EDUCATIONAL COMMENTARY – TUBERCULOSIS PART II: DIAGNOSTIC TESTING FOR THE DETECTION AND TREATMENT OF MYCOBACTERIUM TUBERCULOSIS INFECTIONS

Educational commentary is provided through our affiliation with the American Society for Clinical Pathology (ASCP). To obtain FREE CME/CMLE credits, click on Earn CE Credits under Continuing Education on the left side of the screen.

Learning Objectives

On completion of this exercise, the participant should be able to

- explain laboratory safety requirements for mycobacteria laboratories to process patient specimens and isolate Mycobacterium tuberculosis in culture;
- describe the types of specimens submitted to the clinical laboratory for detection of Mycobacterium tuberculosis;
- explain appropriate digestion and decontamination procedures on specimens submitted for mycobacterial culture, including the purpose of each reagent;
- discuss the methods used for diagnosis of active tuberculosis infections, including advantages and disadvantages of each;
- correlate rapid diagnostic testing results for Mycobacterium tuberculosis, including acid-fast bacilli smears and nucleic acid amplification testing, with relevant clinical history; and
- summarize antimycobacterial susceptibility testing for Mycobacterium tuberculosis, including the antimycobacterial agents used in testing.

Introduction

This Educational Commentary is part II to the previously published commentary, “Tuberculosis Part I: An Old Disease With New Threats.” Part I discussed the different states of tuberculosis (TB) infections including latent vs active infections, screening tests for diagnosis of latent infections, diagnosis of active TB disease, and treatment of Mycobacterium tuberculosis infections. This commentary will go into more depth concerning the clinical laboratory tests used to diagnose and treat active TB infections.

Tuberculosis disease is diagnosed using the patient’s medical history; physical examination; chest x-ray; and laboratory tests, which include detection of Mycobacterium tuberculosis through smears, cultures, and molecular diagnostic methods. The diagnosis of active TB disease requires key epidemiologic risk factors combined with suggestive clinical and radiographic findings, followed by confirmation, which requires a specimen collected from the patient to be sent to a clinical microbiology laboratory for detection and isolation of Mycobacterium tuberculosis.

Early and accurate detection, diagnosis, and reporting of TB cases leading to initiation and completion of treatment for the infection is fundamental to TB control. Laboratory testing has proven to be an essential component of TB diagnosis and control of the disease at a local, state, national, and global level. In the United States, up to 80% of all initial TB-related testing, including acid-fast bacillus (AFB) smear
EDUCATIONAL COMMENTARY – TUBERCULOSIS PART II: DIAGNOSTIC TESTING FOR THE DETECTION AND TREATMENT OF MYCOBACTERIUM TUBERCULOSIS INFECTIONS (cont.)

microscopy and culture, is performed in hospitals, clinics, and independent laboratories outside of the public health system. More than 50% of species identification and antimycobacterial susceptibility testing (AST) for mycobacteria is performed in public health laboratories. Because of this, a network of public and private laboratories is necessary to facilitate timely laboratory testing and flow of information. Public health laboratory professionals are key players in the public health sector as leaders in developing communication among laboratory professionals, clinicians, and TB experts to promote TB control.

Seven types of tests for the diagnosis of TB disease and detection of drug resistance are performed in clinical laboratories (Table 1). These tests facilitate decisions about initiating treatment for TB, infection control measures, and reporting case information to the public health department for investigation. Acid-fast bacillus smears and nucleic acid amplification tests (NAATs) can be performed with rapid turnaround times from specimen collection. In patients with a positive result on an AFB smear from sputum, a NAAT is performed to provide rapid confirmation that the infecting organism is from the *M tuberculosis* complex (MTBC). The NAAT is performed in addition to a mycobacterial culture and AFB smear microscopy because the NAAT is not sensitive enough to replace mycobacterial culture for diagnosis and does not produce colony growth, which is necessary for phenotypic AST.

