

Section II.

Initial Evaluation of Suspected Tuberculosis

Pathogenesis of Tuberculosis

Transmission, Infection and Proliferation

Transmission of tuberculosis (TB) occurs when infected patients expel small droplets containing tubercle bacilli into the air (when they cough, sing or speak) and a susceptible person inhales the bacilli and becomes infected. These tiny droplet nuclei (1-5 μm in diameter) float in air, the fluid evaporates and the living tubercle bacillus may remain airborne for long periods until inhaled.

Initially, there is rapid inflammation at the alveolar site where the tubercle bacillus is deposited, usually in the subpleural and in the mid-lung zones where greater air flow favors bacilli deposition. The initial inflammation does not usually inhibit the growth of the organism—bacilli are engulfed by alveolar macrophages, and therein replicate and destroy the host cell in the process. Tubercle bacilli drain via lung lymphatics to the hilar lymph nodes, then the thoracic duct, and ultimately may gain entry to the systemic venous circulation where they circulate and can disseminate, causing additional local foci of infection, particularly seeding the apices of the lungs, kidneys, bone growth plates and vertebrae.

Host Immune Response

After a period of 6 to 12 weeks, cellular immunity directed to the tubercle bacillus develops. Stimulated by antigens from the organism, T-lymphocytes become specifically sensitized and activated; these in turn activate macrophages that become capable of antibacterial action against the tubercle bacillus. The cellular immune reaction is the basis for the tuberculin skin test (TST) and the blood based assays currently in use, and for the characteristic pathologic lesion (the granuloma) that is typical of tuberculosis infection.

The granuloma is composed of a roughly spherical collection of lymphocytes, macrophages and epithelioid cells with a small area of central caseous necrosis. In up to 90% of individuals who become infected with the tubercle bacilli, these small granulomas remain localized and quiescent, become encapsulated with fibrous tissue and may ultimately show calcification of the central caseum, a highly acidic milieu which inhibits mycobacterial proliferation.

Inflammation, Necrosis and Cavity Formation

Primary tuberculosis is progressive disease without a period of latency in the weeks to months following primary infection. It occurs most commonly in infants with immature immune systems, elderly people with waning immunity and HIV-infected persons. The site of disease reflects the path of infection, appearing as enlarged hilar or mediastinal lymph nodes and lower or middle lung field infiltrates on chest X-ray (CXR).

Reactivation tuberculosis occurs more than a year and sometimes decades after primary infection; in this type, the site of disease is most commonly the apices of the lungs but may also include other sites seeded years earlier by the primary infection.

As disease progresses in the lung, the caseous material in the center of the granuloma may undergo liquefaction. This leads to the release of proteolytic enzymes and cytokines (including TNF- α) which cause tubercle bacilli to proliferate and produce more antigen at the site, causing an increased cellular immune response, and lesion enlargement. Ultimately, the necrotic zone may rupture into a neighboring bronchus, the liquefied debris drains into the bronchus and the site of necrosis is replaced by air, resulting in a small tuberculous cavity. This liquefied material (sputum) may be expectorated by the host, leading to the release of infectious droplet nuclei and the potential for further tuberculosis transmission.

Within the host, the liquefied material may travel endobronchially to other regions of the lungs, spreading disease and causing inflammatory tissue damage to other lobes. Examination of sputum and CXRs are the recommended evaluation tools because of these pathologic and clinical characteristics.

Physical Evaluation of Adults and Children

Examination of individuals suspected of having active TB should include:

- A medical evaluation, including a detailed history of the current illness
- Past Medical history
- Symptom review
- Social history
- Physical examination

In addition:

- If there is no documentation of prior test for TB infection, order one unless cultures for *M. tuberculosis* (*M. tb*) are already positive.
- Ask patients about history of TB treatment. If the patient has previously been treated, determine the drugs used, the duration of treatment, the history of adverse reactions, the reasons for discontinuing treatment and the previous drug susceptibility results.
- Ask female patients whether they may be pregnant; order a pregnancy test for women with menses more than 2 weeks late. Offer pregnant women who are HIV negative or whose HIV status is unknown HIV counseling and testing, unless HIV testing has been done within the past 6 months. Manage pregnant women with TB disease according to the guidelines on p. 56.
- If the patient is a child under 18 years of age, identify the source patient's sputum culture and susceptibility results to ensure treatment is appropriate. If the source patient has not been identified, initiate a source case investigation (see p. 167).

- Search the TB Registry to see if the patient has a record of TB disease or latent tuberculosis infection (LTBI)
- Ask about the following risk factors for multidrug-resistant tuberculosis (MDRTB):
 - Previous (especially incomplete) treatment for TB
 - Close contact with a person who has MDRTB
 - Previous hospitalization in a facility with an outbreak of a drug-resistant strain of TB (especially if housed on the ward where the outbreak occurred)
 - Incarceration in the New York State prison system since 1990
 - Country of origin

If a patient has one or more risk factors, other anti-TB medication should be considered in addition to isoniazid, rifampin, pyrazinamide and ethambutol.

Note: There is nothing about the initial clinical presentation of patients with MDRTB to distinguish them from patients who have a susceptible strain.

