, String Test, and Fine Needle Aspiration

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The diagnostic gold standard for active tuberculosis (TB) is the detection of *Mycobacterium tuberculosis* (MTB) by culture or molecular methods. However, despite its limited sensitivity, sputum smear microscopy is still the mainstay of TB diagnosis in resource-limited settings. Consequently, diagnosis of smear-negative pulmonary and extrapulmonary TB remains challenging in such settings. A number of novel or alternative techniques could provide adjunctive diagnostic use in the context of difficult-to-diagnose TB. These may be especially useful in certain patient groups such as persons infected with human immunodeficiency virus (HIV) and children, who are disproportionably affected by smear-negative and extrapulmonary disease and who are also most adversely affected by delays in TB diagnosis and treatment. We review a selection of these methods that are independent of nucleic acid amplification techniques and could largely be implemented in resource-limited settings in current or adapted versions. Specifically, we discuss the diagnostic use and potential of serologic tests based on detection of antibodies to MTB antigens; interferon gamma release assays using site-specific lymphocytes; detection of lipoarabinomannan, a glycolipid of MTB, in urine; the string test, a novel technique to retrieve lower respiratory tract samples; and fine needle aspiration biopsy of lymph nodes.

Globally, an estimated 9.4 million new cases of active tuberculosis (TB) occur each year [1]. The vast majority (~80%) live in resource-limited settings; ~1.4 million cases are associated with human immunodeficiency virus (HIV), and almost 1 million occur in children [1, 2]. Early diagnosis and treatment of TB leads to reduced morbidity, mortality, and is especially important in immunocompromised persons and young children who have a much higher risk of development of TB, accelerated disease progression, and TB-associated mortality compared with immunocompetent adults [3–5]. However, detection of TB in these patient groups, who live mostly in resource-limited settings, is particularly challenging, resulting in diagnostic delay and increased mortality.

The gold standard for TB diagnosis is either isolation of *Mycobacterium tuberculosis* (MTB) by culture or detection of MTB-specific nucleic acids by molecular methods [6, 7]. However, in addition to requiring laboratory infrastructure, culture methods have a long

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turnaround-time, taking weeks to months, and molecular methods have high cost and technology requirements [8–10]. Therefore, despite its limited sensitivity of 50% or less to detect acid fast bacilli (AFB) in sputum samples [11–13], microscopy is still the most widely used rapid method for diagnosing TB, and often the only diagnostic test available in resource-limited settings. Furthermore, all 3 modalities require either sputum, which may be inadequate due to nonproductive cough or inability to cough, or another specimen from the site of disease, which may not be easily accessible for sampling. For these reasons, diagnosis of pulmonary TB in patients with either negative sputum smears or inadequate sputum production, as well as the diagnosis of extrapulmonary TB, is often difficult, especially in resource-limited settings.

Rates of smear-negative pulmonary and extrapulmonary TB are higher in HIV-infected compared with uninfected persons with TB [12, 14–18]. Therefore, TB case detection rate can be as low as 20%–35% of all TB cases in settings with high HIV prevalence and limited laboratory infrastructure [12, 13]. The increased mortality rates in HIV-infected compared with uninfected persons are particularly high for those with smear-negative pulmonary and extrapulmonary TB [3]. In these patients, severe immunosuppression and delayed diagnosis are additional contributors of such excess mortality [3]. Similar issues exist for children in whom TB diagnosis is equally challenging due to a high proportion of sputum smear and culture negative disease [19, 20]. The objective of this article is to summarize findings of 5 novel or alternative methods that were shown to have potential adjunctive value to conventional diagnostic tests in patient groups in whom TB is particularly challenging to diagnose. We focus on tests that have been evaluated in human studies, are independent of nucleic acid amplification techniques, and for the most part can be easily implemented in resource-limited settings.

SEROLOGIC TESTS FOR THE DIAGNOSIS OF TB

Detection of serum antibodies (Abs) to MTB antigens (TB serology) offers an alternative method for diagnosing TB. Serology does not require a specimen from the site of disease and can be scaled up into a rapid, robust, inexpensive format requiring little laboratory infrastructure. It is therefore an especially attractive option for resource-limited settings and could ultimately serve as a point-of-care test. Many mycobacterial antigens have been evaluated for the serodiagnosis of TB and have been extensively reviewed elsewhere, although data in pediatric populations are very limited [21–24]. Although most antigens do not seem to be ideal candidates for TB serology, some seem to have value in adult TB cases that are often difficult to diagnose with conventional methods alone, such as TB in the setting of HIV coinfection. The goal of this section is to discuss some of the challenges and potential advantages of TB diagnosis based on serology.