### Table 1. Clinical laboratory tests for *Mycobacterium tuberculosis*.

<table>
<thead>
<tr>
<th>Test</th>
<th>Time Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acid amplification test, detection</td>
<td>1 d</td>
</tr>
<tr>
<td>Nucleic acid amplification test, resistance markers</td>
<td>1-2 d</td>
</tr>
<tr>
<td>Acid-fast bacillus smear microscopy</td>
<td>1 d</td>
</tr>
<tr>
<td>Culture growth</td>
<td>≤6-8 wk (average 10-14 d)</td>
</tr>
<tr>
<td>Liquid (broth-based) medium</td>
<td>(average 3-4 wk)</td>
</tr>
<tr>
<td>Solid (agar- or egg-based) medium</td>
<td></td>
</tr>
<tr>
<td>Identification of <em>Mycobacterium tuberculosis</em> complex by DNA probe</td>
<td>1 d&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First-line antimycobacterial susceptibility testing (liquid medium)</td>
<td>1-2 wk&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Second-line and novel compound AST</td>
<td></td>
</tr>
<tr>
<td>Liquid (broth-based) medium</td>
<td>1-2 wk&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Solid (agar- or egg-based) medium</td>
<td>3-4 wk&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>After detection of growth in culture

### Laboratory Safety

The serious nature of TB and the airborne route of infection require critical safety measures when working with patient specimens. Patients with active TB disease can be heavily infected, increasing the risk of human-to-human transfer. If special safety precautions and appropriate personal protective equipment are used, the chance that the laboratorian will be infected is minimal. The Centers for Disease Control and Prevention (CDC) recommends the use of Biosafety Level 3 (BSL-3) practices for those laboratories performing manipulation and propagation of *M tuberculosis*. Biosafety Level 3 standards include...
requirements for BSL-1– and BSL-2–level laboratories with additional criteria for use of PPE, environmental controls, biological safety equipment, and disinfectants.

A BSL-3 laboratory has special engineering and design features. In addition to standard clinical laboratory safety practices, all personnel working in a BSL-3 laboratory must adhere to special safety practices, equipment, and facility requirements. Personal protective equipment includes gloves; laboratory coats or gowns that close in the back rather than front; and eye/face protection, including goggles, masks, face shields, other splash guards, or all. Respirator masks such as N95 should be used when processing, propagating, and manipulating cultures. The N95 respirators will filter at least 95% of airborne particles. The use of hair caps and shoe covers is also recommended.3,4

Regulations have been established governing the ventilation and exhaust systems used in BSL-3 laboratories. Facility requirements include the physical separation of the mycobacteria laboratory from access corridors with self-closing, double-door access, no recirculation of exhaustive air, and negative airflow into the laboratory. The negative air flow will assure that any particles that may become airborne will be kept in the mycobacteria laboratory. The mycobacteria room should have 6 to 12 room air changes per hour, which will effectively remove 99% or more of airborne particles within 30 to 45 minutes. Increasing the frequency of room air changes may disrupt biological safety cabinets (BSCs) and is not recommended. Exhaust air from the laboratory should be dispersed away from occupied areas and must be filtered with high-efficiency particulate air (HEPA) filtration. These regulations are in place to limit exposure to the mycobacterial organisms that are able to float on the air currents and remain suspended in air for long periods of time because of their high-lipid cell wall. The mycobacteria laboratory must also have controlled access and the ability to decontaminate all waste and laboratory clothing before laundering.3,4

All procedures involving the manipulation of patient specimens and cultures must be conducted within a Class II or Class III BSC. Before and after completion of work in the BSCs, the area should be disinfected. Surface areas should be decontaminated using mycobactericidal agents. Appropriate disinfectants include 10% bleach, 70% ethanol, and phenolic solutions. All work with specimens should be performed over disinfectant-soaked pads or towels, and specimens should be decanted into a splash-proof container with disinfectant. Because mycobacteria are small, lightweight organisms that remain suspended in air currents, ultraviolet (UV) light that is UV-C band (254nm wavelength) is used to disinfect work areas when not in use. Ultraviolet light will kill airborne mycobacterial organisms. In addition, self-contained carriers in centrifuges are used to control aerosols when processing patient specimens. Mycobacterial specimens are centrifuged at high speeds, which can potentially collapse the specimen containers. The use of self-contained carriers prevents aerosolization in the event that a specimen
container should break in the centrifuge. Once centrifugation is complete, containers are opened only in
the BSC.3,4 It is important to ensure that smears prepared for staining are heat fixed inside the BSC
before they are removed for staining.