- Patients aged 18 to 64 years, including those without behavioral risk factors for HIV, should be counseled and offered HIV testing unless they have (1) a positive HIV antibody test or (2) a negative result to an HIV antibody test given less than 6 months previously.
- Patients younger than 18 years of age or who are 65 years and older should be counseled and offered testing if they have behavioral risk factors for HIV and have no documented positive HIV test.
- According to New York State law, adolescents over age 13 may be tested for HIV without parental consent. Parental consent for HIV testing is advised for patients younger than 18 years of age, although these patients may be tested without parental consent at the discretion of the physician-in-charge of the chest center. For young children, the results of maternal HIV testing can be used to determine the child's HIV status. In New York State, maternal HIV testing is universal through testing of cord blood.

- Individuals who are HIV negative and who remain at risk for HIV infection during TB treatment should be retested during the course of treatment.
- If ethambutol is being considered for treatment, a baseline visual acuity exam and Ishihara's test for color blindness should be performed.
- If an aminoglycoside or capreomycin is being considered for treatment, a baseline audiogram should be performed.

Radiographic Evaluation

- A baseline chest X-ray (CXR) should be obtained for all patients, except those who show proof of a CXR, taken in the past month, which can be filed in the chest center. An oral report alone is not acceptable.
- Children younger than 5 years of age should undergo both posterior-anterior and lateral CXR. (For children with an equivocal posterior-anterior CXR, computed tomography of the chest (CT) with contrast can assist in the evaluation of possible adenopathy.)
- All other individuals should receive a posterior-anterior CXR only; additional views should be ordered at the physician's discretion.
- Pregnant women who are being evaluated for active TB disease should undergo CXR without delay, even during the first trimester. A lead shield should be used.
- Patients suspected of having extrapulmonary TB should also undergo CXR to rule out pulmonary TB. (Diagnosis and treatment of extrapulmonary TB is discussed p. 71.)
 - If the CXR is abnormal (including pleural TB), smears for acid-fast bacilli (AFB), as well as cultures and drug susceptibilities, should be ordered from sputum samples collected on 2 to 3 separate days.
 - Three sputa should be obtained from patients who are HIV positive and other patients who are immunosuppressed, even if they have a normal CXR.
- Sputa should be obtained for all patients with suspected pulmonary or pleural TB.

Microbiologic Evaluation

The diagnosis of TB disease is mainly bacteriologic in adults. In children it is usually epidemiologic and thus indirect. In addition to AFB smear and culture results, there are new rapid diagnostic tests which may be helpful. Once the diagnosis of TB is established, several molecular techniques (genotyping) are available for distinguishing among strains of *M. tb* complex.

If the patient is suspected of having TB disease, treatment of TB should not be delayed while the laboratory diagnosis is being established.

Specimen Collection

Sputum should always be obtained in adults and children who are suspected of having pulmonary TB.

- Three sputum samples should be collected over 2 to 3 separate days for AFB smears and drug susceptibilities. At least 1 of them should be an early morning specimen.
- Unless failure of treatment or relapse is suspected, obtain only 1 sputum sample in the chest center if several samples have recently been obtained by a hospital, or if the patient's sputum cultures have already converted to negative.
- In addition to common specimens such as sputum (natural or induced) and gastric aspirate, the following specimens are appropriate for laboratory submission:
 - Urine
 - Stool
 - Cerebrospinal fluid (CSF)
 - Pleural, peritoneal or pericardial fluid
 - Bronchial washings
 - Material from abscesses
 - Endometrial scrapings
 - Bone marrow
 - Other biopsy specimens

All specimen collection procedures that produce aerosols that may contain *M. tb* (e.g., sputum induction, bronchoscopy) should be performed in properly ventilated areas or booths by personnel using adequate respiratory protection.

Sputum collection. Patients should be instructed on the proper method of sputum collection (the material brought up from the lungs after a productive cough is what is desired, and not nasopharyngeal discharge and saliva).

- The sputum should be collected in a sterile, wide-mouthed specimen container with a tightly fitting screw-top lid. Alternatively, commercially available sputum collection devices using a 50-ml plastic, disposable centrifuge tube can be used.
- Specimens should be clearly labeled with patient-identifying information and the date of collection. The container should be placed in a paper bag and refrigerated until transported to the laboratory. A TN50 form should be filled out properly and sent with the specimen to the Public Health Laboratory. The form is available at www.nyc.gov/html/doh/downloads/pdf/tb/tb-form-tn50.pdf
- Patients who have difficulty producing sputum should undergo sputum induction by inhalation of an aerosol of sterile hypertonic saline (3%) or sterile water produced by a nebulizer that causes coughing. The procedure should be done in areas with adequate environmental controls such as a hood or booth fitted with a high-efficiency particulate air (HEPA) filter to prevent transmission, and patients undergoing the procedure should be attended by qualified personnel using appropriate respiratory protection (see p. 132).
 - Aerosol-induced specimens may appear thin and watery and should be clearly labeled as “induced sputum” so it will not be discarded by the laboratory as an inadequate specimen.
 - Among younger children, especially children under 5 years of age, sputum is difficult to obtain; most children are sputum smear negative. In children who are able to produce a specimen, however, it is worth sending it for AFB smear microscopy and mycobacterial culture.