For decades researchers have attempted to develop serodiagnostic tests that can detect TB and distinguish it from latent TB infection (LTBI). However, commercially available serodiagnostic tests thus far are limited by a lack of sensitivity and specificity, especially in early stages of TB and HIV-associated TB (reviewed in [25, 26], [27–29]). Depending on antigens included in serologic test, as well as study design, subjects, and site, sensitivity estimates range from 10% to 90%, and specificity estimates range from 47% to 100% [26]. Some of the reasons for the limited sensitivity are that (1) these assays are based on antigens that predominantly elicit Ab responses in HIV-uninfected patients with advanced TB, such as the 38 kDa protein (reviewed in [23]); (2) early stages of non-HIV TB in HIV-uninfected patients are likely associated with low mycobacterial burden resulting in low Ab titers to most antigens [8, 30]; (3) most HIV-infected TB patients appear to develop Ab responses to a smaller repertoire of MTB antigens than HIV-uninfected TB patients [31, 32], and such antigens have not been included in commercial tests; and (4) the Ab profiles in TB patients are heterogeneous [33, 34].

In addition to insufficient sensitivity, a lack of high specificity has also been a major problem when evaluating serologic tests for TB in cross-sectional studies of TB suspects in contrast to case-control studies (reviewed in [23, 26]). This is not surprising because case-control design is typically associated with some degree of bias resulting in overestimation of diagnostic test accuracy [35, 36], especially when comparing cases of advanced disease to healthy controls from nonendemic regions [26]. Plausibly, the specificity of serology for TB may be compromised by Ab reactivity attributable to LTBI, disease caused by nontuberculous mycobacteria (NTM), or receipt of the Bacillus Calmette-Guérin (BCG) vaccine (consisting of an attenuated strain of live *Mycobacterium bovis*). However, studies have shown that some promising antigens, such as the malate synthase (MS; also referred to as GlcB) and the MPT51 protein, which share considerable genetic homology with some other mycobacteria, do not elicit Ab responses in persons with LTBI, irrespective of HIV status, or in BCG-vaccinated healthy volunteers; and elicit only marginally positive Ab responses in some patients with NTM disease [30, 37]. In contrast, Ab responses to these proteins were detected in several TB suspects, most of them HIV-infected, who were subsequently diagnosed with community-acquired pneumonia (based on clinical improvement on antibiotics) [30]. Similarly, Ab responses, even to the more MTB-specific antigens ESAT-6, CFP10, and Rv0222, have been detected in a considerable number of Gambian TB suspects ultimately diagnosed with respiratory diseases other than TB but not in healthy BCG-vaccinated volunteers from Denmark [38]. In a separate study, Danish and Brazilian TB contacts with tests indicating LTBI showed much lower Ab levels to ESAT-6 and CFP10 compared with their Ethiopian counterparts [39]. When interpreting such data, one has to keep in mind that studies from

TB-endemic settings have shown that between 8% and 44% of AFB smear-negative TB patients who ultimately had positive mycobacterial cultures showed symptomatic response to a trial of antibiotics and were initially diagnosed with a respiratory disease other than TB [40]. Furthermore, Ab responses to certain mycobacterial proteins have been detected months to years prior to the diagnosis of TB in persons infected with HIV [41–43]. Thus, it is conceivable that the detection of Ab reactivity in some TB suspects could be due to undetected early disease and reflects a true serologic response to MTB.

Despite the limitations of current serologic tests for TB, recent studies have demonstrated that some could serve as adjunctive diagnostic tests in HIV-infected patients with smear-negative pulmonary TB or extrapulmonary TB [44, 45]. In contrast to paucibacillary TB in HIV-uninfected patients, Ab responses to proteins such as MS and MPT51 are especially strong in most patients with HIV-associated TB, regardless of smear-positivity and level of immunosuppression [30, 44, 45]. More importantly, among US HIV-infected TB suspects, a positive Ab response to the MPT51 protein was strongly and significantly associated with a culture-confirmed diagnosis of TB [30]. Other antigens, such as the recently identified proteins Rv0222 and PPE, also elicit strong Ab responses in HIV-infected TB patients and appear to be promising candidates for TB serodiagnostic tests in TB-endemic regions with high HIV prevalence [38, 43].

