# Diagnosis of HIV-Associated Tuberculosis


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**Abstract** Of the estimated 1.2 million tuberculosis (TB) cases among people living with HIV (PLHIV), less than half are diagnosed and reported to health authorities. This is a key reason why TB remains the leading cause of death among PLHIV. Systematic screening approaches coupled with improved diagnostics are critical to reducing the gap and have begun to emerge over recent years. This chapter reviews current approaches to screening for and diagnosing HIV-associated TB, including drug-resistant TB, in adults. The chapter is organized into three parts: Part I provides an overview of World Health Organization (WHO)-recommended tools to facilitate TB screening and diagnosis among PLHIV, Part II provides a selective overview of tools and tests currently in the later stages of the TB diagnostic pipeline and Part III provides a clinically-oriented, step-wise approach for diagnosing TB in PLHIV in resource-limited settings.

**Keywords** HIV · Tuberculosis · Screening · Diagnosis · Point-of-care · Drug susceptibility testing · Microscopy · Culture · Xpert · LAM

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## Introduction

Of the estimated 1.2 million tuberculosis (TB) cases among people living with HIV (PLHIV), less than half are diagnosed and reported to health authorities [1]; this is a key factor that contributes to why TB remains the leading cause of death among PLHIV. Better diagnostics are critical to reducing the gap and, after more than 150 years, smear microscopy is finally starting to be eclipsed as the primary diagnostic method for TB diagnosis in high burden countries. Since 2010, the World Health Organization (WHO) has endorsed several new diagnostic tools including (1) Xpert MTB/RIF, a semi-automated molecular assay that has higher sensitivity than smear microscopy and can identify rifampin resistance; (2) Determine TB-LAM, a lateral flow assay that can detect lipoarabinomannan (LAM) in urine of the sickest HIV/AIDS patients in less than 30 min at the bedside [2, 3]; and (3) line probe assays (LPAs) that rapidly identify mutations conferring resistance to first and second line anti-TB drugs in reference laboratories [4]. The devastating toll of TB on PLHIV has also led to guidelines emphasizing the need for systematic screening rather than reliance on passive case detection alone.

This chapter will review current approaches to screening and diagnosis of HIV-associated TB, including drug-resistant TB, in adults. The chapter is organized into three parts: **Part I** provides an overview of WHO-recommended tools to facilitate screening for and diagnosis of HIV-associated TB, **Part II** provides a selective overview of tools and tests currently in the later stages of the TB diagnostic pipeline and **Part III** provides a clinically-oriented, step-wise approach for diagnosing TB in PLHIV in resource-limited settings. Of note, the diagnosis of latent tuberculosis infection (LTBI) is covered separately in the chapter "Recent Advances in the Treatment of Latent Tuberculosis Infection Among Adults Living with HIV Infection", the diagnosis of TB immune reconstitution inflammatory syndrome (IRIS) is covered in the chapter "The Tuberculosis-Associated Immune Reconstitution Inflammatory Syndrome (TB-IRIS)" and the diagnosis of pediatric TB disease is covered in the chapter "HIV and Tuberculosis in Children".

## Part I: Overview of Screening Tools and Diagnostic Tests for HIV-Associated TB

### *Types of Available Tests for HIV-Associated TB and Desired Characteristics*

Tools for identifying patients with HIV-associated TB can be broadly organized into one of two categories: screening (typically non-microbiological assays) and diagnostic (typically microbiological assays) tools.

Screening tools are ideally simple, low-cost and can be used at the point-of-care to differentiate between people living with HIV (PLHIV) with a low probability of hav-

ing active TB who can be safely started on TB preventative therapy and PLHIV with an increased likelihood of having active TB who should undergo further microbiological testing. A positive result, however, does not provide confirmation of TB disease. The WHO has proposed that a screening tool/test for TB should be at least 90% sensitive (to make it very unlikely that those screening negative have TB and can therefore safely start TB preventative therapy) and at least 70% specific (to reduce the number of unnecessary confirmatory tests by limiting false-positive results) [5].

Microbiological tests directly detect the presence of *Mycobacterium tuberculosis* (MTB) in a clinical specimen, providing confirmation of a TB diagnosis in the correct clinical setting. An ideal microbiological test would be rapid, inexpensive, have minimal infrastructure requirements and be available for use (and provide results) at the point-of-care [5]. Traditionally, microbiological assays for TB have included acid fast bacilli (AFB) smear microscopy and culture-based methods. However, rapid tests based on molecular methods (Xpert and Xpert Ultra) or detection of TB antigens (lipoarabinomannan) have emerged from the pipeline. The WHO has proposed that new diagnostic tests for TB should have excellent specificity (>98%) to minimize false-positive results and that the sensitivity should be >80% [5].

Below we outline and discuss WHO-recommended screening and diagnostic tools for TB, highlighting their performance among PLHIV. A discussion of tests available for monitoring response to TB therapy is beyond the scope of this chapter.

## *Tools and Tests for TB Screening*

### Symptom-Based Screening Rules

Because of the non-specific symptoms of TB in PLHIV, often including an absence of cough, many HIV-associated TB diagnoses are missed. Standardized, symptom-based screening can help maximize case detection. In 2011, a meta-analysis evaluating different symptom screening rules for HIV-associated TB found that the presence of any one of four symptoms—cough, night sweats, fevers, or weight loss (of any duration)—had a sensitivity of ~79% and specificity of ~50%. This corresponded to a negative predictive value of >90% when TB prevalence ranged from 5% to 20%. On the basis of this study, in 2011, the WHO recommended screening all PLHIV for TB using this screening rule at every clinical encounter, regardless of reason for presentation [6]. PLHIV who screen positive should undergo further microbiological testing, ideally with sputum Xpert, while those testing negative should be evaluated for initiation of TB preventative therapy [7]. More recently, a meta-analysis found that the symptom screen was associated with poor sensitivity among PLHIV receiving antiretroviral therapy (ART) (51%) compared to those who were ART-naive (89%) [8]. It also found that specificity among ART-naive patients was only 28%. These data highlight the urgent need for improved TB screening tools.

The clinical application of this screening rule within a TB diagnosis algorithm as well as its limitations are further described in **Part III**, step 2.

## *Radiologic Screening Tools*

### Chest X-Ray

Chest X-ray has long been a mainstay of TB diagnostic algorithms. There is no single chest radiographic pattern that is pathognomonic for TB, especially in PLHIV where significant variation in radiographic patterns across CD4 strata are observed. This is because more advanced immunodeficiency is associated with an impaired local tissue inflammatory response and results in reduced consolidation, fibrosis and cavitation [9]. PLHIV with a greater degree of immunosuppression are more likely to demonstrate a lower lobe and miliary pattern; however, those on ART and with well-controlled disease may manifest more typical patterns (as seen in HIV-negative persons), such as upper lobe infiltrates with or without cavitation. The diagnostic performance of chest X-ray for detecting HIV-associated TB is dependent on the definition applied to determine an 'abnormal chest X-ray' as well as the average CD4 count of the population in which a study is being conducted. It is well-recognized that those with pulmonary TB (PTB) may have completely normal chest imaging (up to 30%) [10–13]. Thus, a normal chest-X-ray does not exclude the diagnosis of active TB disease. Chest X-rays are non-specific as a patient may have alternative lung pathology accounting for radiographic lesions and they are also subject to both intra- and inter-reader variability. Radiographic findings in PLHIV with TB are discussed in greater depth in the chapter "Clinical Manifestations of HIV-Associated Tuberculosis in Adults".

In PLHIV, chest-X-rays may be complementary to symptom-based screening and serve as an important screening tool for active TB disease. Notably, a meta-analysis found that among patients receiving ART, the addition of chest radiography to the WHO symptom screen increased sensitivity for active TB from 52% (95% CI 38–66) to 85% (95% CI 70–93) [8]; because this results in a substantial improvement in the negative predictive value, TB preventive therapy can be initiated with greater confidence in such patients. Additionally, chest X-rays may provide rapid clues towards a diagnosis in those in whom TB is suspected and Xpert testing (or sputum microscopy) is negative, unavailable or result turnaround time may delay initiation of possibly life-saving therapy (i.e., severely ill patients) [7]. The use of chest X-rays within the TB diagnosis algorithm is discussed in **Part III**, steps 2 and 3.