More detailed information regarding biosafety in microbiology and biomedical laboratories, including
mycobacteria laboratories, can be found at the Centers for Disease Control and Prevention website

Clinical Specimens

Pulmonary Tuberculosis

Patients with suspected pulmonary TB should have a sputum specimen collected and sent to the clinical
laboratory for an AFB smear and mycobacterial culture. The sputum may be obtained spontaneously by
coughing or induced, but care should be taken to get secretions from the lower respiratory tract. At least
5 to 10 mL is optimal. Three specimens should be collected 8 to 24 hours apart to increase the chance of
isolation. Early-morning sputum specimens are preferred, as they are the most concentrated. On at least
one of the sputum specimens, a NAAT should be performed.2

Sputum specimens have the same diagnostic yield as bronchoscopy specimens (bronchoalveolar lavage
or brushings), are less invasive for the patient, and have lower direct costs.5 Bronchoscopy specimens
can be obtained from patients when attempts to obtain a sputum specimen are unsuccessful, in patients
with suspected TB who have negative mycobacterial AFB smears and cultures, and when a diagnostic
bronchoscopy is required for another reason.

Tissue (pleural) biopsy specimens can aid a definitive diagnosis of pulmonary TB when other testing of
the sputum and/or bronchoscopy specimens is not diagnostic. Biopsy specimens allow for
histopathologic examination and microbiology testing, including culture.

Extrapulmonary Tuberculosis

The diagnostic approach for extrapulmonary TB should be guided by signs and symptoms localized to the
area of disease involvement. Radiographic imaging aids in identification of the infected body system.6 In
patients with neurologic signs and symptoms, a lumbar puncture should be performed to collect a
cerebrospinal fluid specimen. If tuberculosis meningitis is suspected, a cerebrospinal fluid specimen
should be analyzed for cell count, protein, and glucose concentrations as well as by AFB smear and
culture.

Biopsy specimens from the lung, bone marrow, bones, joints, bowel, liver, brain, and other tissues allow
for histopathologic examination, AFB smear, and mycobacterial culture. Liver specimens are generally
associated with the highest yield for diagnosis of extrapulmonary TB.\(^6\) If pleural effusion, ascites, or pericardial effusion is suspected, the fluid should be collected for AFB smears and culture. A pleural biopsy specimen can also be submitted to aid in the diagnosis of pleural effusion.

**Specimen Processing**

If \( M\) \( tuberculosis\) is suspected as the cause of an infection, a mycobacterial culture with AFB smear must be ordered to recover the organism. Mycobacteria grow more slowly than many bacteria found in nonsterile specimens such as respiratory secretions. The presence of other bacteria in respiratory and other non-sterile specimens requires the use of decontamination agents such as sodium hydroxide to kill the non-mycobacterial organisms. If decontamination techniques are not used, the non-mycobacterial organisms in the specimen could overgrow the media and make it impossible or extremely difficult to recover the mycobacteria species. In addition, clinical specimens such as sputum can contain mucus (mucin) or other organic debris surrounding the bacteria in the sample. For decontamination agents to act on the non-mycobacterial organisms, the organisms must be released from the mucus or organic debris that surround them. The use of a mucolytic agent, such as \( N\)-acetyl-\( L\)-cystine (NALC), to break down mucus in the specimen increases the recovery of mycobacterial organisms and allows the decontaminant to act on the non-mycobacterial organisms. Mycobacteria are often present in very small numbers in clinical specimens. Centrifugation is performed to concentrate the organisms that may be in small numbers. The concentrated specimen can then be used to make smears for AFB staining and inoculating culture media. Centrifugation is performed even if the specimen does not require decontamination.\(^4\)

**Rapid Diagnostic Tools**

**Acid-fast Bacilli Smear Microscopy**

The slow growth rate of mycobacteria makes direct detection by microscopy of primary importance. Detection of AFB via microscopic examination of stained specimens is the most rapid and inexpensive TB diagnostic tool. Smears can be prepared directly from clinical specimens or from concentrated preparations of clinical specimens. Concentrated smears have a greater ability to detect the mycobacteria and should be included when an AFB smear is ordered. It is important to recognize that smears positive for AFB may be nontuberculous mycobacteria or \( M\) \( tuberculosis\). In addition, organisms such as \( Nocardia\) species and \( Rhodococcus\) can stain at least partially AFB positive. Identification requires culture, NAAT, or both.

The Gram stain is not a useful tool for the detection of mycobacterial organisms due to the increased lipid content of the cell wall. When Gram-stained, \( Mycobacterium\) species will take up the basic dye
irregularly, giving a beaded appearance, or may not stain at all. Specific staining techniques have been developed to detect mycobacteria. Because of their high lipid content, the cell walls of mycobacteria are able to bind to fuchsin dye so that it is not decolorized with acid alcohol. Carbolfuchsin and fluorochrome dyes are used in the acid-fast stains to bind to the mycolic acid in the cell wall and resist decolorization with acid alcohol.