A few studies have demonstrated that Abs to certain mycobacterial proteins, such as ESAT-6, CFP10, MS, MPT51, and PPE55 can be detected months to years prior to the diagnosis of TB in persons infected with HIV [41–43]. This is not surprising because even in immunocompromised patients the progression from LTBI to clinically detectable TB can take months to years [46]. These findings indicate that TB serology could be useful in the identification of disease stages that precede symptomatic and microbiologically positive TB, such as subclinical and incipient TB. Other highly immunogenic antigens in HIV-associated TB are also likely to be elicited prior to clinically detectable TB. However, well-designed, prospective cohort studies are needed to evaluate the prognostic value of a panel of such antigens. The detection of early and often asymptomatic disease stages would be extremely valuable, especially in HIV-infected persons, in whom treatment of subclinical TB suggests an association with reduced mortality [47].

The high level of Ab reactivity to certain proteins in HIV-associated TB is likely due to the impaired granuloma formation and consequent ineffective containment of MTB as evidenced by the significantly lower rate of cavitation but higher rate of lymphadenopathy seen in HIV-infected patients compared with uninfected patients [48–50]. Thus, immunosuppression often results in a high mycobacterial burden and larger proportion of disseminated as well as extrapulmonary TB even when the patients are bacteriologically negative on sputum examination. This is supported by the frequently positive mycobacterial blood cultures or blood-based nucleic acid amplification tests (NAATs) in patients infected with HIV [51–53]. The higher mycobacterial burden combined with the polyclonal B-cell stimulation that accompanies HIV infection likely results in stronger stimulation of the humoral immune responses in HIV-infected patients compared with uninfected TB patients [30, 54, 55].

In summary, despite their limitations, serologic tests could have adjunctive value in the diagnosis of HIV-associated TB, particularly in resource-limited settings. Evaluation of the clinical use of serology for the rapid detection of HIV-associated smear-negative pulmonary and extrapulmonary cases warrants large cohort studies. The observation of higher rates of Ab responses in certain TB suspects—especially those that are HIV-infected and from TB-endemic regions—suggests that Ab reactivity may be identifying a true serologic response to MTB whose prognostic value is yet to be elucidated.

INTERFERON GAMMA ELISPOT ASSAY FOR THE DIAGNOSIS OF TB USING SITE-SPECIFIC LYMPHOCYTES

Interferon gamma (IFN-γ) release assays (IGRAs) have been introduced as an alternative to the tuberculin skin test for the diagnosis of LTBI [56]. Both types of commercially available IGRAs (T-Spot TB, bioMérieux, based on ELISPOT, and QuantiFERON, Celestis, based on enzyme-linked immunosorbent assay [ELISA] technology) measure MTB antigen–specific IFN-γ production produced by T cells that were previously exposed to the pathogen. These assays use unique antigens from the region of difference-1 section of the MTB genome. The standard ELISPOT IGRA, T-SPOT.TB, uses CFP10 (Rv3874) and ESAT-6 (Rv3875). A more recent version of the assay, ELISPOT PLUS, also incorporates Rv3879c [57]. The ELISA IGRA, QuantiFERON-TB Gold In-tube, uses CFP10, ESAT-6, and TB7.7. Both assays are unable to differentiate between TB and LTBI when used on peripheral blood, where low frequencies of MTB-specific T cells are found even in individuals with disease (up to ~0.4% ESAT-6 and CFP10 specific T cells) [58–61]. However, IGRAs may have a use in the diagnosis of TB if disease site-specific lymphocytes are used. MTB-specific T cells are present at a much higher frequency at the site of disease compared with the periphery. They were shown to be ~10-fold higher in the bronchoalveolar lavage (BAL) fluid and pleural effusions, for pulmonary TB and TB pleurisy, respectively, indicating that sampling lymphocytes from the site of disease may enable the diagnosis of TB [58, 60, 62]. MTB-specific T cells have been shown to be concentrated in the following samples from individuals with both smear-negative pulmonary and extrapulmonary TB: cerebrospinal fluid (CSF), ascites, peural effusion, and BAL fluid [58–60, 63, 64]. Such concentration of MTB-specific T cells is generally not seen in individuals with alternative pathologies [58, 60].

