

# Tuberculosis in Antiretroviral Treatment Services in Resource-Limited Settings: Addressing the Challenges of Screening and Diagnosis

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**The high burden of tuberculosis (TB) among patients accessing antiretroviral treatment (ART) services in resource-limited settings is a major cause of morbidity and mortality and is associated with nosocomial transmission risk. These risks are greatly compounded by multidrug-resistant disease. Screening and diagnosis of TB in this clinical setting is difficult. However, progress has been made in defining a high-sensitivity, standardized symptom screening tool that assesses a combination of symptoms, rather than relying on report of cough alone. Moreover, newly emerging diagnostic tools show great promise in providing more rapid diagnosis of TB, which is predominantly sputum smear-negative. These include culture-based systems, simplified versions of nucleic acid amplification tests (such as the Xpert MTB/RIF assay), and detection of lipoarabinomannan antigen in urine. In addition, new molecular diagnostics now permit rapid detection of drug resistance. Further development and implementation of these tools is vital to permit rapid and effective screening for TB in ART services, which is an essential component of patient care.**

The geographical intersection of the HIV pandemic with the existing global tuberculosis (TB) epidemic has led to high rates of HIV-associated TB and related mortality over the past 25 years [1]. During 2008, there were an estimated 1.4 million incident cases of HIV-associated TB worldwide, which accounted for 25% (0.5 million) of global deaths from HIV infection and AIDS [2]. Sub-Saharan Africa bears the brunt of this epidemic, accounting for 4 of 5 cases of HIV-associated TB worldwide [2]. TB incidence rates have increased 3–5-fold in many African countries with a high prevalence of HIV infection, and in the worst affected countries of South

Africa and Swaziland, ~1% of the national population develops TB each year [2]. The region with the second highest burden is southern and Southeast Asia, with 13% of the global caseload.

Extraordinary progress has been made in scaling up access to antiretroviral therapy (ART) in low- and middle-income countries (reaching 5.3 million persons by the end of 2009) [3]. A majority of these persons (3.9 million) were in sub-Saharan Africa. Scale-up of ART in settings with a high burden of TB is associated with a number of opportunities and challenges. ART is a crucial component of case management of HIV-associated TB [4], reducing mortality risk by 64%–95% [5] and halving recurrence rates [6]. In addition, ART has an important role in the prevention of HIV-associated TB, reducing risk in treated cohorts by a mean of 67% (95% confidence interval [CI], 61%–73%) [7]. Furthermore, aggressive ART scale-up could potentially play a key role in the control of this epidemic [8].

However, high rates of TB also present many challenges for ART services: TB is a major cause of morbidity and mortality, concurrent TB treatment and ART is complex [9, 10], and TB is associated with a substantial

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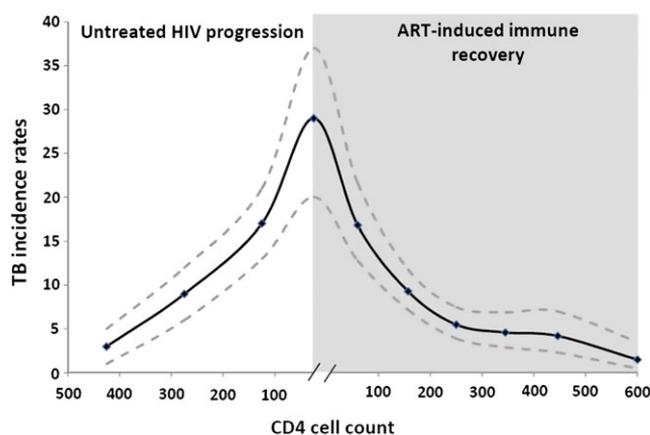
risk of nosocomial transmission [11]. These challenges are amplified greatly by the major difficulties of screening and diagnosing TB in this group of patients. This may, in part, explain why only 4.1% of patients living with HIV infection worldwide were estimated to have been screened for TB during 2008 [2].

In this article, we describe the burden and impact of TB on adults accessing ART programs in resource-constrained settings. We highlight the importance of effective TB screening and reliable diagnosis and review the range of conventional and novel TB diagnostic tools available for use in this context. A majority of the data reported are from sub-Saharan Africa. However, the principles outlined are relevant to other resource-constrained settings.