- Bacterial yields are higher in older children (more than 5 years of age) and adolescents, and in children of all ages with severe disease.
- Induced sputum may be obtained from young children who cannot produce sputum spontaneously.
- Several recent studies have found that sputum induction is safe and effective in children of all ages and the bacterial yields are as good as or better than those from gastric aspirates. However, training and specialized equipment are required to perform this procedure properly.

Gastric aspiration. This method is used for children who cannot produce sputum either spontaneously or with aerosol inhalation. Children who are contacts of a source case susceptible to isoniazid and rifampin will usually have the same susceptibility; treatment should be adjusted accordingly as the concordance between the susceptibility of the source case and the contact is high for drug-sensitive TB. Gastric aspirates may not be needed for such children.

Note: Children not fasting for at least 4 to 8 hours before gastric aspiration and children with a low platelet count or tendency to bleed should not undergo the procedure.

- Gastric aspirates should be obtained from children who are contacts of MDRTB cases as there is not 100 % susceptibility concordance.
- Hospitalize the patient and, for highest yield, take the specimens when the patient awakens in the morning and is still in bed. There is little experience with outpatient collection.

Note: A negative result does not exclude TB—a positive culture occurs in only 25% to 50% of children with active TB.

- Aspirate approximately 50 ml of gastric contents via nasogastric feeding tube on 3 consecutive mornings for maximum smear-positivity (the first aspirate will have the highest yield).
- Send gastric aspirates for AFB smear microscopy and mycobacterial culture. Rapid diagnostic tests should not be used due to poor sensitivity and specificity.

Bronchial washings, bronchoalveolar lavage and transbronchial biopsy. Bronchoalveolar lavage and/or transbronchial biopsy performed with fiberoptic bronchoscopy may be needed to establish diagnosis in some patients.

- The topical agents used to anesthetize the airway lining may be lethal to *M. tb*; these agents should be used judiciously.
- The procedure may cause the patient to continue producing sputum for several days; these specimens should also be collected and examined.

Urine. Collect the first morning, voided-midstream specimen; multiple specimens are sometimes necessary to detect mycobacteria.

- Urine smears are usually negative and therefore performing them may not be cost-effective.
- The patient should not be under treatment with broad-spectrum antibiotics at the time of collection — many antibiotics are concentrated in the urine and may reach levels that inhibit growth of mycobacteria.

Blood. Collect the blood in a heparinized tube (e.g., Isolator® tube) and process with a centrifugation system or inoculate into broth media for mycobacterial blood cultures. Blood collected in ethylenediaminetetraacetic acid (EDTA), the “purple top tube,” is not suitable for mycobacterial culture.

Cerebrospinal fluid. Analyze CSF for protein and glucose, and obtain total white blood cell and differential counts.

- High protein (at least 50% of the serum protein concentration), high white blood cell count and low glucose are typical of meningeal TB.
- Submit a minimum of 5 ml of fluid in a sterile container for mycobacterial culture. AFB smear of CSF is usually, but not always, negative.
- If the laboratory concentrates the fluid before smear and culture, a larger sample (10 ml) can lead to increased yield.

Tissue and other body fluids. Consider invasive procedures to obtain specimens from the lung, pleura, pericardium, lymph nodes, bones and joints, bowel, peritoneum, fallopian tubes,

epididymis and from other involved sites when noninvasive techniques do not provide a diagnosis. Many of these areas are suitable for closed techniques such as percutaneous needle biopsy or aspiration.

- In patients with disseminated disease, consider bone marrow biopsy, lung biopsy or liver biopsy for examination and culture.
- The portion of the specimen placed in solution for histologic examination cannot be used for culture. If the specimen cannot be shipped promptly to the laboratory, refrigerate it until shipped.
- Analyze pleural, peritoneal and pericardial fluids for protein and glucose, and obtain white blood cell and differential counts.
 - High protein, high white blood cell count and low glucose are usually found in tuberculous infections, but neither their presence nor their absence is diagnostic.
 - The number of organisms in the pleural fluid from most cases of pleural TB is low, with positive cultures found in 23% to 67% of cases.
 - Pleural biopsy shows granulomatous inflammation in approximately 60% of patients, and *M. tb* can be cultured from up to 80% of pleural biopsies.
 - The combined yield of AFB stains of pleural fluid and biopsy tissue, coupled with mycobacterial culture of pleural fluid and biopsy tissue, is greater than 90%.

Microscopic Examination (Acid-Fast Bacilli Smear)

The smear is vitally important, both clinically and epidemiologically, to assess the patient's infectiousness because it gives a quantitative estimate of the number of bacilli being excreted. The detection of AFB in stained smears examined microscopically also provides the physician with a preliminary confirmation of the diagnosis.