A number of studies have now reported that the ELISPOT, but not the ELISA platform, may be a useful tool to aid the diagnosis of smear-negative pulmonary TB using BAL and potentially pleural TB using pleural mononuclear cells [58–60, 65–67]. Dheda et al [65] have also demonstrated the potential use of the BAL-ELISPOT platform in individuals infected with HIV living in a highly endemic TB setting, reporting sensitivity of 88.9% and specificity of 94.7%. This is important because individuals infected with HIV frequently present with nonspecific clinical symptoms and abnormal radiological findings. In 2009, a prospective multicenter Tuberculosis Network European Trialsgroup (TBNET) study reported that the BAL-ELISPOT platform was the superior diagnostic tool when compared with the following: peripheral blood mononuclear cells-ELISPOT, tuberculin skin test (TST), and MTB-specific NAATs for the diagnosis of sputum smear-negative TB [68]. A major limitation with the BAL-ELISPOT platform was an inconclusive/indeterminate result in 33% of tests based on their stringently defined assay cutoff. Although the BAL-ELISPOT has been shown to have good discriminatory value and a high specificity in both high- and low-burden settings, the use of an ELISPOT for pleural TB is less clear cut and may only have the potential to act as a diagnostic aid in a low-burden setting [60, 63, 65, 66, 69–71]. Future studies are needed to investigate the use of the ELISPOT platform for other extrapulmonary manifestations of TB such as pericarditis and to confirm initial reports using peritoneal fluid mononuclear cells [72].

Data also suggest that the ELISPOT is potentially a good tool for the diagnosis of TB meningitis using CSF-derived lymphocytes from both HIV-infected and uninfected individuals living in high- and low-burden TB settings [64, 73–76]. TB meningitis occurs in 1%–6.3% of pulmonary TB and in 5%–18% of extrapulmonary TB cases [77–80] with higher frequencies in HIV coinfected individuals [81–83]. It is particularly problematic to diagnose, and as a result delayed therapy increases mortality levels to ~30% [81, 84, 85]. Most recently, Patel et al [73] have reported that the CSF-ELISPOT platform can act as a rapid rule-in test for TB meningitis in a TB-endemic region with high HIV prevalence when used in combination with simple tests to rule out bacterial infection and cryptococcal meningitis (sensitivity, 82%; specificity, 100%; positive predictive value, 100%; negative predictive value, 68%). More importantly, in this large prospective study the performance of the CSF-ELISPOT was compared with a clinical prediction rule (derived from pretreatment clinical and laboratory parameters) and found to provide incremental value and therefore improve on pretest probability [73]. Interestingly, in this study the ELISPOT assay had only ~3% of inconclusive results. The above suggests that the CSF-ELISPOT platform can act as a useful and rapid diagnostic tool to diagnose TBM in an HIV- and TB-endemic setting.

To conclude, reports to date indicate that the ELISPOT IGRA, when performed on disease site-specific lymphocytes, can be a useful tool for the diagnosis of smear-negative pulmonary TB and TB meningitis. Due to the cost, infrastructure, and laboratory requirements needed to perform the assay, in addition to the requirements for BAL, its use in resource-limited settings such as South Africa may be limited to the diagnosis of complex cases in a tertiary care setting.

DETECTION OF LIPOARABINOMANNAN IN URINE FOR DIAGNOSIS OF HIV-ASSOCIATED TB

Mycobacterial antigen detection is an attractive option for TB diagnosis, overcoming many of the limitations inherent in immune-based assays, especially in the context of HIV infection. Compared with sputum, urine is also very practical as a clinical sample for diagnosis. It is easy to collect from both adults and children, quality is likely to be less variable, it contains fewer bacterial contaminants, and it is safer to handle. Thus, mycobacterial antigen detection in urine represents an important potential tool within the developmental pipeline of new TB diagnostics for use in resource-limited settings [86, 87].

A number of mycobacterial antigens can be detected in urine samples of patients with pulmonary TB [88–90]. However, the most promising candidate for diagnostic assays is lipoarabinomannan (LAM)—a heat-stable, major glycolipid constituent of the cell wall of MTB [91]. This antigen is released from metabolically active or degrading mycobacteria and enters the circulation from where it is filtered in the renal tubules. It is detectable in urine of patients with active TB using a simple and rapid sandwich ELISA using polyclonal antibodies [92–94].

