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Laboratory Diagnosis of Tuberculosis in Primary Care

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Introduction

This component of the series on tuberculosis in primary care will focus on laboratory diagnosis. Despite the advances in diagnosis described here, up to 20% of U.S. tuberculosis cases are clinically diagnosed.¹ Recommendations for the core laboratory features of a tuberculosis control program include² the following:

- Rapid microscopic evaluation for acid-fast bacilli;
- Nucleic acid amplification assay (NAA/polymerase chain reaction (PCR)) of collected sample;
- Culture, with detection and subsequent identification of samples within 3 weeks (which presupposes use of automated broth culture methods); and,
- Drug susceptibility testing for both first- and second-line drugs (usually performed at the state lab or national referral center level).

The evidence on laboratory diagnosis of tuberculosis allows a systematic approach to testing in a given patient (Fig 1). Details regarding specimen collection and handling for various anatomic sites are published.^{3,4} Note that while some settings enjoy a high diagnostic yield by laboratory methods, no modality is sufficient in isolation. Smear and culture-negative tuberculosis is a common phenomenon.⁵ CDC surveillance data from 2004 reveals 42% smear-negative and 17% culture-negative rates for pulmonary tuberculosis.¹ Smear-negative index cases contribute to as much as 17% of newly transmitted cases.⁶

Bacteriology and Microscopy

Tuberculosis is caused by a bacillus of the *Mycobacterium tuberculosis* complex. The most common of the group, *M. tuberculosis*, is a respiratory pathogen communicable between infected humans. While different au-

FIG 1. Clinical algorithm for the laboratory diagnosis of tuberculosis.

thors cite a variety of members in the group based upon varying molecular and biochemical criteria, the most commonly accepted grouping includes *M. tuberculosis*, *M. microti*, *M. africanum*, *M. bovis*, and *M. canetti*. The organisms grow slowly and stain acid fast because of a highly

lipid and peptidoglycan-rich cell wall.⁷ Evaluation of sputum smears and tissue samples is accomplished with two main stains: Ziehl–Neelsen and auramine orange fluorescence.³

A large, prospective study was conducted to evaluate the operational clinical performance of various technologies.⁸ Auramine orange fluorescence staining of three sputum samples per patient detected 7 of every 10 cases. The false-positive rate was 2 in 10. In other studies with culture-proven disease, a single sputum sample detected disease in one-half to two-thirds of cases, while three or four specimens detected as many as 9 of every 10 cases.^{9,10} Most of the advantage in testing serial sputum specimens is gained with the second specimen collected. While spontaneous sputum samples are employed in many settings, use of induced sputum sampling confers an advantage.¹¹ Both watery and viscous samples should be tested.

In most cases bronchoscopy for lavage is not required. It may be appropriate when sputum smears are negative in the setting of a known radiographic lesion allowing focused lavage. Induced sputum collection and bronchoalveolar lavage can be used together, detecting 8 of 10 patients.¹² Simple instructions and coaxing can dramatically increase sputum smear yield.¹³

Auramine staining of sputum is superior.^{14,15} It has greater sensitivity with a requirement for fewer screened microscopic fields as a lower magnification can be used. Stain superiority in tissue samples is not clear.^{16,17} Typically, 10^4 to 10^6 organisms/ml are required for detection,^{3,15} although concentration techniques can lower this number.

The hallmark finding on histopathology is the necrotizing or caseating granuloma. When found, tuberculosis should be presumed until disproven. The number of granulomas observed may vary by biopsy site—high numbers in the lung, low numbers in the bone marrow.¹⁸ Nontuberculosis mycobacteria also may cause necrotizing granulomas. HIV status may affect the observed histopathology of biopsy samples.¹⁹

Culture

Culture remains the gold standard for diagnosis. Egg-based plate media such as Löwenstein–Jensen are used, but agar media such as Selective 7H11 and liquid-based media (Becton–Dickinson and Co., BACTEC™ and BACTEC™ MGIT™) now are the standard. Some centers use all of these modalities to capture rare strains.³ Growth in liquid media-automated detection systems is monitored by radiometric, colorimetric, or pressure monitoring. Growth often can be detected within 2 weeks. Typical hold periods are for 4 to 6 weeks. Use of these systems also

allows expeditious drug susceptibility assessment when agents are added into the liquid media.