Fluorochrome stains are a good screening test because they are less time-consuming to perform and more sensitive than the carbolfuchsin stains. The smear is scanned on lower magnification (200x to 400x) with a fluorescent microscope to look for fluorescing bacilli, which are yellow-orange or yellow-green, depending on the stain, against a dark background (Figures 1 and 2). At 200x magnification, at least 30 fields should be examined before a smear is considered negative. At a magnification of 400x, 50 to 55 fields should be examined.3
Carbolfuchsin stains include the Ziehl-Neelsen and Kinyoun stains. The Ziehl-Neelsen stain is referred to as a “hot” stain because heat is used to improve penetration of the stain into the bacterial cell wall. The Kinyoun stain is referred to as a “cold” stain because heat is not included in the procedure. Instead of heat, a surface-active agent in the Kinyoun stain increases the permeability of the dye into the cell wall. The smears should be examined by an experienced microbiologist and 300 oil-immersion fields should be viewed when interpreting carbolfuchsin AFB smears before a smear is considered negative; this is equivalent to three horizontal sweeps of a slide that is 2 cm long and 1 cm wide.\(^4\) The carbolfuchsin stains are considered confirmatory stains because they are more specific; mycobacteria are the only rod-shaped bacteria that will stain a bright red (magenta) (Figures 3 and 4). Disadvantages include that the smears must be read on oil immersion (total magnification of 1000x), they are time-consuming to interpret, and nonviable organisms will not stain.

When interpreting AFB smears, the slide should be scanned for cells showing typical mycobacterial morphology. Acid-fast bacilli are usually slender long or short rods that can be granular or beaded. Acid-fast bacilli can show the formation of serpentine cords within the smear, which is typical of M. tuberculosi. Mycobacteria other than M. tuberculosi can be pleomorphic, thick, or diphtheroid, or may
be very long or coccoid. When interpreting AFB smears, it should be remembered that other organisms such as *Nocardia* species and *Rhodococcus* can stain at least partially AFB positive and should not be misidentified as mycobacteria. The lens should be wiped after reading a positive smear, because the mycobacterial cells can float and contaminate other slides, causing false-positive results. When interpreting direct specimen smears, the number of AFB per field are classified as 4+, 3+, 2+, or 1+. The greater the number, the more infectious the patient. A quantitative report is provided to the clinician, which allows the clinician to follow the disease state of the patient.⁴

A presumptive diagnosis of active TB can be made when AFB are found in the sputum and the patient has clinical symptoms such as history of cough, weight loss, and pulmonary infiltrates.² Acid-fast bacillus
smears are also useful in following treatment. If treatment is effective, smaller numbers of the organism are shed, so the number of organisms shed can be an indicator of treatment success. If organisms fail to decrease after therapy, this may indicate treatment failure due to drug resistance. Negative smears do not exclude TB disease, as patients with TB disease have had negative AFB smears with a subsequent positive culture.

Molecular Diagnostics

The laboratory tests available for diagnosis of active *M tuberculosis* infection have recently evolved, with NAAT molecular methods becoming a mainstay in the diagnostic process. The NAAT can be used to rapidly and reliably detect MTBC from patient specimens in hours, compared with 1 week or more for culture. The CDC recommends that NAAT be performed on at least one respiratory specimen from every patient with signs and symptoms of pulmonary TB when a diagnosis of TB is being considered but is not yet established, and when the test results could alter case management for the patient or TB-control procedures such as contact investigations.7,8

Nucleic acid amplification tests have higher sensitivity than AFB smear microscopy and shorter turnaround times than culture. Diagnosis of pulmonary TB disease is enhanced when NAAT is used in conjunction with the AFB smear. One study found that in AFB smear-positive specimens, the sensitivity and specificity for NAAT were 96% and 85%, respectively. When AFB smear microscopy was negative, sensitivity decreased to 66% and specificity increased to 98%.9 Another study found the sensitivity of AFB smear-positive respiratory samples to be greater than 95%.10 In AFB smear-positive patients, NAAT yields false-negative results only 4% of the time, indicating that it is reliable for excluding pulmonary TB. In AFB smear-negative patients, clinical signs and symptoms must be considered and a positive NAAT result can be used as presumptive evidence of TB and guide therapeutic decisions. If clinical suspicion for TB is low, NAAT should not be performed because false-positive results are frequent.2 An algorithm for interpretation and use of NAAT results in conjunction with AFB smear results can be found at https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5412a1.htm.8