There have been several recent advances in chest radiography. Digital chest X-rays are now available that may be associated with lower radiation doses, more immediate results without the requirement for film, improved image quality, while also allowing for the transmission and storage of images. They are also associated with lower operational costs when compared to film-based X-rays [14]; however, substantial upfront costs have limited their uptake. Furthermore, there are now portable digital X-ray machines that can allow the technology to be decentralized and integrated into mobile screening units/programs. Computer-aided algorithms have been developed to systematically read digital chest X-rays and detect abnormalities

that may be compatible with PTB. A systematic review found that, while available evidence was limited, new computer-aided algorithms are likely as good as novice readers and likely approach the diagnostic accuracy of expert radiologists [15]. HIV prevalence among patients included in the meta-analysis ranged from 33% to 68%, however only one study explicitly reported the sensitivity and specificity of a computer-aided diagnosis (CAD) program for scoring chest X-rays in PLHIV [16]. Among 57 PLHIV in Zambia with Xpert-confirmed pulmonary TB, a CAD score >60 was associated with a sensitivity of 100%, but specificity was only 18% [16]. Automated, computer-aided algorithms are not recommended by the WHO at this time due to insufficient evidence [14], but are due to be formally evaluated by the WHO in the near future.

#### Ultrasound for Extra-Pulmonary TB (EPTB)

Ultrasonography is available as a portable, hand-held device with a number of clinical applications. It can rapidly identify abnormal signs that in high incidence settings may suggest EPTB. There is a standardized protocol for the assessment of HIV-associated TB called FASH (focused assessment with sonography for HIV-associated TB). FASH includes two different types of assessments [17]. The FASH basic assessment attempts to identify the presence of a pericardial effusion (possible pericardial TB), a pleural effusion (possible pleural TB) or ascites (possible abdominal TB). The FASH-plus examine requires greater skill and user experience, but looks for the presence of periportal/para-aortic lymphadenopathy (possible abdominal TB), focal liver lesions (possible liver abscesses due to TB) and focal splenic lesions (possible splenic abscesses due to TB). Several studies have demonstrated the utility of ultrasound to improve and expedite the diagnosis of EPTB, especially abdominal and pericardial disease [10, 18, 19]. One important limitation of ultrasound is its lack of specificity, as findings may be mimicked by other opportunistic infections, Kaposi sarcoma and lymphoma [10].

### *Microbiological Assays (Confirmatory Tests) for TB*

#### Smear Microscopy

AFB smear microscopy remains the most commonly available microbiological test for TB in most low-resource settings as it is simple, rapid and relatively inexpensive. There are two different staining techniques that can be utilized to evaluate for AFB – Ziehl-Neelsen (ZN) staining is used with light microscopy and auramine fluorochrome staining is used with fluorescence microscopy. When available, fluorescence microscopy is preferred over light microscopy as it allows for more rapid scanning of sputum smears at low magnification and has improved sensitivity when compared to light microscopy [20]. Traditional fluorescence microscopy requires

dark room isolation and expensive equipment with ongoing need for replacement bulbs. However, light-emitting diode (LED) fluorescence microscopes are less expensive and have fewer technology requirements. LED fluorescence microscopy is being increasingly utilized in resource-limited settings.

Although widely available, smear microscopy has low and variable sensitivity, particularly for HIV-associated TB. One systematic review found that sensitivity of sputum smear microscopy for HIV-associated PTB ranged from 39% to 76% [21]. The sensitivity of smear microscopy for EPTB varies by sample type, however is generally poor (0–40%) [22, 23], given the often paucibacillary nature of disease. Other disadvantages of smear microscopy include that results are operator-dependent, it cannot differentiate MTB from non-tuberculous mycobacteria (NTM) and it is unable to identify drug resistance.

### Culture

Growth-based detection of MTB remains the gold-standard for the diagnosis of all forms of HIV-associated TB (pulmonary and extra-pulmonary) as it has the highest sensitivity and specificity. Culture can be performed using solid or liquid media. Solid media culture is typically less sensitive and takes longer than liquid media culture, but is less expensive. However, both methods require weeks to provide results, substantial laboratory infrastructure (including biosafety requirements) and highly trained staff. Liquid culture is also prone to contamination and thus rapid specimen transport and quality assurance protocols are crucial. These requirements typically preclude the use of culture-based methods for routine diagnosis of TB in poorly resourced, high burden countries. However, culture-based methods are commonly available at referral laboratories and remain the primary method for drug susceptibility testing, particularly for second-line anti-TB drugs.

### Xpert MTB/RIF Assay

The Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA) is a nucleic acid amplification test (NAAT) that utilizes a semi-automated, cartridge-based system to detect MTB and the presence of RIF resistance within 2.5 h [24]. Single-use plastic cartridges that contain the necessary buffers and reagents for sample processing, DNA extraction and real-time PCR are loaded with a clinical specimen that has been treated with a sample reagent. The cartridge is then loaded into the GeneXpert PCR platform. Five overlapping molecular probes (A-E) that span the entire rpoB core region (81 base pairs) are used to detect the presence of MTB. The probes bind to a matching sequence in the clinical specimen producing a fluorescence signal, indicating the presence of one of the gene sequences. The number of PCR cycles required to detect a minimum fluorescence signal is called a 'cycle threshold ($C_T$)' and the assay will terminate after 38 cycles [24]. When at least two of the five probes produce a positive signal in less than 38 cycles, MTB is detected. The assay

provides one of the following results for TB diagnosis: (1) MTB not detected, (2) MTB detected (high, medium, low or very low), or (3) 'error', 'invalid' or 'no result.' In addition, when MTB is detected, RIF resistance results are also reported as (1) RIF resistance detected, (2) RIF resistance not detected, or (3) RIF resistance indeterminate. The $C_T$ is also reported with a positive Xpert result and provides an approximation of bacillary burden. Studies have found that a $C_T$ value cutoff of ≤28 corresponds to a high bacillary burden and predicts sputum smear-status [25, 26].

Among PLHIV, Xpert has a pooled sensitivity of 97% (95% CI 90–99) for smear-positive PTB and a sensitivity of 61% (95% CI 40–81) for smear-negative PTB [27]; its overall pooled sensitivity is 79% (95% CI 70–86) and pooled specificity is 98% (95% CI 96–99). The sensitivity for EPTB ranges dramatically by sample type (corresponding to disease site) [28]. It performs best on bone/joint, lymph node and urine samples (sensitivity 82–88%), moderately for TB meningitis (sensitivity 71%) and less favorably on pericardial, pleural and peritoneal fluid samples (<31–66%). It should be noted that the sensitivity of urine Xpert (pooled estimate 83%) is among those with genitourinary disease; it has decreased performance when used for testing all PLHIV regardless of symptoms [29–31]. Table 1 summarizes the diagnostic accuracy of Xpert for important non-respiratory samples.

In 2010, the WHO recommended that Xpert replace sputum microscopy as the initial test for the microbiological evaluation of PTB in PLHIV. Subsequent WHO recommendations also endorsed Xpert MTB/RIF as the first line assay for EPTB in PLHIV as well as the first-line diagnostic for the rapid detection of RIF resistance in those with confirmed TB [7].

**Table 1** Pooled estimates of sensitivity and specificity of Xpert MTB/RIF for different forms of EPTB (adapted from Kohli et al.) [28]

| | Number of patients | Number of specimens with culture-confirmed TB | Pooled sensitivity (95% CI) | Pooled specificity (95% CI) |
|---|---|---|---|---|
| **TB of blood** (Disseminated TB) | | | | |
| Blood | 266 | 23 | (Numbers insufficient) | (Numbers insufficient) |
| **TB of genitourinary tract** (renal TB) | | | | |
| Urine | 1199 | 73 | 82.7 (69.6–91.1) | 98.7 (94.8–99.7) |
| **TB of lymph node** (TB lymphadenitis) | | | | |
| Lymph node aspirate | 1710 | 671 | 87.6 (81.7–92.0) | 86.0 (78.4–91.5) |
| Lymph node tissue | 484 | 147 | 84.4 (74.7–91.0) | 78.9 (52.6–91.5) |
| **TB meningitis** | | | | |
| Cerebrospinal fluid | 3774 | 433 | 71.1 (60.9–80.4) | 98.0 (97.0–98.8) |

(continued)