Early developmental versions of such an assay showed moderate sensitivity and specificity and required extensive urine purification that included column chromatography. This rendered them impractical as a simple diagnostic test for resource-limited environments [92, 94]. However, proof of principle data from a simplified version of this assay that required minimal or no specimen processing led to rapid commercialization of this test. This was first developed as the 'MTB ELISA' test (Chemogen Inc) and subsequently marketed as the 'Clearview TB ELISA' (Inverness Medical Innovations).

The first field evaluation of a precommercial prototype of the MTB ELISA test (Chemogen Inc) in Tanzania produced encouraging results among TB suspects that included individuals with and without HIV infection [93]. The sensitivity was 80% for culture-confirmed TB with a specificity of 99% (Table 1). Sensitivity was preserved in patients infected with HIV and exceeded that of sputum microscopy. However, data from 6 further field evaluations using 2 sequential, commercial versions of the assay have yielded disappointing results [95–100] (Table 1). Reasons for these differences are not entirely clear but may relate to lack of standardization of the polyclonal antibody preparations used. Very low sensitivity (6%–21%) for culture-positive TB in

Table 1. Studies Evaluating Commercially Available Assays Detecting Urinary Lipoarabinomannan (LAM) for the Diagnosis of Tuberculosis in Patients With Culture-Confirmed Disease

Study	ELISA version	Country	Study population	Total subjects (n)	HIV+ subjects with culture+ TB (n)	HIV- subjects with culture+ TB (n)	Culture method	Sensitivity of sputum microscopy % (95%CI)	Sensitivity of LAM ELISA in Culture+ TB % (95%CI)			Overall Specificity % (95%CI)
									All subjects	HIV+ subjects	HIV- subjects	
Boehme et al 2005 [93]	MTB ELISA, first prototype (Chemogen Inc)	Tanzania	Out-patient TB suspects	334	85	34	LJ culture	62 (54–70)	80 (72–87)	81 (71–88)	74 (57–86)	99 (94–100)
Lawn et al 2009 [95]	MTB ELISA, second prototype (Chemogen Inc)	South Africa	Active screening of HIV+ out-patients pre-ART	235	58	0	Automated liquid culture	14 (7–25)	33 (22–46)	All: 33 (22–46) CD4>100: 13 (4–33) CD4 50–100: 41 (22–64) CD4<50: 67 (44–84)	N/A	100 (98–100)
Mutetwa et al 2009 [96]	MTB ELISA, second prototype (Chemogen Inc)	Zimbabwe	Out-patient TB suspects	397	140	Not stated	LJ culture	75 (67–81)	44 (36–52)	52 (43–62)	21 (9–37)	89 (81–94)
Reither et al 2009 [97]	MTB ELISA second prototype (Chemogen Inc)	Tanzania	Out-patient TB suspects	291	50	19	LJ and automated liquid culture	70 (58–79)	51 (39–62)	62 (48–74)	21 (8–44)	88 (79–94)
Daley et al 2009 [98]	MTB ELISA, second prototype (Chemogen Inc)	India	Out-patient TB suspects	200	5	40	LJ and liquid culture	79[a] (66–89)	18 (9–33)	20 (1–70)	18 (8–32)	88 (81–92)
Shah et al 2009 [99]	Clearview TB ELISA (Inverness Medical Innovations)	South Africa	In-patient HIV+ TB suspects	499	167	14	Automated liquid culture	42 (36–50)	59 (52–66)	All: 67 (59–74) CD4>200: 55 (41–69) CD4150-200: 14 (4–58) CD4150-200: 56 (30–80) CD450-100: 71 (51–87) CD4<50: 85 (73–93)	14 (3–41)	96 (91–99)
Dheda et al 2010 [100]	Clearview TB ELISA (Inverness Medical Innovations)	South Africa	Out-patient TB suspects	500	44	80	Automated liquid culture	65 (57–72)	13 (8–19)	All: 21 (11–35) CD4<200: 37 (19–59)	6 (3–14)	99 (97–100)

Where 95% confidence intervals (CI) were not calculated in the original manuscripts, these have been calculated using the Wald method.

Abbreviations: ART, antiretroviral therapy; CI, confidence intervals; ELISA, enzyme-linked immunosorbent assay; LJ, Lowenstein–Jensen slopes.

[a] Of all TB diagnoses, not just culture-confirmed.