## HIV PROGRESSION AND TB RISK

HIV infection has a critical impact on the host cell-mediated response to *Mycobacterium tuberculosis* [12]. The risk of TB increases 2–3-fold within the first 2 years of HIV seroconversion [13] and continues to increase as CD4 cell counts decrease (Figure 1) [14, 16]. HIV-infected individuals with *M. tuberculosis* infection have a mean annual risk of developing TB of ~10% per year [17]; however, this risk is highly dependent on the degree of immunodeficiency, the prevailing socioeconomic conditions, and ongoing risk of TB exposure. Rates of HIV-associated TB approach 30% per year among persons with the lowest CD4 cell counts who live in Cape Town, South Africa (Figure 1) [14].

Reported TB incidence rates among patients with advanced HIV-associated immunodeficiency, however, belie a conceptual difficulty with regard to the definition of TB disease. The traditional concept of HIV-infected patients being classified as



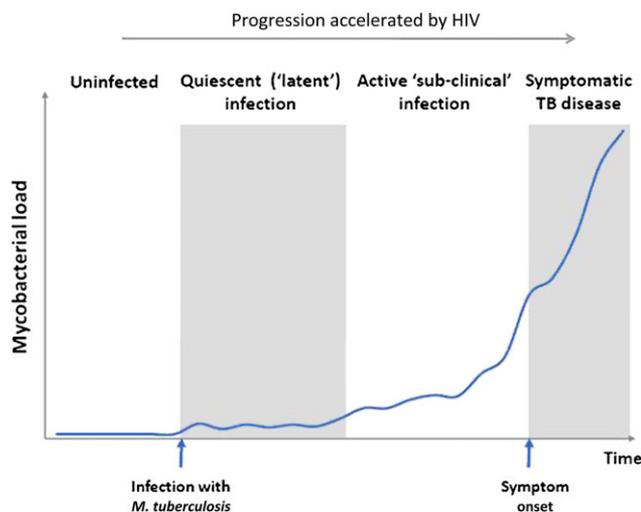
**Figure 1.** Tuberculosis (TB) incidence rates (cases/100 person-years and 95% confidence bands) and CD4 cell counts (cells/ $\mu$ L) in a South African cohort of HIV-infected patients before availability of antiretroviral therapy (ART; unshaded area) and during ART-induced immune recovery (shaded area). The shape of the curve at the time of ART initiation depends on the intensity of TB screening before ART initiation. Figure adapted from Lawn et al (2010) [8], and data originally adapted from Holmes et al (2006) [14] and Lawn et al (2009) [15].

either having active TB or not having active TB has been questioned [18], and the existing paradigm that distinguishes latent TB infection from active TB disease as distinct binary states is thought to be overly simplistic. Our understanding of the host-pathogen dynamics of *M. tuberculosis* infection has fundamentally altered in recent years. It is now thought to be likely that a spectrum of states exists, with different degrees of immune control and mycobacterial load [19, 20] and that HIV infection profoundly shifts this spectrum in favor of bacillary replication (Figure 2) [18].

This concept has important consequences for our understanding of the burden of TB in ART programs. Because this patient population has advanced immunodeficiency, many patients will have prevalent symptomatic TB disease and many others may have subclinical, actively replicating infection that may fall near the limits of detection of TB diagnostic assays.

## CLINICO-PATHOLOGICAL CHARACTERISTICS OF HIV-ASSOCIATED TB

The challenge of TB diagnosis in patients in ART programs relates to the blurring of active disease and latent infection and to the impact of HIV on the clinico-pathological features of TB. The features of TB in HIV-1-infected individuals with well-preserved CD4 cell counts are similar to those of individuals with TB but no HIV-1 coinfection. Progressive immunodeficiency, however, is associated with an increasing frequency of miliary and



**Figure 2.** Total bacillary load of *Mycobacterium tuberculosis* in individuals showing progression from infection to development of symptomatic tuberculosis (TB) disease. After infection, patients may retain good immune control of the infection and low bacillary numbers (quiescent or latent infection). However, subsequent loss of immune control is associated with increasing bacillary load that initially remains as subclinical active infection. At some stage, symptoms develop, leading to presentation of active symptomatic disease. HIV infection greatly accelerates the progression toward active infection and disease. Figure adapted from Lawn et al (2010) [18].

disseminated forms of disease [21, 22], and occult disseminated TB has also been a frequent finding in postmortem studies involving persons who died with HIV infection or AIDS in sub-Saharan Africa [23, 24]. Evidence of the impaired tissue inflammatory response to infection is seen in the radiographic appearances of pulmonary TB, with reduced consolidation, fibrosis, and cavitation [25, 26]. Lack of cavitation in turn results in low numbers of bacilli in sputum specimens; thus, results of sputum smear microscopy for acid-fast bacilli are frequently negative [22, 27].