**Table 1** (continued)

| | Number of patients | Number of specimens with culture-confirmed TB | Pooled sensitivity (95% CI) | Pooled specificity (95% CI) |
|---|---|---|---|---|
| **TB of musculoskeletal system** | | | | |
| Bone or joint fluid | 385 | 58 | 97.2 (89.5–99.6) | 90.2 (55.6–98.5) |
| Bone or joint tissue | 618 | 179 | 82.0 (56.6–94.9) | 91.8 (70.1–98.4) |
| **TB of pericardium** (pericardial TB) | | | | |
| Pericardial fluid | 324 | 76 | 65.7 (46.3–81.4) | 96.0 (85.8–99.3) |
| **TB of peritoneum** (peritoneal TB) | | | | |
| Peritoneal fluid | 712 | 115 | 59.2 (45.2–73.5) | 97.9 (96.2–99.1) |
| **TB pleurisy** (pleural TB) | | | | |
| Pleural fluid | 4006 | 607 | 50.9 (39.7–62.8) | 99.2 (98.2–99.7) |
| Pleural tissue | 207 | 71 | 30.5 (3.5–77.8) | 97.4 (92.1–99.3) |

For all forms of EPTB except pleural TB, solid or liquid mycobacterial culture was used as the reference standard. For pleural TB, either culture or the presence of granulomatous inflammation on histopathological examination defined the reference standard

Unfortunately, cost remains an issue even with subsidized pricing for the GeneXpert platform and Xpert MTB/RIF cartridges (~$10/cartridge). The GeneXpert platform is also sensitive to heat and dust, requires a continuous power supply to operate as well as ongoing maintenance [32]. For these reasons, Xpert testing has mainly been available in higher-level health facilities in high burden countries. Several studies have shown that implementation of Xpert has resulted in increased detection of mycobacteriologically-confirmed TB, reduced time to diagnosis and reduced time to TB treatment. The implementation of Xpert has been associated with a mortality reduction in some settings [33, 34], however, this has not been a universal finding, as the majority of trials did not find a survival benefit associated with its use [35–42].

### Xpert MTB/RIF Ultra (Xpert Ultra) Assay

The Xpert Ultra cartridge utilizes the existing GeneXpert platform, but incorporates two new multi-copy amplification targets (IS6110 and IS1081) and a larger DNA amplification reaction chamber than the original Xpert cartridge. This contributes to an improved lower limit of detection compared to the original Xpert cartridge (16 vs 114 bacterial colony forming units per milliliter), and increased sensitivity [43].

The Xpert Ultra test adds a new result category, 'trace-positive', which corresponds to the lowest bacillary burden for MTB detection. A large multi-country evaluation found that among PLHIV, Xpert Ultra increased the sensitivity for the detection of PTB by 13% (95% CI 6–21) compared to Xpert (90% versus 77%) [44]. However Xpert Ultra was also associated with a small decrease in specificity (2.7%). Specificity was higher when not considering trace results to be positive, and when excluding patients previously treated for TB [44]. Evaluations of Xpert Ultra for EPTB are limited among PLHIV, however a study evaluating its utility for detecting TB meningitis (TBM) found that the sensitivity for probable or definite TBM in PLHIV was 70% (95% CI 47–87), compared to 43% (95% CI 23–66) using either Xpert or culture [45]. On the basis of these early, but highly encouraging results, in 2017 the WHO recommended that the Xpert Ultra cartridge replace the original Xpert cartridge as the first line test for HIV-associated TB (pulmonary and extra-pulmonary samples) [46].

### Lipoarabinomannan (LAM)

LAM comprises a group of lipopolysaccharides within the cell wall of MTB [3]. A commercially available lateral-flow urine assay, called 'Determine TB-LAM' (Alere Inc. Waltham, Massachusetts, USA), was the first truly rapid, inexpensive, point-of-care assay available for the diagnosis of HIV-associated-TB. The assay is a lateral-flow, urine-based, dip-stick assay (henceforth known as 'LF-LAM') that does not have any storage requirements, has minimal training requirements and is capable of providing results within 30 min at the point-of-care [3]. The assay currently costs between $2.50 and $3.00 a test. The sensitivity of LF-LAM strongly correlates with the immune status of HIV-patients as demonstrated by a meta-analysis that showed sensitivity in patients with CD4 count <100 cells/μL was 56% (95% CI 41–70) compared to 26% (95% CI 16–46) in patients with CD4 count >100 cells/μL [47]. Similarly, sensitivity was greater among hospitalized patients than among ambulatory outpatients (53% versus ~20%). Pooled specificity was found to be 92%, but approaches 99% when a rigorous reference standard is utilized [48, 49].

While the LF-LAM assay has only moderate sensitivity among immunocompromised HIV patients, it rapidly detects TB in the sickest patients at the highest risk for poor outcomes [50]. For example, one study found that LF-LAM detected TB in two-thirds of all patients with evidence of mycobacteremia, including all patients dying within 90 days [51]. Furthermore, a meta-analysis among HIV patients found that mortality was 2.5-fold higher among those with a positive versus a negative LF-LAM result [2]. Notably, two randomized trials have evaluated the addition of LF-LAM to the local diagnostic standard of care in sub-Saharan Africa and have demonstrated a mortality reduction associated with its use among those with a CD4 count <100 cells/μL [52, 53]. This mortality benefit likely reflects the ability to more rapidly detect TB and start potentially life-saving anti-TB therapy. LF-LAM was conditionally recommended by the WHO in 2015 for use in PLHIV with signs

and symptoms of TB (pulmonary and/or extra-pulmonary) who either have a CD4 count ≤100 cells/μL or who are seriously ill with any 'danger signs' as defined by the presence of respiratory rate >30, temperature > 39.0° C, heart rate > 120 beats per minute, or inability to ambulate unassisted (independent of CD4 count) [54]. Since 2015, a number of additional studies have reported on the diagnostic performance of LF-LAM among PLHIV; in 2019 the WHO is expected to reappraise the available evidence and issue updated guidance on the use of LF-LAM.

#### Loop-Mediated Isothermal Amplification (LAMP)

TB LAMP (Eiken Chemical Company Ltd. Tokyo, Japan) is a rapid assay that can provide results in less than 1 h. It uses a temperature-independent method for DNA amplification that is easy to use, requires minimal laboratory infrastructure and that can be read using the naked eye under ultraviolet light. However, the assay has several steps and requires trained laboratory personnel. A systematic review was undertaken in 2016 to evaluate its diagnostic performance against smear microscopy as well as Xpert [55]. There was limited data available among PLHIV. Overall, the sensitivity of TB LAMP for pulmonary TB ranged from 64% to 73% and its specificity from 95% to 99% depending on the reference standard used. On the basis of these results, TB LAMP was recommended by the WHO as a replacement for sputum smear microscopy or as a follow-on test after a negative sputum smear result [55]. However, the WHO advised that TB LAMP should not replace Xpert where available, and felt that there was insufficient evidence to recommend the use of TB LAMP for non-respiratory samples or for testing for TB among PLHIV.

### *Detection of TB Drug-Resistance (Drug-Susceptibility Testing)*

Only one-quarter of RIF-resistant (RR) and multi-drug resistant (MDR)-TB cases worldwide are detected each year. The rapid and accurate detection of drug resistance is important to the individual and to public health alike. For the individual, rapid drug susceptibility testing (DST) allows for initiation of the most effective anti-TB regimen as soon as possible, which allows for the highest likelihood of cure. For the community, rapid DST can help to minimize the transmission of drug-resistant TB, help to guide appropriate care for contacts and help prevent the spread of drug-resistant TB. The END TB strategy rightfully calls for universal access to DST [56].

DST is broadly comprised by two major methodologic categories – growth-based (phenotypic) and molecular-based (genotypic). Generally, culture-based DST is thought to be more reliable than molecular methods because an MTB isolate is grown on a culture media containing the critical concentration of a given anti-TB agent. It is therefore typically assumed that if growth of MTB is inhibited by that agent on DST, that same agent should be reliably effective for the patient's isolate

in vivo; however, up to 5% of wild-type strains may be classified as resistant, in part likely due to limitations of critical-concentration methods [57, 58]. Additionally, there are reports of specific rpoB mutations that confer rifampicin resistance not being detected on liquid culture DST [59]. This is compared to molecular methods that detect known mutations for drug resistance. If all resistance mutations are not known or included in the probe, drug-resistance using molecular techniques may be underdiagnosed in a proportion of patients.

One important difference between growth-based and molecular-based DST is the requirement for a pure MTB isolate to be obtained from either solid or liquid culture media before culture-based DST can be performed. When coupled with the further requirement to monitor growth (or lack of growth) in the setting of agar or liquid culture media containing a specific drug, the overall process can take several weeks to months. Molecular methods not only provide for more rapid results, but also offer standardized testing with fewer biosafety requirements; both of which may allow for increased throughput. On this basis, the WHO recommends that molecular methods for TB DST be performed in addition to culture-based DST whenever available [60, 61].