HIV-uninfected patients was a consistent finding among all studies. In contrast, sensitivity was higher among patients infected with HIV (20%–67%). Specificity was variable in both HIV-infected and uninfected patients, with 3 studies from South Africa reporting high specificities of 96%–100% compared with just 88%–89% in 3 other studies from Zimbabwe, Tanzania, and India (Table 1).

With most existing TB diagnostic tools, including sputum microscopy, radiology, and NAATs, test performance is progressively impaired in patients infected with HIV as immunodeficiency advances [4, 101]. However, data from 3 field evaluations of the commercial LAM ELISA assays show exactly the converse: sensitivity was highest in patients with the lowest CD4 cell counts [95, 99, 100] (Table 1). In ambulatory patients screened prior to antiretroviral therapy (ART) and in hospitalized TB suspects, the sensitivities of the assay were 67% and 85%, respectively, for those with CD4 cell counts <50 cells/μL [95, 99]. A high specificity was maintained in both settings. This is consistent with the fact that patients with markedly impaired anti-mycobacterial immune responses tend to have disseminated, multibacillary disease, and the likelihood of mycobacterial antigenuria is therefore increased [102, 103].

So what is the potential clinical use of the urinary LAM ELISA? It is clear that this assay is not useful for investigation of unselected TB suspects. Rather, use appears to be restricted to screening or diagnosis of TB in patients with known HIV infection and advanced immunodeficiency. This includes those enrolling in ART programs and also hospital in-patients. In these patients, the sensitivity of the LAM ELISA is considerably greater than that of sputum smear microscopy and, when the 2 tests are used in combination, there is a significant further increment in overall sensitivity [95, 99, 100]. In view of the very low bacillary burden in sputum, the average time to culture positivity for many of these patients may exceed 3 weeks, even when using automated sputum culture [95]. The LAM ELISA, which can be performed as a same day test, considerably shortens the time to TB diagnosis in those patients who test positive. This may be of real clinical importance in view of the high mortality risk from undiagnosed smear-negative TB in this patient group [104].

For this assay to be of clinical use, high specificity will be important so that it can be incorporated within a screening diagnostic algorithm as a 'rule-in' test for TB. Reasons for the variable specificity observed (Table 1) are unclear and need to be resolved in future studies in different settings. Potential reasons for lower specificity in some studies include deficiencies in the gold standard used for TB diagnosis and contamination of the urine sample with environmental mycobacteria or other bacteria. In addition, the possible contribution of NTM disease has not been adequately addressed. The clinical use of this assay would also be greatly enhanced if a simple inexpensive point-of-care version of this assay were developed. A prototype of such a simple lateral flow test to be used on unprocessed urine samples is currently undergoing evaluation [87].

In summary, a high specificity, moderate sensitivity point-of-care version of the urinary LAM test could be of great benefit in high TB burden settings when use is targeted in HIV-infected patients with advanced immunodeficiency.

STRING TEST: A NOVEL TECHNIQUE TO RETRIEVE LOWER RESPIRATORY TRACT SAMPLES FOR THE DIAGNOSIS OF TB

Obtaining sputum samples in children and HIV/TB coinfected patients can be a challenge because it is common for such patients to have a poorly productive or nonproductive cough [105, 106]. In addition, coinfected individuals, depending on the level of immune suppression, often demonstrate limited organisms in clinical samples [12, 101], challenging organism detection techniques to their limits. Thus, any improvement in quality of a clinical sample will add value to a test applied to the sample.

Over the years, several methods of obtaining quality lower respiratory tract samples for the diagnosis of TB have been explored including induced sputum [107–114], gastric aspirates [115–119], and BAL [115, 120–123]. Although BAL fluid is considered the gold standard biological sample for the diagnosis of smear-negative pulmonary TB, several studies have demonstrated that induced sputum is at least equivalent to BAL [107, 120, 121, 124, 125]. The cost, risks, invasive nature, mandatory precautions, and expertise required for bronchoscopy precludes its application in most resource-limited settings [126–128]. Sputum induction has been successfully applied in resource-limited settings under study conditions [113, 114, 124, 129]. However, it has not gained broad application because its safe use requires infection-control measures, such as air filtration, ventilation, UV radiation, and personal respiratory protection. On top of this, the large numbers of patients and demands made on personnel and time makes implementation an arduous challenge [130, 131].

