

Infectious Diseases (ID) Learning Unit: How Rapidly to Evaluate for Active Tuberculosis Disease in Low-Prevalence Settings

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With declining tuberculosis (TB) incidence in low-prevalence settings, many clinicians are likely unaware that the approach to diagnosing active TB is evolving with newer technologies. Rapid molecular assays are commercially available, and more are likely to enter the market in the coming years. These tests, such as the Xpert MTB/RIF, which can detect TB and drug-resistance in 2 hours, are increasingly used in settings with higher TB prevalence; however, uptake has been slower in low-prevalence settings. Newer algorithms incorporating rapid TB diagnostics have the ability to alter current clinical and infection control practice patterns. In this learning unit, we review current and newly available tests for the detection of active TB disease and their usage in low-prevalence settings.

Keywords. diagnosis; nucleic acid amplification tests; tuberculosis.

In recent years, several advances have been made in tuberculosis (TB) diagnostics. This Infectious Diseases learning unit reviews current and newly available tests for detection of active TB disease and their usage in low-prevalence settings.

TUBERCULOSIS DIAGNOSIS IN THE MODERN ERA

A 35-year-old man from India presents to the emergency room with 3 months of productive cough. A chest x-ray (CXR) shows a left upper lobe cavitory lesion.

Should You Consider Active Pulmonary Tuberculosis Disease in This Patient?

Symptoms consistent with active pulmonary TB disease may include several weeks of cough, fever, weight loss, hemoptysis, chest pain, or night sweats; findings on chest imaging can be varied and range from cavities, to lobar or miliary infiltrates, to lymphadenopathy [1]. Not all patients fit a “classic” profile, but these signs and symptoms in conjunction with either epidemiologic and/or host risk factors should prompt an evaluation for active pulmonary TB disease. Examples of persons at higher risk of being exposed to TB include those from high-prevalence countries and residents and employees of conjugate settings

such as jails, homeless shelters, and healthcare settings [1]. Examples of persons at increased risk of progressing to active TB disease after initial TB infection include those with human immunodeficiency virus (HIV) infection, diabetes mellitus, smokers, and those who are immunosuppressed or taking immune suppressing medications.

What Diagnostic Testing Should You Pursue to Evaluate for Active Pulmonary Tuberculosis Disease?

Providers commonly use smear-microscopy and mycobacterial culture in the evaluation of TB, but they may be less familiar with newer technologies, such as nucleic acid amplification tests (NAATs). Nucleic acid amplification tests are highly specific and allow for rapid detection of *Mycobacterium tuberculosis* (MTB); they have been described as “game-changers” in the fight against TB, and they are being scaled up around the world [2, 3].

Current Centers for Disease Control and Prevention (CDC) guidance recommends that the following diagnostic tests be obtained on all patients being evaluated for active TB disease [4]: (1) smear microscopy to evaluate for presence of acid-fast bacilli (AFB); (2) mycobacterial culture to evaluate for organism growth—the reference standard (and necessary for phenotypic drug susceptibility testing); and (3) NAAT—molecular detection of MTB genetic material. Nucleic acid amplification tests are rapid and have improved sensitivity compared with smear microscopy; some also detect specific mutations that provide information on drug sensitivity, which would otherwise take weeks.

These tests should be performed on samples from the suspected disease site. For active pulmonary TB disease, 3 consecutive sputum samples should be obtained 8–24 hours apart for

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smear microscopy and culture, with at least 1 being an early morning sample [4]. Centers for Disease Control and Prevention recommends a NAAT on the first obtained sputum, but more can be obtained to increase diagnostic sensitivity.

CONVENTIONAL TESTS HAVE LIMITATIONS: SPUTUM SMEAR MICROSCOPY AND CULTURE

A 70-year-old woman from South Africa presents with a 2-month history of cough, fevers, and a right upper lobe infiltrate on CXR. Three sputum specimens are negative for AFB on smear microscopy. Mycobacterial cultures are pending.

Has Active Tuberculosis Disease Been Excluded as the Diagnosis in This Patient?

Smear microscopy, first used by Robert Koch in the 1880s, relies on direct visualization of AFB in specimens. It is widely used but has limitations. The presence of AFB does not differentiate between MTB and other mycobacteria. In addition, the sensitivity of sputum smear-microscopy ranges from 50% to 80%, and it may be even lower in immunocompromised persons who often have paucibacillary (ie, few bacilli) pulmonary disease due to lack of cavity formation [5]. Some studies suggest sensitivities as low as 30% in HIV-infected persons [6]. Ultimately, providers should not presume that negative AFB smears implies a lack of the presence of MTB; NAAT testing in this case would offer improved diagnostic sensitivity while mycobacterial cultures are pending (Table 1).