Guidelines for preparing smears:

- Prepare directly from clinical specimens or from concentrated preparations.
- The acid-fast staining procedure depends on the ability of mycobacteria to retain dye

when treated with mineral acid or an acid–alcohol solution; 2 types of techniques are commonly used for acid–fast staining:

- The carbolfuchsin methods (which include the Ziehl–Neelsen and Kinyoun methods)
- Fluorochrome procedures using auramine–O or auramine–rhodamine dyes
- At least 5,000 to 10,000 bacilli per milliliter of specimen must be present to detect bacteria in stained smears. In contrast, only 10 to 100 organisms are needed to obtain a positive culture.
- Concentrate a liquefied specimen by centrifuging and use the sediment for staining to increase the sensitivity of the test. (The sensitivity of sputum smear is 50% – 80% among patients with pulmonary tuberculosis.)
- Factors influencing the sensitivity of smears include:
 - Staining technique (fluorochrome technique has a higher sensitivity than carbolfuchsin-based techniques)
 - Experience of the microscopist
 - The prevalence of TB disease in the population being tested
- In reading smears, the microscopist should provide the clinician with a rough estimate of the number of AFB detected. (See Table II-1 below for the scale used to quantify organisms on AFB smear.)

Nucleic Acid Amplification

Detecting *M. tb* complex with traditional laboratory culture methods takes 1 to 8 weeks; however, direct molecular methods using nucleic acid amplification (NAA) can detect *M. tb* complex genetic material directly from clinical specimens within 3 to 5 hours.

NAA tests identify genetic material unique to *M. tb* complex directly in pre-processed clinical samples. During 1995 and 1996, the FDA approved 2 rapid diagnostic tests based on NAA assays: (1) the Gen-Probe AMPLIFIED MTD (*Mycobacteria Tuberculosis Direct*) Test and (2) the Roche AMPLICOR MTB Test. In 1998, the FDA approved a modified version of the MTD (Gen-Probe MTD-2) that is faster and more sensitive.

- Both types of the MTD test are FDA-approved for use in respiratory specimens from smear-positive, previously untreated patients with high clinical suspicion for TB. Under these circumstances, sensitivity is 95% and specificity is 98%.
- The MTD-2 is also approved for smear-negative cases when clinical suspicion is high, but the sensitivity decreases to as low as 66%, with specificity remaining close to 100%.
- When used in extrapulmonary specimens, sensitivity may be as low as 64% in smear-negative specimens, but specificity remains close to 100%.
- For AFB smear-positive respiratory specimens, NAA should be used to confirm that a

Table II-1

Quantitation Scale for Acid-Fast Bacillus Smears by Stain Used.*

Carbolfuchsin (x 1,000)	Fluorochrome (x 250)	Quantity Reported
No AFB/300 fields	No AFB/30 fields	No AFB seen
1-2 AFB/300 fields	1-2 AFB/30 fields	Doubtful or Suspicious, repeat test
1-9 AFB/100 fields	1-9 AFB/10 fields	Rare (1+)
1-9 AFB/10 fields	1-9 AFB/field	Few (2+)
1-9 AFB/field	10-90 AFB/field	Moderate (3+)
>9 AFB/field	>90 AFB/field	Numerous (4+)

* Adapted from American Thoracic Society. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. *Am J Respir Crit Care Med* 2000;161:1376-1395.

positive AFB smear represents *M. tb*. If the NAA is negative, it may be appropriate to delay starting of anti-TB therapy and contact investigation until culture results are available. However, if a patient lives in a congregate setting or with young children, it may be justified to start treatment pending culture results. (see p. 156, Table IX-1).

- Recent New York State regulations require laboratories to perform rapid diagnostic tests using NAA methods on initial AFB smear-positive sputa or respiratory specimens.
- For AFB smear-negative respiratory specimens, NAA should be used if the clinical suspicion for TB is high. If the NAA test is positive, diagnosis of TB is presumed, and should be confirmed by culture. If the NAA test is negative, diagnosis of TB may not be excluded, and decisions about treatment must be based on clinical assessment.
- For extrapulmonary specimens, NAA should be used if the clinical suspicion of TB is high; if the NAA is positive, diagnosis of TB is presumed and should be confirmed by culture. As with smear-negative specimens, if the NAA test is negative, diagnosis of TB may not be excluded.
- NAA tests must be interpreted within the context of the patient's signs and symptoms, and should always be performed in conjunction with AFB smear and culture.
- NAA procedures can detect nucleic acids from dead as well as live organisms and, therefore, can remain positive for long periods in patients who are taking anti-TB medications or have completed TB treatment. Thus, this method should be used only for initial diagnosis and not for follow-up evaluation of patients.
- If an NAA test is positive, but the patient has no positive cultures, the treating physician must determine TB diagnosis based on clinical response.

Two laboratories—the NYC Public Health Laboratory and the Wadsworth Center of the New York State Department of Health—provide the MTD test at no charge when TB is newly suspected. The provider or the hospital lab can send the specimens to the appropriate lab. Call the Provider TB Hotline at (212) 788-4162 for more information.

Criteria for Requesting NAA Tests

- High clinical suspicion of TB, previously untreated or less than 7 days of treatment*
- Respiratory specimen, or
- Non-respiratory specimens (request from the lab on a case-by-case basis if clinical suspicion is high)

* Since the NAA test can amplify rRNA from both viable and nonviable organisms, and therefore may detect nonviable tubercle bacilli expelled by an individual being treated for TB, the test result may be positive even though the treatment has decreased the likelihood that the TB is infectious.