## **BURDEN OF TB IN ART PROGRAMS**

### **TB Referrals to ART Programs**

HIV-infected patients typically present to health services in resource-limited settings with advanced immunodeficiency and opportunistic infections [28], with TB being one of the most common presentations [29]. The proportion of patients with TB who undergo HIV testing has increased substantially in recent years [2], being catalyzed by implementation of the provider-initiated HIV testing and counseling strategy in TB programs. As a result, the proportion of patients referred to ART programs who have a known TB diagnosis has increased substantially in some settings; for example, this proportion increased from 16% to 35% of referrals to a South African ART service over a 6-year period [30].

### **Prevalent Undiagnosed TB at Baseline**

In addition to patients with known TB, there is a large burden of undiagnosed TB in other patients. The proportion detected is likely to vary greatly depending on the prevailing TB burden, the degree of immunodeficiency, and the rigor with which patients are screened. The prevalence of TB is established most accurately when all patients are screened for TB regardless of the presence or absence of symptoms and when high-sensitivity investigations, such as automated liquid culture, are used [31, 32]. In 2 studies involving South African cohorts, patients had median CD4 cell counts of  $\sim 100$  cells/ $\mu\text{L}$ , and pulmonary TB was diagnosed in 19% [33] and 25% [34] of patients when sputum samples from all patients were examined using automated liquid culture.

Data from these 2 South African studies are likely to represent the upper end of the range of TB prevalence, but similar studies in ART programs in countries with less severe TB epidemics are lacking. However, patients attending HIV clinics or voluntary counseling and testing services in Southeast Asia have been reported to have a TB prevalence of 6%–15% [35–37]. A systematic review of the yield of TB screening in HIV-infected patients attending ART and medical clinics in resource-limited settings found a median prevalence of 8.2% (range, 1%–25%), detected using a wide range of screening strategies [31]. The yield was strongly associated with TB prevalence in the country and the screening strategy.

### **Incident TB During ART**

TB risk persists during ART. Incidence rates are particularly high during the first few months of ART, although this is strongly dependent on the intensity of screening at baseline [38]. A proportion of patients with TB at baseline are either asymptomatic or have minimal symptoms and are not clinically recognized until immune recovery triggers the development of symptoms during early ART (so-called “unmasking TB”) [39]. Under routine program conditions in a Cape Town ART service, it was estimated that  $\sim 40\%$  of TB cases presenting during the first 4 months of ART were caused by unmasking TB [15]. Consistent with this finding, implementation of an intensive pretreatment screening strategy using liquid culture of induced sputum samples approximately halved the TB incidence rate during this period [38].

TB incidence rates diminish rapidly with ongoing immune recovery during ART. Data from cohort studies in both high-income and resource-limited settings report TB risk reductions of 54%–92% in adjusted analyses [5] and a pooled summary estimate of a 67% reduction (95% CI, 61%–73%) [7]. The reduction in TB incidence rates is time-dependent, with ongoing reductions during the first 2–3 years of treatment [29, 40–43], reflecting the rate of ART-induced immune recovery [15]. TB risk is strongly associated with the current CD4 cell count; an increase in CD4 cell count from  $< 100$  cells/ $\mu\text{L}$  to  $> 500$  cells/ $\mu\text{L}$  is associated with a 10-fold reduction in TB risk [15] (Figure 1). Data are lacking on how TB prevalence changes over time during ART, although it is likely that the highest yield of new cases would be detected by screening persons with poor CD4 cell count recovery.