In 2005, a novel method to obtain lower respiratory tract secretions (LRTS) from patients with suspected smear-negative TB was described [106]. These investigators used a device originally used to sample duodenal contents for the diagnosis of giardiasis, strongyloidiasis, and salmonellosis [132–134]. The device, a weighted gel capsule containing a coiled nylon string, commercially known as Enterotest (HEDECO), has one end of the string protruding through a hole in the capsule. The capsule is swallowed with the trailing string held at the mouth and then taped to the cheek. Peristalsis carries the weighted capsule, which later dissolves, into the duodenum while the string unravels, extending from the mouth to stomach/duodenum. The sting is left in situ for ~4 hours during which it traps sputum along its length as LRTS are carried up by the mucociliary escalator and spontaneously swallowed. Trapped LRTS are

retrieved upon withdrawal of the string [106] and processed similar to a sputum sample for AFB smear and culture.

Three studies in the literature, all from Peru, have reported on the use of this novel method of obtaining LRTS, 2 in the adult population [106, 135] and 1 in the pediatric population [105]. The first was a preliminary report on 160 HIV-infected adults suspected of tuberculosis, who underwent 2 methods of LRTS retrieval, sputum induction (default gold standard) and the string procedure. The string LRTS demonstrated positive TB cultures in 14 of 160 suspects compared with 8 positive cultures with induced sputum (McNemar's test, $P = .03$). It was concluded that the string technique was an effective, safe, and well-tolerated means for the collection of LRTS for the diagnosis of tuberculosis. Particularly noteworthy was that this method of sputum retrieval posed no appreciable risk to other patients and staff, in contrast to sputum induction [106]. Unfortunately, the study did not provide information on the number of patients that were ultimately diagnosed with TB in the face of a negative string sample culture. Furthermore, the study did not attempt to determine any clinical, immunologic, or radiologic characteristic that may be associated with a higher yield with the string sample, thus allowing one to rationalize its use.

Chow et al [105] assessed the acceptability and tolerability of the string test in 22 subjects aged 3–14 years. They demonstrated this technique to be highly acceptable and well tolerated by the majority of subjects and proposed the procedure be tested for its ability to enhance the microbiological diagnosis of tuberculosis in children. Bae et al [135] embarked on an exploratory, proof-of-principle study on 12 subjects with smear-positive disease to test whether modifications to the technique might improve its practical use for outpatient application, without affecting the quality of sample. They demonstrated that reducing the string down time from 4 hours to 1 hour did not compromise the value of the string LRTS and speculated that the down time might be shortened in smear-negative subjects—a hypothesis that requires testing.

In conclusion, this novel method to retrieve clinical samples from the lower respiratory tract can assist in the diagnosis of smear-negative TB. Although studies are limited, the string test appears to be at least as good as induced sputum [106] and, by extrapolation almost as good, if not equal to BAL [105, 120, 121, 124, 125]. The major advantages of the technique are that it is associated with a low risk to patients and does not require any special infection control measures, special equipment, or personnel training and time. Therefore, it can be easily implemented in resource-limited settings.

LYMPH NODE FINE NEEDLE ASPIRATION BIOPSY IN THE DIAGNOSIS OF PEDIATRIC TB

Pediatric tuberculosis continues to be a major cause of morbidity and mortality in resource-limited countries, where children under the age of 15 years account for ~15%–20% of the disease burden [136, 137]. In young children, TB presents a diagnostic challenge, particularly where there is coinfection with HIV. Both are chronic diseases presenting with pulmonary signs and symptoms, as well as peripheral lymphadenopathy. Sputum smear microscopy is positive in only 10%–15% of children with culture yields of ~30%–40% [138]. To complicate matters further, young children are often unable to provide a sputum specimen, and alternative specimens such as induced sputum or gastric aspirates are more difficult to collect [139]. Up to 30% of children with pulmonary TB have extrapulmonary disease, with tuberculous lymphadenitis as the commonest manifestation [140]. These nodes may be sampled as a simple outpatient procedure by fine needle aspiration biopsy (FNAB), providing diagnostic material for mycobacterial culture and sensitivity testing, cytology, as well as NAATs.