Culture

All clinical specimens suspected of containing mycobacteria should be cultured for the following 4 reasons:

- Culture is much more sensitive than microscopy and is able to detect as few as 10 bacteria/ml of material
- Growth of the organisms is necessary for precise species identification
- Current drug susceptibility testing methods require pure culture of the organisms
- Genotyping of cultured organisms may be useful to identify epidemiological links between patients or to detect laboratory cross-contamination

In adults, the sensitivity of sputum culture is 80% to 85% with a specificity of approximately 98%. The sensitivity of sputum culture is much lower in children, although the rate may be higher in HIV-infected pediatric patients, adolescents and children with adult type disease.

Three different types of traditional culture media are available:

- Egg-based (Löwenstein-Jensen)
- Agar-based (Middlebrook 7H10 or 7H11 medium)
- Liquid (Middlebrook 7H12 and other commercially available broths). Liquid systems (BACTEC, MGIT, MB/Bact, Septi-check, ESP) allow for rapid growth—detection of mycobacterial growth within 1 to 3 weeks compared with solid media (3 to 8 weeks growth). Agar media provide an opportunity to examine colony structure and detect mixed cultures. (See p. 32 and p. 33, Table II-2).

Species Identification

The genus *mycobacterium* consists of more than 80 different species of organisms, all of which appear similar on acid-fast staining.

Two identification procedures that examine distinctive molecular characteristics of *Mycobacterium tuberculosis* (*M. tb*) have gained widespread use:

- Nucleic acid hybridization, which uses molecular probes that can hybridize specifically with *M. tb* complex, *M. avium* complex, *M. kansasii* and *M. goodii*. Probes for other specific mycobacterial species are not yet commercially available.
- High performance liquid chromatography, which:
 - Is based on the fact that most *mycobacterium* species synthesize a unique set of mycolic acids as components of the cell wall
 - Can produce a pattern that reliably identifies and distinguishes 50 *mycobacterium* species; however, it cannot differentiate *M. tb* from wild type *M. bovis*, although it can differentiate *bacille Calmette-Guérin* (BCG) strain of *M. bovis* from *M. tb* complex

These assays have sensitivities and specificities approaching 100% when at least 10^5 organisms are present; this requirement is easily met when pure cultures are used. Thus, nucleic acid hybridization is typically used after the organisms are grown in culture.

Drug Susceptibility Testing

To formulate an effective anti-TB regimen, drug susceptibility tests are needed on initial isolates from all patients. These tests should be repeated if the patient continues to produce culture-positive sputum after 2 to 3 months of treatment or develops positive cultures after a period of negative cultures. The critical concentrations used by these different methods are listed on p. 33, Table II-2.

There are 2 laboratory methods used in the United States for detecting mycobacterial resistance: (1) the agar proportion method (also known as the conventional method) and (2) the liquid broth method. A smear-positive specimen may be used for drug susceptibility testing when

a moderately large number of organisms is seen on stained smears—at least 3+ or more (direct method), or growth from a primary culture or subculture may be used (indirect method).

- The agar proportion method allows for the calculation of the proportion of organisms that is resistant to a given drug at a specified concentration.
 - Countable colonies (50–150) are obtained on the drug-free medium.
 - The number of colonies observed on the drug-containing medium is then compared with the number on the drug-free medium.
 - The proportion of bacilli that is resistant to a given drug is determined and expressed as a percentage of the total population tested. (This proportion has been set at 1%. When 1% or more of the mycobacterial population is resistant to the critical concentration of a drug, that agent is not — or soon will not be — useful for therapy.)
- The liquid method provides a rapid alternative to conventional drug susceptibility testing.
- The 2 methods may have different critical concentrations for different drugs; attention to the method used to interpret the results of drug susceptibility testing is important.

Testing of susceptibility to pyrazinamide.

Pyrazinamide testing is different from that of other first-line drugs. Activity must be measured at pH 5.5 rather than pH 6.8, the usual pH of the growth medium. As a compromise between testing at the pH for optimum pyrazinimide activity vs. optimum growth, pH 6.0 has been chosen for testing pyrazinimide in liquid and solid media.

If an isolate shows resistance to pyrazinimide, especially if the isolate is resistant to pyrazinimide alone, the identity of the isolate should be confirmed since *M. bovis* and *M. bovis* BCG are naturally pyrazinimide-resistant, whereas the majority of *M. tb* isolates are pyrazinimide-susceptible. This is especially important if the laboratory identifies isolates only to the level of the *M. tb* complex.

Table II-2

Drug Concentrations* for Various Methods Used by New York City Reference Laboratories for *M. tb* Complex Susceptibility Testing