## *Culture-Based Methods for DST*

Phenotypic methods, or culture (growth)-based DST remain the gold standard for DST. There are multiple methods available and in clinical use. Critical concentrations, not minimum inhibitory concentrations (MIC), are used to determine the susceptibility or resistance of anti-TB agents for a given culture isolate. The critical concentration is defined as the lowest concentration that reliably inhibits >99% of wild-type MTB complex strains in vivo, while also not inhibiting strains considered to be resistant [62]. The critical concentration varies slightly between culture media and in 2018 the WHO published standard critical concentrations for most first-, second- and third-line agents [62].

Solid media-based DST (the indirect agar proportion method) most commonly utilizes Lowenstein-Jensen, Middlebrook 7H10 or 7H11 agar. Using this technique, a culture isolate is directly inoculated into a quadrant of the plate. Three quadrants contain a specific anti-TB agent at its critical concentration, while one quadrant without a drug serves as a control. After 21 days colony counts are taken and if the number of colonies in a drug-containing quadrant is >1% of the colonies in the control quadrant, the isolate is considered to be resistant to that drug.

Liquid media-based DST has faster turnaround time when compared to solid media-based techniques, with results available in as little as 7–10 days after inoculation. There are several commercially available platforms, but WHO critical concentrations are only available for the MGIT 960 platform (Becton Dickinson, Sparks, MD). The MGIT 960 platform can provide DST for first- and second-line agents. The method is based on fluorescence that is produced from the MGIT medium when bacterial growth results in reduced oxygen. The amount of fluorescence generated

is then converted to growth units (GU), where greater GU corresponds to more growth. If a drug containing tube yields a GU < 100 at the end of incubation then the organism is considered susceptible, while a GU ≥100 is considered resistant.

## *Molecular Methods for DST*

There are several benefits associated with molecular methods compared to growth-based methods. The most important is the short turnaround time for DST results, which may be as few as 1–2 days as compared to at least several weeks associated with culture-based methods. Additionally, unlike culture-based methods, molecular methods can be run on smear-positive/ culture-negative specimens, as well as fixed pathology specimens. In general, there are two broad categories of molecular methods available for DST: sequencing and non-sequencing based techniques. Currently, non-sequencing methods predominate especially in low- and middle-income settings. However, sequencing-based methods are expected to become more simplified and increasingly affordable, which will likely translate to increased availability over the next several years.

### Xpert and Xpert Ultra

As noted previously, the Xpert assay is able to rapidly detect RIF resistance in clinical specimens in which MTB is confirmed and results are provided within 2.5 h. It is recommended by the WHO as the first-line assay for the rapid detection of RIF-resistance and has become the most widely available assay for TB DST globally. Its pooled sensitivity and specificity for the detection of RIF-resistance in patients with HIV-associated TB is 95% (95% CI 90–97) and 98% (95% CI 97–99), respectively [27].

The Xpert Ultra cartridge utilizes a new melt curve analysis to detect RIF-resistance and data to date suggest that the Xpert Ultra cartridge provides similar (non-inferior) diagnostic accuracy for the detection of RIF resistance compared to the traditional Xpert cartridge [44]. As described in **Part III**, the detection of RIF resistance by Xpert and Xpert Ultra testing should prompt further DST for first and second line anti-TB agents (injectable agents and fluoroquinolones).

### Line Probe Assays

Line probe assays (LPA) are a type of molecular test that permit the detection of *M. tuberculosis* complex, as well as mutations associated with TB drug resistance. LPA involves a multi-step process that includes: (1) DNA extraction, (2) PCR-based amplification of known resistance determining regions using primers, (3) reverse hybridization of amplicons to probes affixed on the assay strip and (4) colorimetric

detection of captured hybrids allowing for visualization of bands. LPAs can be performed on DNA extracted from clinical specimens (direct method) or from culture isolates (indirect method). LPAs can detect specific mutations known to be associated with drug resistance but can also indirectly indicate drug resistance when a mutation is present in one of the target regions, resulting in the amplicon not hybridizing with a wild-type probe [63].

The WHO has made formal recommendations for two commercially available LPAs that detect drug resistance associated with RIF and INH (first-line agents). These include the GenoType MTBDRplusv2.0 (Hain Lifescience, Nehren, Germany) and the Nipro NTM + MDRTB Detection Kit 2 (Nipro, Tokyo, Japan). The diagnostic accuracy of both assays for the detection of RIF and INH resistance directly on smear-positive sputum samples was evaluated and found to be comparable (~97–98%, ~95% sensitive for RIF and INH resistance, respectively; ~98% specific for RIF and INH resistance) [60]. However, the sensitivity of both assays for indirect testing of MTB culture isolates was lower, ~90–91% [60]. On the basis of these results, the WHO recommended that for persons with sputum smear-positive disease or any culture-isolate positive for MTB complex, either LPA (MTBDRplusv2.0 or NTM+MDRTB Detection Kit 2) may be used as the initial test for the rapid detection of RIF and INH resistance in addition to conventional culture-based DST [60]. There is limited data available that specifically evaluate the performance of these LPAs among PLHIV, however one study suggested that the MTBDRplusv2.0 had excellent sensitivity for the detection of RIF resistance (>90%), but only moderate sensitivity for the detection of INH resistance (~70%) [64]. Incomplete sensitivity for INH resistance likely reflects the fact that additional resistance conferring mutations are not included in the assay.

The GenoType MTBDRsl (Hain Lifescience, Nehren, Germany) version 1.0 was the first commercially available LPA able to rapidly detect mutations associated with resistance to second-line agents, thus allowing for the diagnosis of MDR-, pre-extensively drug resistant- (XDR) and XDR-TB [61]. The assay can detect the presence of MTB complex, mutations associated with fluoroquinolones (ofloxacin, levofloxacin, moxifloxacin, gatifloxacin) and second-line injectable agents (kanamycin, amikacin, capreomycin). The pooled sensitivities and specificities of the version 1.0 assay for second-line TB drugs are shown in Table 2.

The manufacturer has subsequently introduced a newer generation of the GenoType MTBDRsl assay (version 2.0) that detects additional resistance mutations as well as all identified by the version 1.0 assay. There is limited published data on its diagnostic accuracy specifically among PLHIV, however, in one study testing 268 respiratory isolates from a high burden HIV-associated TB setting, the sensitivity of the version 2.0 assay for fluoroquinolones (100%; 95% CI 96–100) and second-line injectable agents (89%; 95% CI 79–96) was excellent and was associated with a specificity >98.5% for all agents with the exception of capreomycin (95.9%) [65].

In patients with either confirmed RR-TB or MDR-TB (detected using Xpert, LPA or culture-based methods), the WHO recommends that the MTBDRsl assay may be used as the initial test (in addition to culture-based DST) to rapidly detect

**Table 2** Pooled sensitivity and specificity estimates of GenoType MTBDRsl v1.0 for fluoroquinolones and second-line injectable agents using conventional culture-based DST reference standard[a]

| | Number of patients | Pooled sensitivity (95% CI) | Pooled specificity (95% CI) |
|---|---|---|---|
| **Fluoroquinolones, direct testing** | 1771 | 86.2 (74.6–93.0) | 98.6 (96.9–99.4) |
| Ofloxacin | 1667 | 90.9 (84.7–94.7) | 98.9 (97.8–99.4) |
| Moxifloxacin | 821 | 95.0 (92.1–96.9) | 99.0 (97.5–99.6) |
| **Fluoroquinolones, indirect testing** | 2223 | 85.6 (79.2–90.4) | 98.5 (95.7–99.5) |
| Levofloxacin[b] | 169 | 80.0–100[b] | 96–100[b] |
| Ofloxacin | 1927 | 85.2 (78.5–90.1) | 98.5 (95.6–99.5) |
| Moxifloxacin | 419 | 94.0 (82.2–98.1) | 96.6 (85.2–99.3) |
| **Second-line injectable agents, direct testing** | 1639 | 87.0 (38.1–98.6) | 99.5 (93.6–100) |
| Amikacin | 1491 | 91.9 (71.5–98.1) | 99.9 (95.2–100) |
| Capreomycin | 1027 | 76.6 (61.1–87.3) | 98.2 (92.5–99.6) |
| Kanamycin | 1020 | 78.7 (11.9–99.0) | 99.7 (93.8–100) |
| **Second-line injectable agents, indirect testing** | 1921 | 76.5 (63.3–86.0) | 99.1 (97.1–99.7) |
| Amikacin | 1301 | 84.9 (79.2–89.1) | 99.1 (97.6–99.6) |
| Capreomycin | 1406 | 79.5 (58.4–91.4) | 95.6 (93.4–97.3) |
| Kanamycin | 1342 | 66.9 (44.1–83.8) | 98.6 (96.1–99.5) |

[a]For the MTBDRsl v2.0 there was insufficient data to undertake a meta-analysis or compare direct and indirect testing

[b]Insufficient data precluded pooled estimates; numbers represent ranges from study point estimates

resistance associated with fluoroquinolones or second line injectable agents on (1) sputum samples (irrespective of smear status—*direct testing*) or (2) cultured isolates of MTB complex from any respiratory or non-respiratory samples (*indirect testing*) [61].