M. tuberculosis grows at 37°C with a 5 to 10% CO₂ mixture. It can be distinguished from some atypical pathogens by its absence of growth at room temperature, beading when stained from media, and propensity to form cords on 7H11 agar.²⁰ With a generation time of over 12 hours, sample contamination and overgrowth by conventional bacteria is a continuing challenge. Biochemical assays may be used to distinguish *M. tuberculosis* from other members of the complex when molecular methods are not specific, such as growth in niacin, positive nitrate reduction test, and high-performance liquid chromatographic analysis.³ False-positive culture results may occur from laboratory contamination, or misidentification of an atypical mycobacterium. Pretreatment with a fluoroquinolone may delay diagnosis by more than 2 weeks, including delays in culture positivity.^{21,22}

The Orange County study outpaced CDC data on culture yields. Solid media detected 3 of every 4 cases, but the BACTEC™ 12B detected 19 of every 20 cases (SENS 95%).⁸ Mean culture turnaround times were 18 days for BACTEC™ 12B, and over 30 days for the solid media. No single modality captured all infections in this study.

Culture yields in extrapulmonary tuberculosis are significantly lower than from sputum samples. Improvements in sample processing for use in PCR have increased culture yields, resulting in detection of less than 10² organisms/mL.^{23,24} Pleural culture yields range from 23 to 67% from fluid, and 90 to 97% from biopsy.²⁵ Despite high yields, finding providers to perform pleural biopsy is problematic.

All new culture isolates should be submitted for drug susceptibility testing. This requires submission to referral centers, or special requests of commercial laboratories.

Molecular Methods: NAA/PCR

NAA assays allow rapid turnaround time (24 to 48 hours) when samples are smear positive and can be done on smear-negative specimens, but with less sensitivity and specificity. They are performed directly on specially processed specimens to provide simultaneous detection and identification. Culture of the organism is still necessary to confirm the identification and to perform drug-sensitivity tests. Molecular targets usually include both an insertion sequence and a 16S rRNA sequence. A recent comprehensive review on PCR utility is available.²⁶ Direct PCR tests must be explicitly ordered in most circumstances because of the added costs of the amplification technique.

Two FDA-approved molecular tests are available for rapid diagnosis: a revised Amplified Mycobacterium Tuberculosis Direct Test (MTD) by Gen-Probe, and the Mycobacterium Tuberculosis Test (MTT) by Ampli-cor.²⁷ The Orange County study detected 9 of every 10 cases in 1 to 2 days with the revised MTD.⁸ PCR in sputum should be used to confirm diagnosis on a smear-positive specimen, though it has been used in primary diagnosis.²⁸

Results in extrapulmonary testing demonstrate decreased sensitivity. One method incorporating both *devR* and *IS6110* primers yielded positive results in two-thirds of cases of lymph node tuberculous disease.²⁷

These are amplification tests. With positive culture material, a nonamplified sequence test can be performed. PCR sequences for *M. avium intracellulare* and *M. kansasii* also are available.

Molecular Methods: Inflammatory Marker Measurements

Inflammatory marker measurements exploit differences in the predominating immune response to different pathogens. Adenosine deaminase (ADA) and interferon gamma (IFN- γ) sometimes are used to diagnose tuberculous effusions.²⁹ Other inflammatory markers also have been studied.³⁰ These assays, too, may be compromised in immunocompromised patients.³¹ Combined PCR, ADA, and culture techniques have been used.^{32,33}

QuantiFERON[®]-TB Gold Test (QFT-G) is discussed for LTBI elsewhere in this compendium. It has been studied in large cohorts,³⁴⁻³⁶ including HIV patients.³⁷ Relative anergy during active disease can occur, though it may outperform TST.^{38,39} Other assays are under development.⁴⁰

Fluid Cell Counts and Chemistries, Nonspecific Changes

Fluid analysis may provide helpful clues in the diagnosis of tuberculosis. Involved pleural, pericardial, and CSF fluid may manifest neutrophilia, although a lymphocytosis eventually predominates. Protein is elevated and glucose levels may be low or low normal. Lactate dehydrogenase usually is elevated as effusions most often are exudative. Persistent sterile pyuria in an ill patient should raise suspicion for tuberculosis. Detailed descriptions of extrapulmonary tuberculosis findings are published.⁴¹

A wide range of nonspecific findings also may suggest tuberculosis.

These changes are more prevalent with worsening disease.⁴² In pulmonary disease, up to three-quarters of moderately ill patients will have anemia with a low serum iron and total iron binding content, and erythrocyte sedimentation rate elevation. Half to two-thirds of patients will have a peripheral neutrophilia, monocytosis, and thrombocytosis. Rarer findings include lymphopenia, leukopenia, folic acid deficiency, macrocytic anemia, pancytopenia, and thrombocytopenia. Miliary disease may manifest an elevation of alkaline phosphatase, normal or mildly elevated hepatic transaminases and adrenal insufficiency.⁴³

Summary

Laboratory diagnosis of tuberculosis requires a multimodality approach, as well as recognition that empiric therapy often is appropriate. New technologies such as proteomics and microarrays may soon find their place in the evaluation of patients.^{44,45} However, sputum and tissue analysis remain the cornerstone of laboratory diagnosis. Providers must be aware of how their laboratories process specimens, and which tests are an automatic part of evaluation. A direct PCR often must be requested separately.

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