Drug	Broth-based Systems ¹			Solid Media-Agar Proportion Method			Other		
	MGIT (Fluorescence)	Bactec (Radiometric)		Conventional Methods ¹		MIC-Bactec 7H12 Broth ²			
		NYC-PHL	NYS	NJMRC	7H10 Agar	7H11 Agar	Susceptible	Intermediate	Resistant
First-line Drugs				PHL	NYS	NJMRC	NJMRC		
Isoniazid	0.1	0.1 ³	0.1 ³	0.2 ³	0.2 ³	0.2 ³			
Isoniazid (high)	NT	0.4	0.4	1.0, (5.0)	1.0	1.0			
Rifampin ⁴	1.0	2.0	0.5, 2.0	1.0 ³	1.0 ³	1.0			
Pyrazinamide	100.0 ³	100.0 ³	300.0	-	-	-			
Streptomycin	-	-	-	2.0	2.0	2.0			
Streptomycin (high)	4.0	6.0	5.0	10.0	10.0	4.0			
Ethambutol	5.0 ³	7.5	5.0	5.0	5.0	7.5			
Other Drugs									
Amikacin					2.0, 4.0	6.0			
Capreomycin				10.0	10.0	10.0			
Ciprofloxacin ⁵				2.0	-	-			
Clofazimine							≤0.12	0.25	≥0.5
Cycloserine				30.0	30.0	60.0			
Ethionamide				5.0	5.0	10.0			
Kanamycin				6.0	5.0	6.0			
Levofloxacin ⁵							≤1.0	2.0	≥4.0
Linezolid							≤4.0	8.0	≥16.0
Moxifloxacin ⁵							≤1.0	2.0	≥4.0
Ofloxacin ⁵				-	2.0, 4.0	-	≤2.0	4.0	≥8.0
Para-aminosalicylic acid				2.0, 10.0	2.0	8.0			
Rifabutin ⁶				0.5	0.5, 1.0, 2.0	-			

Abbreviations: PHL = MGIT = Mycobacterial Growth Indicator Tube; NJMRC = National Jewish Medical & Research Center; NYS = New York State; New York City Public Health Lab

* Concentration in micrograms per milliliter

¹ Broth-based testing or conventional method testing: any drug resistance found for either method usually means the drug should not be used in the treatment regimen

² MIC-minimal inhibitory concentration-the lowest drug concentration that produces inhibition of more than 99% bacterial growth *in vitro*. Interpretation is based on susceptible, intermediate or resistant strain, and is reflected by that concentration of drug tested at NJMRC.

³ Critical concentration of the drug in this medium is the MIC concentration that inhibited the growth of all wild strains. The critical concentration to separate a susceptible from a resistant strain is reflected by the highest MIC found for the wild *M. tb* strain.

⁴ Rifampin is the class agent for rifapentine and results for rifampin reflect rifapentine susceptibility.

⁵ Fluoroquinolone testing – each laboratory generally tests one member of the class.

⁶ Some investigators also test a higher concentration (usually 1.0 or 2.0 mg/ml) of rifabutin.

Source: Drug susceptibility in the chemotherapy of mycobacterial infections. Leonid B. Heifets. CRC Press, Inc 1991

All susceptibility testing reports should include the method used, the name of the drug, the concentration tested and the result (susceptible or resistant for the liquid method, susceptible or percent resistant for the agar proportion method). Clinician concerns about discrepancies between susceptibility test results and clinical response or status must be communicated back to the laboratory as part of an effective quality assurance program.

Genotyping

Genotyping or DNA fingerprinting of *M. tb* is used to determine clonality of bacterial cultures. Briefly, cultured organisms are heat-killed and their DNA is isolated, cut with specific restriction enzymes, separated in an agarose gel by electrophoresis, transferred to a membrane and probed for specific genetic sequences. A standardized protocol has been developed to permit comparison of genotypes from different laboratories around the world.

Genotyping is useful for:

- Confirming laboratory cross-contamination
- Investigating outbreaks of TB
- Evaluating the efficacy of contact investigations
- Determining whether new episodes of TB are due to reinfection or reactivation

There are 3 methods of genotyping that are currently being used by the BTBC to determine the relatedness of specific *M. tb* strains: (1) restriction fragment length polymorphism (RFLP); (2) spoligotyping; and (3) variable-number tandem repeats of mycobacterial interspersed repetitive units. Since 2001, initial isolates of all culture-positive TB patients in NYC have had genotyping performed by RFLP and spoligotyping analysis.

Restriction fragment length polymorphism (RFLP). *M. tb* complex contains a conserved sequence of DNA called IS6110. Usually there are several copies (generally ranging from 5-20 copies) of this stretch of nucleotides in each strain of *M. tb* complex. When the genome is digested by a specific enzyme and then treated with probes that attach specifically to IS6110 sequences, the digested DNA appears on an electrophoresis gel in distinct bands corresponding to DNA fragments of various sizes that contain the IS6110 element in the genomic DNA. Since the number of these IS6110

sequences varies from one strain to the next, *M. tb* complex strains can be distinguished from one another by the number and size of the fragments of DNA that were created by the enzymatic digestion and visualization of the probes. This process has been standardized to allow universal comparisons of patterns; however, nomenclature may differ across laboratories.

Spoligotyping. Spacer oligonucleotide (spoligotyping) takes advantage of the properties of the direct repeat (DR) region of the *M. tb* complex genome. The DR region consists of a number of copies of repeated sequences consisting of 36 base pairs (bp) interspersed with non-repetitive spacer elements that are each 35 to 41 bp long; there are 43 known spacer elements (spacers). Differences between strains arise by variation in the number and identity of these spacers.

Spoligotyping employs a filter membrane to which short sequences of DNA corresponding to each set of the 43 known spacers are attached. The entire DR region of an isolate to be tested is amplified by a polymerase chain reaction (PCR) and is radiolabeled so that hybridization to the filter shows a pattern of spots corresponding to those spacers that are present in the isolate's genome. Comparison of these patterns enables differentiation between strains.