## Part II: Novel Approaches to Diagnosis of HIV-Associated TB

### *Overview*

There are considerable ongoing efforts to develop TB tests that are faster, cheaper, simpler and can be performed on samples that are easier to collect than sputum. These range from discovery phase studies that seek to identify and validate novel biomarkers in blood, urine and breath, to the development and evaluation of new technologies to facilitate sample processing and analysis [4, 66]. In this section, we will provide a selective overview of tools in the TB diagnostic pipeline that are either at the later stages of development or have later phase clinical data published

and focus on tests and platforms that we anticipate will improve the diagnosis of HIV-associated TB in the near future.

For the most up-to-date information on the TB diagnostics pipeline, the Foundation for New Innovative Diagnostics (FIND) has developed an online, interactive diagnostics pipeline that shows the current status and estimated release dates of various diagnostic tools and assays. Please visit: **https://www.finddx.org/tb/pipeline/**

## *Tools and Tests for TB Screening*

### Clinical Prediction Scores

Clinical prediction scores may combine symptoms as well as easily obtained clinical information (body mass index, vital signs, ART status) with routinely available laboratory tests (hemoglobin, CD4 cell counts) to direct diagnostic testing for HIV-associated TB. Notably, two clinical prediction scores have been studied among ambulatory HIV patients screening positive using the WHO symptom screen [67, 68]. Both studies propose that a defined cutoff could be used to safely reduce the overall number of patients requiring further TB testing without missing a large number of TB cases. One of the clinical scores utilized ART status (ART >3 months vs. pre-ART or ART <3 months), body mass index, CD4 count and the number of WHO symptoms present (1 versus >1 symptom). When used among those with a positive WHO symptom screen, a cutoff score of 3 had a sensitivity and specificity for HIV-associated TB that was 92% and 34%, respectively and would have resulted in a >30% reduction in need for further TB testing while missing <10% of all TB diagnoses (predominantly among those on ART and with higher CD4 cell counts) [68].

### C-Reactive Protein (CRP)

CRP is an acute phase reactant that is detectable in serum and can be rapidly measured at the point-of-care [69]. A systematic review among predominantly ambulatory PLHIV found that the sensitivity and specificity of CRP (cutoff: 10 mg/L) for the detection of confirmed pulmonary TB was 93% (95% CI 88–98) and 60% (95% CI 40–75), respectively [70]. Prospective studies in Uganda and South Africa have demonstrated that point-of-care CRP testing has similar sensitivity when compared to the WHO symptom screen (~90%), but has significantly improved specificity (59–72%) [69, 71]. Dependent on the CRP cut-off level used, the specificity associated with CRP is 21–58% higher than that of symptom screening. While these results must be further validated, the results suggest that use of CRP in place of the WHO symptom screen as part of intensified case finding for PLHIV would detect a similar number of HIV-associated TB cases, while

significantly reducing the number of patients requiring further TB investigations by >50%. Indeed, one study among ambulatory PLHIV showed that CRP-based TB screening followed by confirmatory testing with LF-LAM (if CD4 count < 100), Xpert and a single liquid culture, would increase case detection relative to the currently recommended strategy [72].

## *Microbiological Assays (Confirmatory Tests) for TB*

### Next-Generation LAM Assays

Several urine-based assays that detect the presence of LAM are undergoing development and evaluation. They aim to retain the point-of-care quality of the currently available LF-LAM assay while improving upon sensitivity that would expand utility beyond only the sickest HIV patients [4]. One test, the SILVAMP TB LAM assay (FujiFilm Global, Tokyo, Japan), had a sensitivity of 70.4% compared to 42.3% using the LF-LAM assay without a significant difference in specificity, when retrospectively testing 968 urine samples from PLHIV in South Africa. Among those with a CD4 count ≤100 cells/μL, the SILVAMP TB LAM had a sensitivity of 84.2% versus 57.3% using the LF-LAM assay [73]; it also demonstrated useful sensitivity in those with CD4 counts 101–200 cells/μL—60.6% compared to 26.4% using LF-LAM. prospective evaluations of its performance will be undertaken in 2019.

### Xpert Omni

In 2019, a new Xpert platform called Xpert Omni is expected to be introduced that may allow for truly point-of-care detection of MTB and the presence of RIF resistance within 2 h. The single module unit is lightweight (~1 kg), portable, battery-powered (up to 12 h rechargeable battery life) and is designed to allow for testing in more extreme clinical settings. Its initial cost is expected to be ~$5,000 per device and it will require special cartridges which will be ~$1.50 more expensive than traditional Xpert cartridges (to allow for the incorporation of wireless near-field communication) [74]. It is currently undergoing feasibility studies and is expected to become commercially available in 2019.

### Nucleic Acid Amplification Tests (NAAT) Other Than GeneXpert

Since the introduction of GeneXpert, there have been many companies that have sought to develop competing rapid NAAT-based assays for the diagnosis of TB. Some of the assays furthest along in development and evaluation include the

Genedrive MTB/RIF assay (Epistem Ltd., UK), TrueNat MTB RIF assay (Molbio, Goa, India), TRCReady (Tosoh Bioscience, Tokyo, Japan), EasyNAT TB assay (Ustar Biotechnologies Ltd., Hangzhou, China), RealTime MTB (and MTB RIF/INH) assay (Abbott, Chicago, USA), and the FluoroType MTB assay (Hain Lifescience, Nehren, Germany). Of these, only the TRCReady assay represents a stand-alone, semi-automated NAAT similar to Xpert; however, it does not provide simultaneous RIF resistance detection. While some of these assays are already commercially available and even in use in countries such as India and China, there are minimal published data to recommend their routine use in PLHIV [4].

## *Molecular Methods for DST*

### Xpert Xtend XDR

A new cartridge utilizing the GeneXpert platform called the Xpert Xtend XDR will test for resistance associated with isoniazid (INH) as well as fluoroquinolones and injectable aminoglycosides. The Xtend XDR cartridge is expected in 2019 and may potentially allow for decentralized, rapid detection (results available within 90 min) of resistance associated with second-line agents. An initial prototype demonstrated promising results [58].

### Sequencing

Next generation sequencing (NGS) is the latest advance in the rapid detection of TB-associated drug resistance. It can be used to perform targeted and whole genome sequencing. Non-sequencing, molecular methods such as Xpert and LPAs may miss important resistance-conferring mutations if not encapsulated within the target probe(s) or may detect mutations that do not confer resistance, resulting in false-negative and false-positive results, respectively. One major advantage of NGS is its ability to identify all known mutations simultaneously.

Studies have demonstrated that NGS has good concordance with culture-based methods and NGS can be performed directly on smear-positive clinical specimens [75–78]. NGS may ultimately one day allow for more individualized treatment regimens based on knowledge of the most effective anti-TB drugs for each person. However, a number of challenges face the implementation and scale-up of NGS, especially in resource-limited settings. These include the ability to reliably extract sufficient mycobacterial DNA from clinical samples for sequencing, the cost of sequencing platforms and laboratory infrastructure requirements, as well as the need for improved means to process and analyze large amounts of raw data [4].

## Part III: A Suggested Step-Wise Approach to Diagnosing HIV-Associated TB for Clinicians with a Focus on Resource-Limited Settings

### *Overview*

Recent WHO guidelines highlight the shift towards active case finding among PLHIV as well as new diagnostic tools for rapid TB detection and DST. In the subsequent sections, we present a suggested step-wise approach for the diagnosis of HIV-associated TB using current WHO recommendations and the revised 2018 Global Laboratory Initiative (GLI) model TB diagnostic algorithms for PLHIV as a framework (Figs. 1–3) [79].