## **POTENTIAL BENEFITS OF TB SCREENING AND DIAGNOSIS**

There is a very strong rationale for screening and rapid TB diagnosis among patients accessing ART services (Table 1). Efficient baseline screening is associated with a substantial reduction in the risk of incident TB and associated morbidity during the initial months of ART [38]. TB is the most common reported cause of death in ART programs and frequently remains undiagnosed [28]. Postmortem data are few; however, in a series of 25 patients receiving ART who died after hospital admission in Johannesburg, South Africa, disseminated mycobacterial disease was found in 19 [44]. Similarly, in another South African hospital, approximately half the deaths among HIV-infected adults were associated with culture-confirmed TB [45]. Studies are needed to determine whether rapid screening and diagnosis of TB in this patient group improves survival. In the absence of effective diagnostic tools, an alternative approach may be to provide empirical TB treatment to persons at highest risk of TB and associated mortality [46]. Studies exploring such a strategy are needed, and one is being planned by the AIDS

**Table 1. Summary of Potential Benefits of Baseline Screening and Rapid Diagnosis of Tuberculosis (TB) Among Patients Entering Antiretroviral Treatment (ART) Services**

Potential benefit	Reference
Reduction in morbidity	[38]
Reduction in mortality	[28, 44]
Reduced hazard of nosocomial TB transmission	[11]
Reduced risk of unmasking TB immune reconstitution disease	[38, 39]
Facilitation of more efficient clinical decision making, reducing delays in ART initiation	...
Reliable exclusion of active TB may permit use of isoniazid preventive therapy	[7]

Clinical Trials Group (ACTG5274 REMEMBER trial; Mina Hosseinipour, personal communication).

TB in patients accessing ART services presents a serious infection-control hazard [11]. This hazard is likely to be highest among newly enrolling ART-naive patients who have a high prevalence of undiagnosed disease and who often spend considerable time with groups of peers preparing for treatment in over-crowded facilities. Multidrug-resistant (MDR) TB presents a particularly important threat [11, 47]. Nosocomial transmission was thought to be a key factor in the 2006 outbreak of extensively drug-resistant TB (XDR-TB) among patients accessing ART from a district hospital in rural KwaZulu Natal, South Africa [48]. Screening and rapid diagnosis leading to early commencement of TB treatment are central to reducing the period of infectiousness and transmission risk.

In a small minority of patients, the clinical presentation of unmasking TB during the initial months of ART may be severe, with immune reconstitution disease causing considerable immunopathology and clinical compromise [39, 49, 50]. In such cases, effective screening and early TB diagnosis and initiation of TB treatment may diminish the risk of these severe forms of disease.

World Health Organization (WHO) guidelines recommend that TB should be diagnosed and treated before initiation of ART [51]. Difficulties in diagnosing TB may therefore contribute to delays in initiation of ART, and this may be associated with considerable mortality risk [52, 53]. Thus, rapid and effective TB screening and diagnostic strategies are needed to permit efficient commencement of TB treatment and ART. Similarly, implementation of isoniazid preventive therapy in patients with advanced immunodeficiency has been greatly undermined by the difficulties of reliably excluding active TB. More reliable screening methods may facilitate greater uptake in this patient group in whom there is some evidence of an additive preventive effect when combined with ART [7].

## SCREENING AND DIAGNOSTIC METHODS

### Symptom Screening

The WHO previously recommended a cough duration of 2–3 weeks as a symptom screen for TB [51, 54]. This is now recognized as inadequate for HIV-associated TB, with the sensitivity

frequently being found to be <50% in this patient group [32, 52, 53]. Screening tools that combine multiple symptoms have much higher sensitivity, albeit with low specificity. A meta-analysis of ~10 000 HIV-infected patients actively screened for TB was conducted to identify an optimum symptom screening algorithm [55]. The optimum algorithm identified patients with at least 1 of 4 common symptoms (current cough, night sweats, weight loss, or fever) with a sensitivity of 79% and a specificity of 50%. These values are very similar to those found in TB screening studies in 2 ART services in South Africa [33, 34]. This new screening tool has been included in the WHO 2010 guidelines on intensified case finding and isoniazid preventive therapy [56] and represents an important step toward more standardized and effective screening. However, in ART programs in which the prevalence of TB is as high as 20%–25%, there is a strong argument for microbiological screening of all patients regardless of the presence or absence of symptoms [31, 33, 34]. However, the overall clinical benefits and associated costs are as yet unknown.

### Sputum Smear Microscopy

TB diagnosis in resource-limited settings remains heavily reliant on smear microscopy using direct Ziehl–Neelsen staining of sputum. Although this method is highly specific, fast, and relatively inexpensive, it is operator dependent and its use is greatly impaired in the context of HIV infection. A concentration of ~10 000 bacilli per mL of sputum is required for a smear result to be positive; the higher the bacillary concentration is above this threshold, the greater the likelihood of positivity. However, lack of pulmonary cavitation and the resulting low bacillary concentrations in sputum means that sputum microscopy results are usually negative in more than half of patients with HIV-associated TB [27, 32]. Advancing immunodeficiency is associated with increased likelihood of negative smear results; in TB screening studies in South African ART cohorts, >80% of culture-proven TB cases were sputum smear–negative [33, 34].