There are several molecular detection methods used in the United States for detection of MTBC, including the Amplified *Mycobacterium tuberculosis* Direct Test (Hologic), Cepheid GeneXpert (Xpert) MTB/RIF, line probe assays, and real-time PCR and DNA sequencing.11 Molecular tests performed in clinical laboratories can be either laboratory-developed tests or US Food and Drug Administration (FDA)-approved tests. Every laboratory performing testing should provide a description of the type of molecular method, laboratory-developed or FDA-approved, method description (DNA, RNA, or nucleic acid), and the sensitivity and specificity of the test for both AFB smear-positive and AFB smear-negative specimens. The reporting language should be standardized to avoid confusion or clinical misinterpretation of data,
which could delay proper patient treatment decisions. Suggested terminology has been created by the Association of Public Health Laboratories Tuberculosis Subcommittee and can be found at https://www.aphl.org/aboutAPHL/publications/Documents/ID_2017Apr-MTBC-Suggested-Reporting-Language.pdf.11

Nucleic acid amplification testing has significantly increased capabilities for earlier detection of MTBC, but it does not replace the AFB smear or culture. The smear results are used to qualify the sensitivity of the NAAT and to indicate the relative infectiousness of the patient. Culture is considered the criterion standard for diagnosis of TB disease and is also required for AST and genotyping. Nucleic acid amplification test methods detect MTBC, which includes the human pathogens *M tuberculosis*, *M africanum*, and *M bovis*. However, NAAT cannot differentiate between these species; if speciation is required, a culture must be performed. Nevertheless, NAAT should be available in the clinical microbiology laboratory and standard practice for patients with suspected TB.

**Culture for Identification of *Mycobacterium tuberculosis***

Two categories of media are routinely used to isolate mycobacteria in the clinical laboratory: solid and liquid media. It is recommended that both liquid and solid mycobacterial cultures be used, rather than performed alone, for every specimen obtained from a patient with suspected TB disease.² Any organism isolated in mycobacterial cultures should be identified according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and as directed in the American Society for Microbiology *Manual of Clinical Microbiology*.⁴,¹² Once growth is detected in culture, the sample is often sent to a reference laboratory for species identification and AST.

The two types of solid media are egg-based media and agar-based media. Lowenstein-Jensen medium is the most common egg-based medium used and contains malachite green, which inhibits the growth of contaminating bacteria and fungi. Average time to detection of *M tuberculosis* growth is long, requiring an incubation protocol of up to 8 weeks. Common agar-based media include Middlebrook 7H10 and Middlebrook 7H11. The average time to detection of growth is earlier than for egg-based media, with a recommended incubation period of 6 weeks for *M tuberculosis*.⁴

Liquid media enhance the rate of growth of mycobacteria. Use of a liquid medium has been shown to reduce the time to isolation of mycobacteria to about 10 days, significantly less than the 17 days or longer on typical solid media. Isolation and susceptibility testing of *M tuberculosis* can be reported in an average of 4 weeks’ time when liquid media are used. The CDC recommends the use of liquid media for detection and AST in the United States due to the significant increase in positive culture rates and time saved to diagnosis.⁴
After processing, specimens should be inoculated onto at least one type of solid medium and a liquid culture medium. Solid media should be examined once or twice weekly for growth. Liquid media should be examined at least every 2 to 3 days; some automated platforms provide real-time monitoring with immediate notification of growth detection. Optimal temperature for growth of *M. tuberculosis* is 35°C to 37°C, and growth on solid media is enhanced with 5% to 10% CO₂ and high humidity. Most solid media require 8 weeks of incubation before they can be reported as negative for growth. Most commercial liquid media require a maximum of 6 weeks. Smear-negative specimens require longer time for culture positivity than smear-positive specimens, and should be incubated for at least 6 weeks before reporting them as negative for growth. Once growth is detected in culture, additional testing and characteristics guide identification of the mycobacterial species. Characteristics such as growth rate, colony morphology including texture, shape and size, and pigment production or photoreactivity give a preliminary subgroup. Once placed into a subgroup, the species can be identified by biochemical tests or alternative methods such as NAAT or matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS).