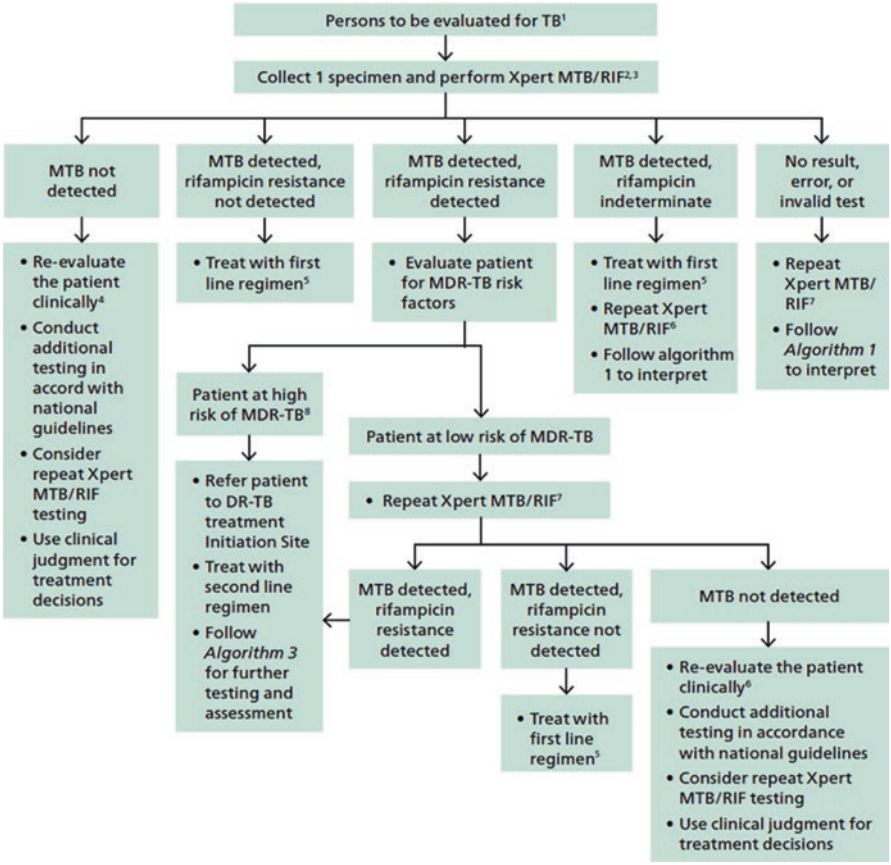


**Fig. 1** WHO recommended algorithm for evaluating persons for TB (Xpert as the initial test)

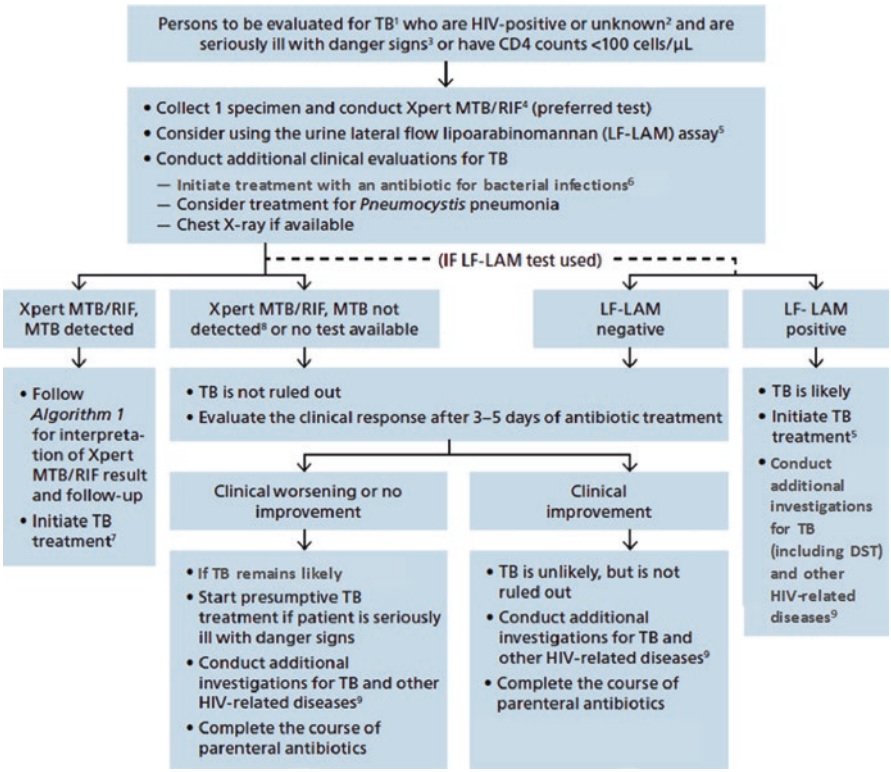


**Fig. 2** Algorithm for evaluating PLHIV for TB among those who are seriously ill with danger signs or have CD4 count ≤100 cells/μL

## Step 1. Who Should I Screen for HIV-Associated TB?

*Current WHO recommendation*: All patients with confirmed HIV (or an unknown HIV status) should be screened for TB at each health care encounter.

*Further information*: There are typically two broad approaches to identifying people with HIV-associated TB - passive and active case finding. Passive case finding is reliant upon symptomatic TB patients to self-present to a health-care setting followed by a health worker recognizing that their symptoms may be due to TB and ordering TB testing [80]. This approach on its own has led to substantial underdiagnosis of HIV-associated TB globally for several reasons. These include that patients with early TB disease may not be symptomatic (or symptoms may be nonspecific) and that health workers often fail to order TB testing even when indicated. In contrast, active or intensified case finding (ICF) in either facility- or community-based settings involves screening everyone within a high-risk group, such as PLHIV,

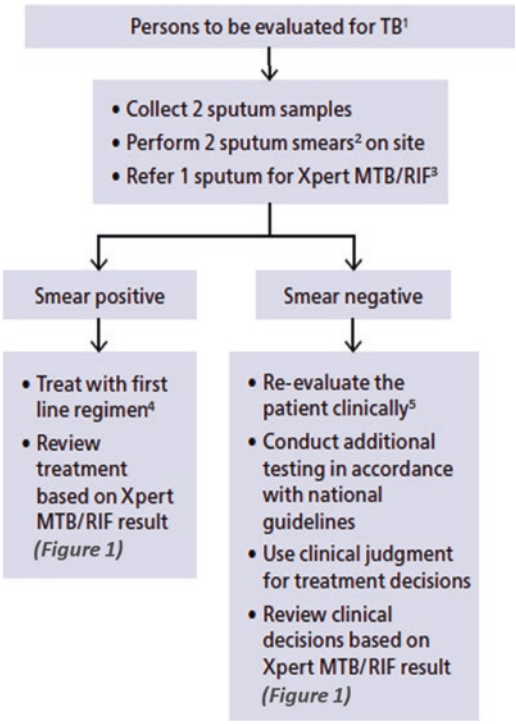


**Fig. 3** Alternative algorithm for evaluating persons for TB where molecular testing is not readily available (sputum microscopy as the initial test)

followed by confirmatory diagnostic testing for those who screen positive. The goals of ICF are not only to identify more people with TB but also to identify them earlier in order to reduce morbidity and community transmission [80].

**Relevant guidelines:**

- Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: WHO; 2016.
- Guidelines for intensified tuberculosis case-finding and isoniazid preventative therapy for people living with HIV in resource-constrained settings. Geneva: WHO; 2011.
- Systematic screening for active tuberculosis: Principles and recommendations. Geneva: WHO; 2013.

### Step 2. How Should I Screen for HIV-Associated TB?

*Current WHO recommendation*: A four-part symptom screen should be used: current cough, fever, weight loss or night sweats. For PLHIV on ART, chest radiography may be considered in addition to symptom screening. Chest radiography (when

available) is also recommended as a screening tool in addition to symptom-based screening in all PLHIV with a CD4 count <100 cells/μL or those presenting with 'danger signs,' regardless of symptoms (Fig. 2).

*Further information*: PLHIV who have a negative symptom screen with or without a negative chest X-ray are unlikely to have active TB and should be offered TB preventive therapy, regardless of ART status. In addition to the above symptom screen (with or without chest radiography), all PLHIV should have a careful history and vital signs obtained and physical exam undertaken to determine: 1) if there are 'danger signs' present (defined as any one of the following: respiratory rate > 30, temperature > 39.0C, heart rate > 120 beats per minute, or unable to ambulate unassisted) that would suggest a need for referral to a higher level of clinical care and 2) if there are any signs or symptoms that might suggest EPTB (Step 5).