Although newer technologies are clearly needed, efforts have been made to improve the performance of smear microscopy. In a meta-analysis, sputum processing with bleach or sodium hydroxide and centrifugation was associated with a mean increase in the sensitivity of smear microscopy of 13% [57]. Fluorescence microscopy also increases sensitivity by 10% and retains

specificity similar to that of conventional Ziehl–Neelsen staining [58]. This technology permits more efficient reading of slides, which is critical to improving laboratory performance. Although traditional fluorescence microscopes are expensive, cheaper fluorescence microscopes with light-emitting diodes are equally sensitive and were endorsed by the WHO in 2009 [59]. In addition, the WHO made policy changes in 2007 to permit TB diagnosis to be based on 1 rather than 2 positive sputum smear results [51]. The number of sputum specimens needed to be examined before a diagnosis of smear-negative TB could be made was also reduced from 3 to 2.

### **Chest Radiography**

The pulmonary radiographic features of TB are markedly affected by HIV coinfection and progressive immunodeficiency [25, 26]. In addition, the high cost, limited availability in many settings, and difficulties in interpretation by health care workers substantially undermine the usefulness of radiography in TB diagnosis in patients accessing ART services. In a study involving such patients in Cape Town, the sensitivity of any radiographic abnormalities consistent with TB was just 68% and the specificity was 53% [60]. Thus, one-third of patients found to have sputum culture–positive TB by active screening had completely normal chest radiograph findings.

However, chest radiology has an important role in diagnosis of other HIV-related pulmonary diseases, which are also common in this patient group. When used in combination with symptom screening algorithms to rule out TB, the addition of radiography may increase sensitivity further and, therefore, may have use in screening before use of isoniazid preventive therapy [55].

### **Sputum Culture**

Automated liquid culture systems are the gold standard for TB diagnosis and are of great usefulness in this patient population in whom most TB is sputum smear–negative. Liquid culture is considerably more rapid and has a greater yield than does culture using solid media. In 2007, these assays were recommended by the WHO to be used in combination with antigen-based species confirmation for TB diagnosis and for drug-susceptibility testing in low- and middle-income countries [61]. However, these culture systems are expensive and prone to contamination. Despite its invaluable role observed in TB diagnosis among patients accessing ART services [33, 34], culture-based diagnosis is unavailable in most resource-limited settings. Thus, alternative inexpensive, noncommercial culture and drug-susceptibility testing methods were also endorsed by the WHO in 2009 for use as an interim solution in resource-constrained settings [59]. These include microscopically observed drug susceptibility (MODS) and the nitrate reductase assay.

The sensitivity of culture when screening patients for pulmonary TB before ART depends substantially on the number of sputum samples analyzed. In a study using automated liquid

culture of sputum samples from patients starting ART in Cape Town, 2 samples were collected [34]. The incremental yield of the second sample was 22% [62], which is similar to the 17% incremental yield observed a study screening for HIV-associated TB in Southeast Asia [36]. In the latter study, a third sputum culture identified 10% of the overall yield of pulmonary TB cases, indicating that a large majority of cases could be diagnosed on the basis of culture of 2 samples.

The time to culture positivity is prolonged in this patient population, reflecting low concentrations of mycobacteria in sputum. Even with use of automated liquid culture, the mean time to positivity was >3 weeks in a study in Cape Town [34]. Such delays may contribute to morbidity, mortality, nosocomial TB transmission, and delayed decision-making regarding ART initiation. The time to positivity of the MODS assay appears to be less dependent on sputum smear status, with the median time to positivity for sputum smear–negative sputum samples reported to be 7 days, compared with 6 days in sputum smear–positive samples [63]. The usefulness of MODS should be evaluated in this patient population.

### **Testing Nonpulmonary Clinical Samples**

A comprehensive evaluation of the additional value of extrapulmonary samples was made in a study in Southeast Asia in which patients (median CD4 cell count, 281 cells/μL) attending HIV clinics were intensively screened for TB, with collection of 3 sputum samples together with stool, urine, blood culture, and (when possible) lymph node aspirates [36]. TB was diagnosed in 14% of patients overall; 86% of these diagnoses were made using sputum samples, and the additional yield of 14% of diagnoses were made using culture of nonpulmonary samples. The greatest yield from nonpulmonary samples was from lymph node aspirate samples, which provided an incremental yield of 6% when used in addition to liquid culture of 2 sputum samples [36].