Variable-number tandem repeats of mycobacterial interspersed repetitive units (MIRU). This is a high-resolution, automated typing technique that involves multiple PCR assays and focuses on 12 defined regions of the TB genome (called loci) that contain variable number of repeats of genetic elements, known as MIRU. The repeated units are 51 to 77 bp long, and the number of repeated units in a locus is determined by the size of the PCR products, which have specific primers that hybridize to the contiguous MIRU regions. The number of repeated units represents a specific allele for each locus and the variation at the 12 MIRU loci generates an allele profile for each strain of TB; the resulting 12-digit output allows for easy comparison of results across laboratories.

The level of strain differentiation provided by this technique is intermediate between that of RFLP and spoligotyping, and thus may have higher or lower utility in some areas than others, depending on the diversity of strains in the population. TB isolates from NYC have only been sent for MIRU since 2004.

These comparisons can be done manually for small databases. But for large databases, some form of shorter designation is necessary to refer to specific patterns. When RFLP and spoligotyping are used together, differentiation between *M. tb* strains can be achieved with a high degree of accuracy. The use of genetic fingerprinting has increased our understanding of the epidemiology of TB transmission, rates of laboratory contamination (see below) and the role of reinfection vs. reactivation among those who relapse.

False-Positive Results

The definitive diagnosis of TB depends on the isolation and identification of the etiologic agent *M. tb* from clinical specimens. The methods currently used may at times lead to a false-positive *M. tb* culture.

A false-positive *M. tb* specimen is a positive culture that is not the result of disease in a patient but is due to either contamination of a clinical device, clerical error or laboratory cross-contamination during processing.

Laboratory cross-contamination is the inadvertent transfer of bacilli from a specimen or culture to another specimen or culture not containing bacilli. This occurs in almost every mycobacteriology laboratory, yet it is difficult to confirm. The reported rate of false-positive cultures varies considerably. The use of highly sensitive culture systems, using both solid and liquid media, may detect a relatively small inoculum. In addition, *M. tb* is relatively stable in the laboratory environment and can remain viable for long periods.

Identifying cross-contamination affords the opportunity to:

- Correct equipment or procedural errors responsible for the false-positive cultures
- Correct an erroneous diagnosis and stop needless therapy
- Avoid unnecessary source/contact investigations, cost of incentives and DOT
- Remove an erroneously diagnosed patient from local and national surveillance systems

Our ability to identify false-positive cultures has greatly improved through the use of DNA

analysis by both spoligotyping and the IS6110-based RFLP methods on isolates from all culture-positive cases of tuberculosis in NYC.

Objectives of False-Positive *M. tb* Specimen Investigations

- Identify patients with false-positive *M. tb* cultures
- Discontinue unnecessary treatment if indicated
- Identify the most likely source/mechanism for every confirmed false-positive event
- Determine the impact of misdiagnosed TB based on false-positive cultures, including the extent of unnecessary patient treatment, hospitalizations, tests and examinations, contact investigations and other BTBC activities
- Disseminate information on rate/source/mechanism of the false-positive event and/or laboratory cross-contamination to participating laboratories

Methods Used to Identify False-Positive *M. tb* Cultures

The retrospective identification of false-positive *M. tb* cultures is achieved by both active and passive surveillance. Active surveillance is accomplished through the following:

1. Review of patients with single, positive cultures

- Cases of TB with only a single positive culture (SPC) for *M. tb* are identified bimonthly. BTBC physicians review each patient with an SPC to determine if the patient's clinical presentation is consistent with TB. Patients with a clinical picture inconsistent with TB are referred for a false-positive culture investigation.
- Patients are investigated under 1 or more of the following circumstances:
 - The patient has a normal CXR with a positive *M. tb* sputum culture.
 - No anti-TB medications were given or anti-TB medications were started after culture result became available (at least 14 days after the date of collection of the positive *M. tb* culture).

- CXR did not improve after the patient received 2 or more months of anti-TB medications.
- Three or more sputum samples were taken and only 1 result was culture positive for *M. tb*.

2. Cases identified through the Molecular Epidemiology Database

Identical matches of spoligotype and/or RFLP can trigger potential false-positive culture investigations by:

- Identifying matching spoligotypes on a batch of specimens analyzed
- Identifying spoligotypes consistent with laboratory proficiency strains
- Periodic queries of identical DNA patterns (both spoligotype and RFLP) in the molecular epidemiology database

3. Clinician referral

- An investigation of potential false-positive cultures can also be initiated by physicians and laboratories. Justification should be provided regarding the need for such an investigation, including a summary of the patient's overall clinical status and the reason the physician or laboratory believes an investigation is warranted.

Interpreting Results of the False-Positive Investigation

If DNA analysis does not identify a match of isolates within concurrent processing or does not indicate contamination with a proficiency, or laboratory, strain, then cross-contamination may be ruled out unless the treating provider requests further investigation into non-laboratory contamination causes of a false-positive result. In that case, further investigation may be warranted to rule out mislabeling or another source of false-positive results.

If DNA analysis identifies a match of isolates with the exact spoligotype and RFLP pattern within concurrent processing, or if DNA indicates contamination with a laboratory proficiency strain, the treating physician may be requested to re-evaluate the patient in light of the laboratory findings to decide on the patient's diagnosis. The processing laboratory also is informed of the findings and asked to investigate the matter within the laboratory.