*Limitations of currently recommended strategy*: There are several limitations of the WHO standard symptom-screening rule that has kept it from being widely implemented in high burden settings. It is not objective in that it relies on patients' self-reported symptoms. Additionally, and more pragmatically challenging, it has low overall specificity (~50%) [8], and even poorer specificity among ART-naive PLHIV (~28%) [8]. As many clinicians and policy makers point out, universal application of this recommendation would result in a large proportion of PLHIV requiring additional TB investigations, of whom only a small number might have TB. This may stretch the resources of HIV/AIDS programs as well as delay and reduce the number of patients initiated on TB preventative therapy. Furthermore, it demonstrates poor sensitivity among those receiving ART (~50%) [8]. The WHO symptom screen therefore falls short of the WHO proposed cutoffs for a screening tool - >90% sensitivity and > 70% specificity [5]. Thus, there is significant interest in developing improved screening strategies that might help better identify PLHIV who should be prioritized for TB testing.

**Relevant guidelines:**

- Chest radiography in tuberculosis detection – summary of current WHO recommendations and guidance on programmatic approaches. Geneva: WHO; 2016.
- Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: WHO; 2016.
- Guidelines for intensified tuberculosis case-finding and isoniazid preventative therapy for people living with HIV in resource-constrained settings. Geneva: WHO; 2011.
- Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. Geneva: WHO; 2018.
- Systematic screening for active tuberculosis: Principles and recommendations. Geneva: WHO; 2013.

**Step 3: Whom Should I Investigate Further for Pulmonary TB?**

*Current WHO recommendation*: All PLHIV who screen positive using four-part symptom screen should be investigated for active TB. Furthermore, anyone with clinical exam findings or radiology (chest X-ray, ultrasound [when undertaken]) findings potentially consistent with PTB should also be further investigated for active TB, regardless of symptoms.

**Relevant guidelines:**

- Chest radiography in tuberculosis detection – summary of current WHO recommendations and guidance on programmatic approaches. Geneva: WHO; 2016.
- Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: WHO; 2016.
- Guidelines for intensified tuberculosis case-finding and isoniazid preventative therapy for people living with HIV in resource-constrained settings. Geneva: WHO; 2011.
- Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents. Geneva: WHO; 2007.
- Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. Geneva: WHO; 2018.
- Systematic screening for active tuberculosis: Principles and recommendations. Geneva: WHO; 2013.

**Step 4. How Should I Test for PTB?**

*Current WHO recommendation*: Xpert MTB/RIF (Xpert Ultra if available) should be used as the initial diagnostic test for PTB (Figs. 1 and 2). In addition, the LF-LAM assay should be performed in all PLHIV who are severely ill or have CD4 count <100 cells/mm$^3$ to enable rapid diagnosis and treatment initiation (Fig. 2). Where Xpert MTB/RIF is not readily available, sputum microscopy should be used as the initial diagnostic test for PTB (Fig. 3).

*Further information:*

*Xpert (Ultra) for PTB*: For patients with suspected PTB, one fresh sputum sample should be collected and tested using Xpert (or preferably Xpert Ultra) (Fig. 1). If the initial Xpert test result is negative, but the clinical suspicion for PTB remains high, undertaking repeat Xpert testing on a newly collected, fresh sputum specimen may be considered as this has been associated with up to a 20% increase in diagnostic sensitivity for smear-negative disease [81]. It is not yet clear if there is increased

diagnostic yield associated with undertaking repeat sputum Xpert Ultra testing if the first Xpert Ultra test is negative. When Xpert Ultra testing is utilized, the WHO recommends that for PLHIV a trace positive result be regarded as a true positive result and that these patients be initiated on anti-TB therapy [46].

*Smear microscopy for PTB*: Where Xpert testing is not available for the investigation of PTB, it is recommended that microscopy (LED fluorescent microscopy preferred) be performed on two sputum samples to evaluate for the presence of acid-fast bacilli (Fig. 3). Same-day microscopy involves collecting two spot sputum samples at the initial health center visit and is the recommended approach as it is more patient-friendly and retains similar sensitivity and specificity when compared to multiple day sputum collection [82, 83]. Use of a concentrated sputum sample does not appear to increase sensitivity and is not recommended because it increases resource requirements [84]. A positive sputum AFB microscopy result should be confirmed as MTB (when possible) as this may represent non-tuberculous mycobacteria (NTM); however, this should not delay treatment, especially if the patient is at risk for further clinical deterioration.

*Culture-based methods*: When the results of rapid tests are negative, culture-based methods should be considered where resources permit. A recent study demonstrated considerable incremental yield with the addition of a single liquid culture when Xpert results are negative [72].

*TB-LAMP*: Where available, TB-LAMP may be used as a replacement test for sputum-smear microscopy for the diagnosis of PTB only, or may be considered as a follow-on test in those testing sputum-smear negative (see Fig. 3).

**Relevant guidelines:**

- Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: WHO; 2016.
- Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis. Geneva: WHO; 2011.
- GLI model TB diagnostic algorithms. Geneva: WHO; 2018.
- Same-day diagnosis of tuberculosis by microscopy. Geneva: WHO; 2011.
- Xpert MTB/RIF implementation manual – technical and operational "how-to". Practical considerations. Geneva: WHO; 2014.

### Step 5: Whom Should I Investigate Further for EPTB and How Should I Test for EPTB?

*Current WHO recommendation*: All PLHIV with signs or symptoms of EPTB should be investigated for TB using microbiological tests. If PLHIV have respiratory symptoms or chest radiograph abnormalities, sputum-based testing with Xpert should be performed (Fig. 1). Even in PLHIV without respiratory symptoms, sputum-based testing will yield some TB diagnoses. If the results of rapid sputum-

based testing (Xpert or microscopy) are negative, or PLHIV are unable to produce sputum, microbiological testing should be undertaken on non-respiratory samples corresponding to the extra-pulmonary manifestation most strongly suspected using Xpert (or Xpert Ultra). In PLHIV with signs and symptoms of TB who either have a CD4 count ≤100 cells/μL or are seriously ill (independent of CD4 count), the LF-LAM assay should be performed in parallel with sputum Xpert testing for the diagnosis of disseminated TB; its use should especially be considered in those unable to produce sputum (Fig. 2) [54].

*Further Information:*

*Overview of EPTB*: EPTB is defined as any case of TB that involves an organ or anatomic site other than the lungs. EPTB is common among PLHIV, especially those with severe immunosuppression (present in up to 90%). Disseminated and extra-pulmonary disease is associated with significant morbidity and mortality [1]; therefore, timely diagnosis is crucial. Unfortunately, the diagnosis of EPTB remains challenging given its non-specific presentations and traditional difficulty in obtaining non-respiratory samples.

*Clinical and radiological features of EPTB*: TB can involve almost any anatomic site, but patients will often have local signs and symptoms related to the site of their disease with or without constitutional symptoms. EPTB clinical manifestations are reviewed in greater detail in the chapter "Clinical Manifestations of HIV-Associated Tuberculosis in Adults". Clinicians should have heightened suspicion for EPTB in PLHIV presenting with a positive symptom screen as well as dyspnea (possible TB pleural effusion and/or TB pericarditis), enlarged cervical/axillary lymph nodes (possible TB lymphadenitis), headache or altered mental status (possible TB meningitis). The WHO has previously outlined a pragmatic clinical approach to help identify cases of EPTB by "looking and listening" for signs of four common forms of EPTB, including TB lymphadenitis, pleural TB, TB pericarditis and TB meningitis (Table 3) [85].

As discussed in **Part I**, ultrasonography may also help rapidly and inexpensively detect pleural or pericardial effusions suggesting pleural and pericardial TB, respectively [17]. The abdomen is the most frequent site of TB disease dissemination beyond the chest cavity and almost any structure (i.e., peritoneum, gastrointestinal tract, lymph nodes) or solid organ (spleen, liver, pancreas) may be involved. Intra-abdominal findings on ultrasonography, especially ascites, diffuse lymphadenopathy or splenic or liver micro-abscesses should result in microbiological TB investigations.

*Overview of diagnosing EPTB*: When EPTB is suspected on the basis of clinical or radiologic features (chest X-ray or ultrasound), rapid investigations to confirm a TB diagnosis should be undertaken to allow for the prompt initiation of TB therapy. If empiric TB treatment is initiated on the basis of high clinical suspicion (for example: the patient is symptomatic, has compatible ultrasound findings and is at high risk for clinical deterioration), clinical specimens should still be obtained for TB confirmation and DST.