Although mycobacterial culture remains the reference standard for the diagnosis of active TB disease, MTB is slow growing, and results may not return for up to 8 weeks. Standard phenotypic drug susceptibility testing requires MTB growth, further adding to treatment delays. Given the need to start TB therapy, initiate isolation, and begin contacting tracing, decisions often cannot wait upon culture. In addition, providers

should be aware that no currently available TB diagnostic test (eg, smear microscopy, NAAT, or culture) has perfect sensitivity; as such, negative TB diagnostic tests cannot completely exclude active TB disease, and final clinical decisions must consider all available clinical, radiographic, and microbiological information.

WHAT IS NEW?

Nucleic Acid Amplification Testing

A 25-year-old man with untreated HIV infection presents with productive cough, fever, and weight loss. A CXR shows nodular infiltrates in the bilateral apices. Sputum for smear microscopy is negative. A NAAT (eg, Xpert MTB/RIF) is positive for MTB. Culture results are pending.

Does This Patient Have Active Tuberculosis Disease?

Nucleic acid amplification tests have high specificity for MTB; a positive NAAT generally indicates a diagnosis of active TB disease in patients presenting with signs or symptoms consistent with TB (Table 1). Nucleic acid amplification tests also have a lower limit of detection than smear microscopy (ie, improved sensitivity), making them useful for rapidly diagnosing smear-negative active TB disease before culture results are available [4]. Nucleic acid amplification tests were incorporated into CDC guidance for active TB disease diagnosis in 2000, but uptake in the United States has been low due to lack of provider awareness, costs, and availability [4, 7].

Multiple NAATs are approved by the US Food and Drug Administration (FDA) for TB detection and have been available in the United States for years; the Amplified *Mycobacterium tuberculosis* Direct Test ([MTD] Gen-Probe, San Diego, CA) is approved for use in both smear-positive and -negative respiratory samples, whereas the Amplicor *Mycobacterium tuberculosis* Test ([Amplicor] Roche Diagnostics, Basel, Switzerland) is only approved for smear-positive samples. Utilization of these tests has been limited by the need for specialized laboratories to perform the assays. Several public and private laboratories also offer their own in-house validated NAATs, and the sensitivity and specificity of these tests can be heterogeneous.

Most recently, the Xpert MTB/RIF assay ([Xpert] Cepheid Inc., Sunnyvale, CA) has been approved by the FDA for use in the United States [8]. This fully automatized cartridge-based NAAT detects TB and also rifampin resistance in approximately 2 hours. It is easy to use, does not require specialized laboratories or training, and has excellent performance with high specificity [9]. The sensitivity of Xpert is superior to smear microscopy, but it is not as good as mycobacterial culture, which remains the reference standard [10]. Therefore, a culture should always be performed concomitantly. In systematic reviews across settings, Xpert sensitivity exceeds 98% in smear-positive samples, and it will additionally identify approximately 50%–70% of smear-negative active pulmonary TB cases

Table 1. Interpretation of Rapidly Available Diagnostic Tests for Active Pulmonary Tuberculosis Disease When Mycobacterial Culture Is Pending

Test	Result			
	+	–	–	+
AFB smear microscopy	+	–	–	+
NAAT	+	+	–	–
Interpretation	TB*	Probable TB*	Possible TB**	Unlikely TB***

Abbreviations: AFB, acid-fast bacilli; MTB, *Mycobacterium tuberculosis*; NAAT, nucleic acid amplification test; TB, tuberculosis; +, positive; –, negative.

*A positive NAAT is suggestive of the presence of MTB, particularly in the setting of a positive AFB smear result. It warrants initiation of therapy for active TB disease while awaiting culture results for confirmation and additional drug sensitivity testing. Interpretation of a positive NAAT with negative mycobacterial culture results warrants consultation with a TB expert.

**In the setting of negative AFB smears and NAAT, a clinical diagnosis of TB may still be made based on consideration of other microbiologic and laboratory test results and the clinical presentation. The decision to initiate empiric TB treatment should be made in consultation with a TB expert, as should the decision to continue therapy if culture results are negative.

***AFB visualized in sputum by smear microscopy is unlikely to represent MTB if the NAAT is negative. The decision to initiate empiric TB therapy in this setting should be made in consultation with a TB expert.

(smear-negative, but later MTB culture-positive) [9, 10]. In low-prevalence, high-resource settings, some authors have suggested lower Xpert sensitivity among smear-negative pulmonary TB cases (approximately 28%), potentially due to patients presenting earlier in their illness and with minimal disease [11]. By contrast, however, a more recent large-scale study comparing Xpert sensitivity among those with smear-negative pulmonary disease in the United States and those in higher prevalence settings found similar sensitivities (approximately 59% vs 53%, respectively) [12]. This study also found obtaining 2 Xperts can increase the tests' overall sensitivity; therefore, providers may consider obtaining more than 1 test if suspicion for active pulmonary TB is high. Xpert's specificity is also excellent (99% in meta-analyses) [9]. However, clinicians should be aware that there may be decreased specificity in patients with recently treated active TB disease, because Xpert positivity does not distinguish live from nonviable bacteria; further research is still needed to elucidate the overall impact of prior treatment on false-positive results [13].