*Mycobacterium tuberculosis* is a slow-growing organism; colonies are usually isolated in 2 to 3 weeks on conventional egg-based media at 35°C to 37°C with increased CO₂. Automated liquid-based media decrease the time for detection by 1 to 2 weeks compared with solid media. *Mycobacterium tuberculosis* colonies are classically described as being “rough and buff”: colonies of this organism appear small, dry, and rough and are buff-colored, resembling bread crumbs (Figure 5). After isolation of growth on media, colonies are stained by carbolfuchsin methods to determine acid fastness. *Mycobacterium tuberculosis* appears as acid-fast rods and may form serpentine cords, a unique characteristic among the mycobacteria. DNA probes can be used to identify MTBC, but for species identification, biochemical testing is needed. *Mycobacterium tuberculosis* does not produce a heat-stable catalase and is niacin-accumulation and nitrate-reduction positive. The most useful biochemical test for identification of *M tuberculosis* from other species of MTBC is the niacin-accumulation test, because *M tuberculosis* is the only member of the complex that is positive. Pyrazinamidase is also positive for *M tuberculosis* and can be used for speciation of MTBC.

Figure 5. *Mycobacterium tuberculosis* colony morphology on Lowenstein-Jensen agar
**Mycobacterium tuberculosis** Drug Susceptibility Testing and Resistance Detection

The CDC recommends AST on all *M. tuberculosis* isolates.² Solid media or liquid media may be used. Time required is at least 7 days for liquid media and at least 1 month for solid media. The breakpoint between a resistant and susceptible strain is the critical concentration; this is the level of drug in the culture medium that inhibits 95% of the wild-type TB strains that have not been exposed to the drug, but does not suppress the growth of strains that are resistant to the drug (which were established via clinical treatment failure). The CLSI recommends testing a full panel of the first-line drugs (rifampin, isoniazid, pyrazinamide, and ethambutol) because this combination of tests gives clinicians comprehensive information related to the four-drug antituberculosis therapy currently recommended for most patients in the United States.¹³ Identification of resistance to rifampin or another first-line drug should prompt susceptibility testing for the second-line drugs, including amikacin, capreomycin, a fluoroquinolone, and ethionamide. Other drugs that may be tested include cycloserine, para-aminosalicylic acid, rifabutin, linezolid, and clofazimine.¹³

Phenotypic culture-based AST is considered the gold standard but can take 2 weeks or more for return of results. Rapid molecular tests have been developed that can be performed within hours to detect resistance to rifampin and isoniazid. Detection of rifampin resistance or susceptibility is helpful in prescribing empiric antituberculosis treatment at the time of diagnosis. If resistance is detected, it is a good indicator of multidrug-resistant *M. tuberculosis* (MDR-TB) in locations where rifampin monoresistance is uncommon. However, positive predictive value is low in areas where rifampin resistance is not common. In these areas, confirmation of a positive test result for rifampin resistance is recommended. Rapid molecular AST for rifampin with or without isoniazid is recommended for subgroups in which drug resistance is more likely. Examples of patient populations for testing include those who have a positive NAAT or AFB smear result and meet one of the following criteria: (1) have been treated for tuberculosis in the past; (2) were born in or have lived at least 1 year in a country outside the United States with at least a moderate TB incidence (≥20 per 100,000) or a high primary MDR-TB prevalence (≥2%); (3) are contacts of patients with MDR-TB; or (4) are infected with HIV.² Molecular AST should only be performed on the respiratory specimens of persons who are either AFB smear positive or NAAT positive for MTBC.

**Conclusion**

Patients exhibiting relevant clinical symptoms (e.g., fever, cough, shortness of breath, night sweats, chills, weight loss) and epidemiologic factors should be evaluated for TB. Diagnosis of TB is definitively established by isolation of *M. tuberculosis* from a body secretion (e.g., sputum, bronchoalveolar lavage, pleural fluid) or tissue (e.g., lung biopsy). Additional rapid diagnostic tools include the AFB smear and
NAAT. Positive NAAT with or without AFB smear positivity is considered sufficient for diagnosis of TB, but radiographic evidence is important to support the diagnosis.\(^2\) Detection of resistance to rifampin and isoniazid via molecular methods can provide guidance for initial therapy decisions, but AST should be performed on all \(M\) \(tuberculosis\) isolates to inform directed therapy decisions regarding patient treatment, as MDR-TB infections are increasing.

**References**