### **Antigen Detection**

Although current commercially available serological tests for TB are of little diagnostic value [64], mycobacterial antigen detection is theoretically more attractive, overcoming many of the limitations inherent in immune-based assays. A simple, commercially available assay is able to detect lipoarabinomannan (LAM) excreted in the urine of patients with TB. Although the sensitivity has been disappointing in HIV-uninfected patients [65], moderate sensitivity and high specificity has been observed in HIV-infected patients in South Africa [34, 66, 67].

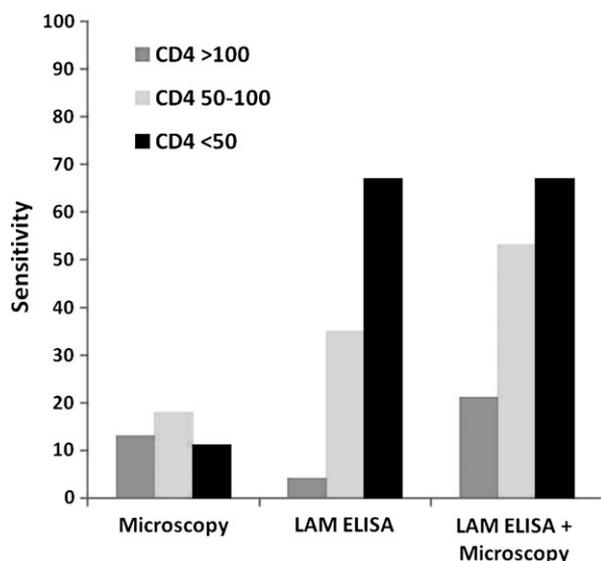
In each of these studies [34, 66, 67], the sensitivity of the LAM enzyme-linked immunosorbent assay (ELISA) exceeded that of sputum smear microscopy, and there was an incremental yield when these tests were used in combination. The sensitivity of the LAM ELISA was highest among patients with the lowest CD4 cell counts (Figure 3). In ambulatory patients screened before ART and in hospitalized HIV-infected patients with suspected

TB who had CD4 cell counts <50 cells/ $\mu$ L, the sensitivities of the assay were 67% and 85%, respectively [34, 64]. Very high specificity was observed in both studies. Greater sensitivity at lower CD4 cell counts is consistent with the fact that patients with markedly impaired antimycobacterial immune responses tend to have disseminated, multibacillary disease, and thus, the likelihood of mycobacterial antigenuria is likely to be increased.

A simplified lateral flow version of this assay (dip-stick) is currently being evaluated [65]. If the sensitivity and specificity of this is at least comparable to that of ELISA, it could be used as a simple and cheap point-of-care test incorporated in a diagnostic screening algorithm for outpatients accessing ART or for HIV-infected inpatients. This would reduce the mean time to TB diagnosis by  $\sim$ 3 weeks in approximately half the patients with TB who had CD4 cell counts <100 cells/ $\mu$ L in a South African ART program [34].

### Nucleic Acid Amplification Tests (NAATs)

NAATs represent the most promising development for rapid diagnosis of TB and rapid drug-susceptibility testing [68]. However, their technical complexity has hindered widespread implementation in resource-limited settings. Moreover, their usefulness when applied directly to smear-negative sputum samples has been limited to date. However, considerable progress has been made in developing simplified versions with higher sensitivity for smear-negative disease. A manual NAAT using loop-mediated isothermal amplification with a simple visual



**Figure 3.** Sensitivity of a commercially available enzyme-linked immunosorbent assay (ELISA) to detect lipoarabinomannan (LAM) in urine samples for diagnosis of tuberculosis (TB) in a cohort of patients accessing antiretroviral treatment (ART) in a South African township. The sensitivity of sputum microscopy and the LAM ELISA are shown individually and combined (either positive), compared with a gold standard of automated liquid culture of 2 sputum samples. Data are stratified by CD4 cell count. Data are from Lawn et al (2009) [34].

colorimetric read-out showed high specificity but only moderate sensitivity for smear-negative culture-positive disease [69]. A revised prototype is undergoing further evaluation in peripheral laboratory facilities in resource-constrained settings.