The World Health Organization (WHO) recommends Xpert as the initial diagnostic test in adults and children suspected of having active TB disease with either multidrug-resistant TB or HIV-associated TB [9]. Large investments have consequently occurred to implement this technology in high-prevalence settings; for example, in South Africa, Xpert is largely replacing smear microscopy [3]. The global deployment of Xpert (and other new technologies) for active TB disease diagnosis is in contrast to low-prevalence nations such as the United States, where uptake has been modest despite longstanding CDC guidance to utilize NAAT testing [7]. Although many health departments and hospitals offer Xpert or other NAATs, providers are often unaware of their availability. In addition, whereas some laboratories are able to perform Xpert testing on several specimen types after in-house validation of testing procedures, currently Xpert has FDA approval only for use in sputum specimens. This is in contrast to WHO endorsement for usage on multiple other specimen types (it should be noted that NAAT performance may differ in extrapulmonary specimens, compared with pulmonary samples) [9]. Cost constraints may also limit availability, particularly for laboratories with low testing volumes. Despite evidence that Xpert utilization (ie, 1 sputum sent for Xpert testing in conjunction with 3 sputa for smear and culture) in the United States would be highly cost-effective from a health system perspective, offering NAATs is not always affordable or feasible for laboratories [14].

WHAT IS COMING?

More than 30 new TB diagnostic technologies are under development or are being evaluated [15]. Examples include the lateral-flow detection of lipoarabinomannan, which offers a point-of-care approach using urine specimens and is now WHO-recommended in immunosuppressed HIV-infected

patients as an adjunctive means of rapidly diagnosing active TB disease [16]. Newer molecular assays with improved limits of active TB disease detection are also being developed with sensitivity that may approach that of culture [17].

Researchers are also exploring new usages of emerging technologies. Studies suggest that incorporating Xpert into US hospital infection control algorithms could reduce durations of inpatient airborne isolation by up to 24–48 hours compared with serial evaluation of multiple sputa by smear microscopy [18, 19]. In 1 recent study, the sensitivity of a single Xpert test in the US setting was 82%, compared with 62% for 3 AFB smears [12]. In addition, a single Xpert had a negative predictive value of 99.7% for detecting smear-positive (ie, infectious) active pulmonary TB disease, whereas 2 Xperts had a negative predictive value of 100%. Although not sufficient to exclude a diagnosis of active TB disease, these results suggest that testing of 1 or 2 sputa with Xpert would more rapidly identify the same “smear-positive” cases that are the focus of current isolation policies (which advocate 3 separate sputum samples for smear microscopy). The FDA has now approved the use of 1 or 2 Xpert tests to remove patients from airborne isolation as an alternative to examination of serial sputum smears [8]. However, similar to sputum smear microscopy, clinical decisions regarding the need for continued isolation should take into account (1) all clinical and laboratory data and (2) the overall likelihood of infectious active pulmonary TB disease.

WHAT ABOUT INTERFERON-GAMMA RELEASE ASSAYS AND THE TUBERCULIN SKIN TEST?

A 27-year-old man from Mexico presents with a 2 cm cavitary lesion in his left lower lobe. An interferon-gamma release assays (IGRAs) is positive. Smear microscopy, Xpert, and culture on 3 sputa are negative. His sputum grows *Coccidioides immitis*.

Does This Patient's Positive Interferon Gamma Release Assays Mean He Has Active Tuberculosis Disease?

Interferon-gamma release assays and tuberculin skin test (TST) are indirect immune-based tests that do not differentiate between active disease (replicating bacilli) and latent infection (little metabolic activity). Therefore, a positive test does not confirm a diagnosis of active TB disease. In addition, because the sensitivity of these tests in active TB disease is between 70% and 90%, negative results do not exclude TB diagnosis [20]. Providers should primarily use these tests for targeted latent TB screening in persons at high risk for TB infection or progression; they should not rely on TST and/or IGRAs to diagnose active TB, nor should they use them as “triage” tests before considering additional evaluation. All patients with signs and/or symptoms of active TB warrant evaluation with AFB smear, NAAT, and mycobacterial culture, irrespective of their TST and/or IGRA results. This patient likely has latent TB infection, but his acute presentation is most consistent with *Coccidioides* infection.

CONCLUSIONS

Current United States-based recommendations are to obtain samples for smear microscopy, culture, and NAAT testing (such as Xpert) in persons thought to have active TB disease. Nucleic acid amplification tests are rapid, available commercially and through public health departments, and can identify more cases of active pulmonary TB disease than smear microscopy alone. The sensitivity of available NAATs remains less than that of mycobacterial culture, and culture currently remains the reference standard for diagnosis of active TB disease. However, newer diagnostic technologies are emerging and will continue to alter the landscape of TB diagnosis domestically and globally.

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