FNAB is a safe, minimally invasive procedure that does not require hospital admission and can be easily performed by healthcare professionals trained in this technique [141]. It is performed under light sedation for amnesia in children under the age of 6 years and without sedation in older children. Two needle passes are performed with a 22 or 23 gauge needle, slides are prepared for cytology, and the residual material in the needle and syringe is rinsed directly into a mycobacterial growth indicator tube (eg, MGIT, Beckton Dickinson,) or mycobacterial transport medium [142]. The transport medium is inexpensive, easy to prepare, and does not require refrigeration. The mycobacteria remain viable for up to 7 days at room temperature, making it ideal for use in peripheral clinics without laboratory infrastructure [143].

Using a combination of cytomorphology and identification of the mycobacterial organisms on Ziehl-Nielsen (ZN) stains or Papanicolaou-induced autofluorescence, a rapid and accurate diagnosis of mycobacterial infection may be made on the lymph node aspirates. Papanicolaou-induced autofluorescence of mycobacteria using light-emitting diode (LED) technology is an additional simple and inexpensive, although not specific, method of identifying mycobacteria on routine cytology smears. This low-cost LED microscopy makes it ideal for use in low-resource countries [144].

A prospective study of 200 children at Tygerberg Hospital, Cape Town, South Africa showed that in high-risk populations, FNAB using a combination of cytomorphology, autofluorescence, and ZN staining provides a rapid and definitive diagnosis of mycobacterial infection, allowing initiation of therapy pending culture and sensitivity testing [145]. A recent retrospective study in this same population showed FNAB to provide a better yield and a more rapid time to diagnosis than other respiratory specimens. This study compared FNAB to gastric aspirates, induced and expectorated sputum, and nasopharyngeal aspirates. FNAB was positive for microscopy and/or culture (bacteriologic diagnosis) in 60.8% compared with any

of the respiratory specimen in 39.2% of cases ($P < .001$). Mean time to bacteriologic diagnosis with FNAB was 7.1 days (95% confidence interval [CI], 4.1–10.1) compared with 22.4 days (95% CI, 15.8–29.1) for any respiratory specimen ($P < .001$) [139]. FNAB showed also a better yield than respiratory specimens (gastric aspirates and/or other respiratory specimens), even when all these specimens were combined.

Despite providing a rapid diagnosis of mycobacterial infection, cytomorphology and identification of the organism using ZN staining or Papanicolaou-induced autofluorescence are limited by not being specific for MTB. In addition, they do not provide information on drug susceptibility, which is increasingly important in this era of drug-resistant TB. Culture, on the other hand, may take up to 6 weeks to provide a diagnosis. NAATs combine speed with a species-specific diagnosis, and FNAB is the ideal method to obtain a pure uncontaminated sample. The results of NAATs from lymph node FNABs to date have been highly variable, as highlighted in a recent systematic review, with sensitivities reported between 2% and 100%, and specificities ranging from 28% to 100% [146]. Open tube formats such as gel electrophoresis are technically challenging, permit cross contamination, and are not ideal for use in resource-limited countries.

A method to improve polymerase chain reaction (PCR) yields of FNAB is high-resolution DNA melt (HRM) analysis. This is a simple closed tube technique that by using a fluorescent dye enables identification of specific PCR amplicons according to the temperature at which their DNA dissociates into single strands. A recent study in Cape Town, South Africa, used HRM on FNAB specimens placed in the mycobacterial transport medium noted above and achieved a sensitivity of 51.9% and a specificity of 94% [147]. This species-specific diagnosis of mycobacterial lymphadenitis permits early institution of appropriate therapy while continuing with culture in the negative cases.

In summary, FNAB can substantially improve the diagnosis of TB in children, particularly in TB-endemic countries with high HIV prevalence. It can be easily performed in resource-limited settings where improvement of pediatric TB diagnosis is most needed. As technology is advancing, the yield of FNAB will likely improve even further.

SUMMARY OF ADJUNCTIVE METHODS FOR THE DIAGNOSIS OF TB

To the extent that TB manifestations vary according to the host immune status, so too TB diagnostic tests have differential performance characteristics. Although not able to stand alone and unlikely to replace any of the current diagnostic gold standards for TB, the alternative methods discussed here have adjunctive value in the context of difficult-to-diagnose TB. Such adjunctive tests could be especially useful in HIV-infected persons and children, who are disproportionably affected by smear-negative pulmonary as well as extrapulmonary TB. One or a combination of these tests is likely to enhance the TB case detection rate in these patient groups, particularly in resource-limited settings. Whether the broad use of such alternative methods can ultimately improve outcome in certain populations remains to be determined in randomized clinical trials.

Notes

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