The most important development to date is a sensitive and specific fully automated and commercially available NAAT assay, which has been developed for use outside reference laboratory centers [70, 71]. The Xpert MTB/RIF assay (Cepheid) uses a series of molecular beacons and real-time polymerase chain reaction technology to detect *M. tuberculosis* and the *rpoB* rifampicin resistance mutation. The cartridge-based system dispenses with the need for prior sputum processing and requires minimal laboratory expertise; furthermore, results are available <2 hours, permitting a specific TB diagnosis and rapid detection of rifampicin resistance. A large multicountry evaluation found excellent performance characteristics, including sensitivities of 72.5%, 85.1%, and 90.2% for sputum smear-negative disease when processing 1, 2, or 3 sputum specimens, respectively [71].

However, when used to screen for TB in patients starting ART, the Xpert MTB/RIF assay may be stretched to its limits of detection [62]. Laboratory evaluations have shown a limit of detection (as defined by 95% sensitivity) of 131 bacilli per mL of sputum [70], whereas automated liquid culture systems have a sensitivity range of 10–100 organisms per mL. It will be important to assess the sensitivity of the Xpert MTB/RIF assay among patients commencing ART in whom bacillary numbers in sputum are often very low. Multiple samples may be needed to achieve adequate sensitivity, which will increase costs. However, the simplicity of use of this technology means that the machines could be located at ART clinics and used by health care personnel after minimal training. Such field evaluations of this technology are ongoing.

### Interferon- $\gamma$ Release Assays

Interferon- $\gamma$  release assays detect interferon- $\gamma$  production from peripheral blood mononuclear cells after in vitro stimulation with antigens, such as ESAT-6 and CFP-10 (immunodominant antigens expressed by members of the *M. tuberculosis* complex) [72]. These assays have a sensitivity of 80%–90% among patients with confirmed TB but are unable to distinguish between active and latent disease, resulting in very poor specificity as a TB diagnostic in populations in settings with a high TB burden where prevalence of latent infection is high. These assays therefore have no role for TB diagnosis in resource-limited settings.

### Rapid Detection of Drug-Resistant TB

The global emergence of the MDR-TB epidemic provides a particular threat to patients in ART services [11], as evidenced by the devastating outbreak of XDR-TB in rural KwaZulu Natal, South Africa, during 2005–2006 [48]. The outbreak was believed to have been caused by nosocomial transmission in patients

accessing ART at a district hospital. Expanded capacity for TB screening and rapid phenotypic or genotypic resistance testing is urgently needed.

To enhance capacity for rapid diagnosis of MDR-TB, in 2008, the WHO approved use of line probe assays for the rapid molecular detection of drug resistance in smear-positive specimens or culture isolates [73, 74]. Two commercial line probe assays have shown high accuracy when applied to culture isolates, and one of these, the GenoType MTBDR*plus* assay (Hain Lifescience GmbH), has also shown very good performance characteristics when applied directly to smear-positive sputum specimens [75, 76].

In 2009, the GenoType MTBDR*sl* assay (Hain Lifescience GmbH) became available; it is also able to detect resistance to fluoroquinolones, aminoglycosides, and ethambutol in culture isolates or smear-positive sputum specimens [77]. When used together with the GenoType MTBDR*plus* assay (which detects resistance to rifampicin and isoniazid), the combined results potentially provide a means of rapid detection of XDR-TB. Use of such molecular assays reduces the time to diagnosis of MDR-TB and XDR-TB from weeks or months to days. However, line probe assays are technically demanding and require complex infrastructure and highly trained staff; thus, their use is limited to centralized laboratories. Their impact on patient outcomes has yet to be demonstrated. Simplified automated NAATs, such as the Xpert MTB/RIF assay, may provide a simpler means for rapid detection of drug resistance.

## CONCLUSIONS

TB represents a huge challenge to ART services in resource-limited settings, and this requires the implementation of routine, systematic, and effective screening. Progress has been made in developing a better screening algorithm, and there is a developmental pipeline of very promising new diagnostic technologies that require evaluation in this specific patient population. Newer culture-based systems, simplified NAATs, and urinary antigen detection may each play an important role, depending on the setting, local laboratory capacity, and human resources available. Implementation of these new tools is vital to permit rapid and effective screening for TB in ART services, which must be regarded as an essential component of patient care.

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