**Table 3** 'Look and listen for' signs of possible EPTB

| |
|---|
| • **Possible TB lymphadenitis** |
| – Cervical (typically unilateral) or axillary lymphadenopathy |
| • **Possible pleural TB** |
| – Absent breath sounds |
| – Reduced chest wall movement |
| – Dullness to percussion |
| • **Possible TB pericarditis** |
| – Distant heart sounds |
| – Peripheral edema and/or abdominal distension |
| – Jugular venous distension |
| • **Possible TB meningitis** |
| – Neck stiffness |
| – Confusion |
| – Atypical eye movements |

The WHO recommends that Xpert should be the initial test for the investigation of all forms of EPTB. The most common forms of EPTB are listed in Table 4 along with associated clinical samples that might be obtained and submitted for further microbiological testing when those forms of EPTB are clinically suspected. The approach to microbiological testing for EPTB is described below.

*Sputum-based testing for those able to produce sputum*: A large proportion of patients with extra-pulmonary disease also have concomitant pulmonary disease [29]. For PLHIV with suspected EPTB, those who are able to produce a sputum sample should still undergo initial testing with sputum Xpert testing (Figs. 1 and 2) or sputum AFB microscopy (and culture) testing where Xpert testing is unavailable (Fig. 3).

*Obtaining non-respiratory clinical specimens*: When the diagnosis of EPTB is suspected but cannot be made via sputum-based methods (either sputum testing negative, or patient is too sick/unable to provide a sputum sample) then further non-respiratory samples should be obtained and submitted for rapid microbiological testing. The obtainment of clinical samples should be guided by which clinical site/organ is suspected to be involved, as well as what investigations are locally available (Table 4). When multiple anatomic sites are thought to be involved, the least invasive clinical specimen that can be obtained for microbiological testing should be prioritized.

*Xpert MTB/RIF (and Xpert Ultra) for EPTB*: The diagnostic sensitivity of Xpert for non-respiratory samples is summarized in Table 1. While evaluations to-date are limited, Xpert Ultra is expected to improve detection of EPTB in PLHIV. As above, a 'trace-positive' Xpert Ultra result in PLHIV should be regarded as a true-positive result [46].

*LF-LAM assay*: When LF-LAM results are positive, an additional microbiological test that provides drug susceptibility testing results should be performed if possible (steps 6 and 7).

*Microscopy and culture for EPTB*: In PLHIV with suspected EPTB for which Xpert and LF-LAM testing is either negative or unavailable, smear microscopy and

**Table 4** Forms of EPTB and associated clinical samples for TB testing

| EPTB form | Sample |
|---|---|
| Bacteremia | Blood |
| Genitourinary TB | Urine, semen (men), organ biopsy |
| Lymphadenitis | Fine needle aspirate of affected tissue, excisional biopsy |
| Meningitis | Cerebrospinal fluid, tuberculoma biopsy |
| Pericarditis | Pericardial fluid, pericardial biopsy |
| Peritonitis | Ascitic fluid (paracentesis), peritoneal biopsy |
| Pleurisy (pleural TB) | Pleural fluid (thoracentesis), pleural biopsy |
| Skeletal (bone/joint) | Synovial fluid (arthrocentesis), bone biopsy |

culture may be considered on EPTB samples (Table 4). Smear microscopy is often of limited value given the pauci-bacillary nature of most EPTB samples and the clinical utility of culture-based methods is greatly diminished, especially among sick hospitalized patients given prolonged time-to-positivity and these patients' predisposition to rapid clinical deterioration without appropriate treatment.

**Relevant guidelines:**
- Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: WHO; 2016.
- GLI model TB diagnostic algorithms. Geneva: WHO; 2018.
- Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents. Geneva: WHO; 2007.
- The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV. Geneva: WHO; 2015.
- Xpert MTB/RIF implementation manual – technical and operational "how-to". Practical considerations. Geneva: WHO; 2014.

### Step 6: Whom Should I Test for Drug Resistance?

**Current WHO recommendation:** All PLHIV with confirmed TB should undergo rapid DST for RIF. Patients with HIV-associated TB and evidence of RIF resistance should have further DST undertaken for other first-line drugs and at least for fluoroquinolones and second-line injectable agents.

**Relevant guidelines:**
- Framework of indicators and targets for laboratory strengthening under the End TB Strategy. Geneva: WHO; 2016.
- WHO treatment guidelines for drug-resistant tuberculosis, 2016 update. October 2016 revision. Geneva: WHO; 2016.

### Step 7: How Should I Test for Drug Resistance?

*Current WHO recommendations*: Xpert (or Xpert Ultra) should be used for first-line DST to evaluate for RIF-resistance. In PLHIV with confirmed RR-TB or MDR-TB, further DST should be undertaken for other first-line drugs and at least fluoroquinolones and second-line injectable agents using LPA or other molecular methods (where available), in addition to culture-based methods.

*Further information*: For PLHIV, the WHO recommends universal access to rapid drug-susceptibility testing (DST) for at least RIF and if RIF-resistance is present, further DST for fluoroquinolones and second-line injectable agents. This allows for the prompt identification of RR-TB, MDR-TB and XDR/pre-XDR TB.

**Relevant guidelines:**

- Framework of indicators and targets for laboratory strengthening under the End TB Strategy. Geneva: WHO; 2016.
- The use of molecular line probe assays for the detection of mutations associated with resistance to fluoroquinolones (FQs) and second-line injectable drugs (SLIDs). Policy guidance. Geneva: WHO; 2016.
- The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin. Geneva: WHO; 2016.
- WHO treatment guidelines for drug-resistant tuberculosis

### Step 8: For Whom Should I Consider Initiation of Empiric TB Therapy?

*Current WHO recommendations*: Every effort should be made to confirm the diagnosis of TB. When sputum Xpert (or smear microscopy) testing is negative, or in settings where TB investigations are limited, empiric TB therapy should be considered in those who are seriously ill due to suspected TB.

*Further information*: Whenever possible, all attempts should be made to make a microbiological diagnosis of TB as outlined in steps 4 and 5, before initiating empiric TB therapy. However, there are circumstances when empiric therapy (the administration of TB therapy without microbiological confirmation of TB) might be warranted. According to the current WHO algorithm for ambulatory HIV patients [7], empiric therapy might be considered in those for which TB is still felt to be likely despite negative Xpert testing (on respiratory and/or non-respiratory samples) or negative sputum microscopy (if Xpert testing is unavailable). However, if the patient's clinical stability will allow for further TB investigations (i.e., repeat sputum testing, repeat chest imaging, abdominal ultrasound and extra-pulmonary sampling) these should be preferentially pursued before initiating empiric therapy. A multi-country trial among ambulatory PLHIV with CD4 counts <50 cells/μL randomized patients to either ART plus isoniazid preventive therapy (IPT) or ART plus active TB treatment after systematic TB screening and further TB investigations were negative [86]. No difference in 24-week mortality between the two arms was found; this suggests that empiric TB treatment does not improve outcomes in ambu-

latory PLHIV if TB investigations are negative and that IPT can be safely initiated even in those with severe immunodeficiency if TB symptom screening and/or subsequent TB investigations are negative.

In hospitalized PLHIV or those who are seriously ill as defined by the presence of one or more danger signs (Fig. 2), if one or more Xpert tests (or sputum smear microscopy where Xpert is not available) and a LF-LAM test (where available) are negative, empiric therapy should be started when the patient fails to clinically improve on broad-spectrum antibiotics within 3–5 days and TB remains clinically suspected. This approach is supported by studies demonstrating that among seriously ill hospitalized patients with smear-negative, but suspected TB, early empiric therapy was associated with reduced hospitalization and improved survival at 8 weeks [87, 88].

The initiation of empiric TB therapy may be necessary in settings where there are limited or no TB investigations routinely available. In patients who are seriously ill due to suspected TB (based on compatible clinical history, exam and/or imaging findings) a clinician at their discretion may choose to start empiric TB-therapy. In such settings, clinical prediction scores may be helpful in assessing which patients should be started on empiric TB therapy (see **Part II**). For example, one study among HIV inpatients with cough (of any duration) and at least one WHO danger sign found that a clinical prediction rule using only clinical, laboratory and radiographic characteristics might have utility for determining who may benefit from empiric TB initiation [89]; a cutoff score of 3 or 4 was associated with a sensitivity of 87–90% and a specificity of 45–59% for culture-confirmed TB and thus might be used to guide initiation of empiric therapy.

**Relevant guidelines:**

- Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: WHO; 2016.

## Conclusions

As more than half of incident TB cases in PLHIV remain undiagnosed and unreported, significant challenges remain in the diagnosis of HIV-associated TB. However, there is much reason to be excited about new and imminent diagnostic tools and tests. With improved implementation of currently recommended WHO universal screening and testing strategies for HIV-associated TB, the diagnosis gap can be greatly reduced allowing for significant progress to be achieved towards improved individual patient outcomes among PLHIV and enhanced TB control.

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