A suspected false-positive culture is considered to be confirmed as false if there is a spoligotype/RFLP match between the suspected false-positive and another isolate processed concurrently or with a laboratory proficiency strain.

A suspected false-positive culture is considered to be unlikely if there is no DNA fingerprint match between the suspected false-positive and any other isolate(s) processed concurrently or with a laboratory proficiency strain. If the physician treating the patient feels that TB is not the correct diagnosis, that physician must present other clinical information to support his or her decision not to treat the patient for TB disease.

Other Laboratory Tests

The following laboratory tests should be ordered for all patients:

- Complete blood count including platelets.
- Chemistry panel (blood urea nitrogen, creatinine, uric acid, and liver function tests: SGOT/AST, SGPT/ALT, alkaline phosphatase, and total direct bilirubin)
- Viral hepatitis screen
- HIV testing and counseling, and, if HIV-positive, CD4 lymphocyte count (if not done within previous 6 months)

Classification of Suspected Tuberculosis Patients

Patients highly suspected of having current TB disease and expected to evolve as a Class III, active disease should be classified as Class V (High). (This classification would include, for example, a patient whose CXR shows a cavitory lesion and infiltrates typical of active pulmonary TB. In contrast, patients suspected of having old, healed TB and expected to evolve as a Class IV, or non-TB, patient should be classified as Class V [Low]. This would include, for example, a patient who has a positive test for TB infection and has only nodules or linear shadows on CXR.)

All patients initially classified as Class V (High) or Class V (Low) should be reported to BTBC Surveillance Office as a "suspect" and should be reclassified within 4 months of the initiation

of TB evaluation based on their clinical improvement, AFB culture and/or CXR results. (See p. 114).

Tuberculosis in Childhood

In the United States, most children are asymptomatic when they are diagnosed with TB and present as part of an investigation of contacts of an adult case. The test for TB infection plays a very important role in the diagnosis of TB in children. Older children and adolescents who are symptomatic may present with the protean symptoms of TB—fever, weight loss and night sweats. Younger children may have disseminated disease, meningitis or a “pneumonia” that is unresponsive to antibiotics.

Children usually have paucibacillary pulmonary disease, as cavitating disease is relatively rare (about 6% of cases or fewer) in those younger than 13 years of age. In contrast, children develop extrapulmonary TB more often than adults do. Severe and disseminated TB (e.g. TB meningitis and disseminated TB) occur more frequently in young children (less than 3 years) and can occur relatively quickly once the child is infected.

The following areas of evaluation and presentation of disease merit special attention in children:

Medical Evaluation

Obtain a detailed history and physical examination

- Review family history and include history of possible contacts, including non-household care givers, visitors and foreign travel. Specific information about contact may allow retrieval of the isolate and its susceptibilities.
- Enquire about missed milestones, behavioral changes, headache, gastrointestinal disturbance, weight loss, lack of weight gain and night sweats.
- Do a thorough exam, beginning with weight and height.
- Always keep in mind extrapulmonary sites when evaluating children for TB, such as peripheral lymph nodes, central nervous system, bones and joints, liver and spleen (in addition to evaluation for chest disease).

Chest X-ray in Children

CXR is useful in the diagnosis of TB in children; they should receive a posterior-anterior and lateral CXR which should be read by a radiologist experienced in pediatric radiography. In the majority of cases, children with pulmonary TB have CXR changes suggestive of TB.

The most common finding is persistent opacification in the lung in conjunction with enlarged hilar or subcarinal lymph glands. A miliary pattern of opacification in HIV-uninfected children is highly suggestive of TB. Patients with persistent opacification which does not improve after a course of antibiotics should be investigated for TB.

Some characteristics of childhood TB include:

- More than half of children with radiologic pulmonary disease are asymptomatic (identified through contact tracing).
- The CXR is typically “sicker” than the child.
- Children of any age with complicated intrathoracic disease and adolescents more typically have adult-type reactivation TB (upper lobe disease which may cavitate) with positive AFB-sputum smear. (However, adolescents with significant radiographic findings, including cavitary disease, may have surprisingly few symptoms.)
- Wheezing is an occasional manifestation of TB in an infant due to endobronchial disease or lymph nodes compressing a bronchus.
- Children should be regarded as infectious if they have sputum smear-positive pulmonary TB or cavitary TB on CXR. Airborne isolation should be initiated (see p. 122).
- If the child is not AFB smear-positive, diagnostic criteria for sputum smear-negative pulmonary TB should include:
 - At least 3 sputum specimens negative for AFB
 - Radiological abnormalities consistent with active pulmonary TB
 - No response to a course of broad-spectrum antibiotics
 - Decision by a clinician to treat with a full course of anti-TB chemotherapy

Congenital and Neonatal Tuberculosis

The distinction between congenital and early (neonatal) TB is primarily epidemiological. Presentation, management and prognosis are similar.

Congenital TB is uncommon, with about 300 reported cases in the English language literature (only 29 cases from 1980 to 2000); however, the incidence is probably underestimated due to the difficulty in making the diagnosis. It is important to keep a high index of suspicion, as fewer than 50% of the mothers of children with congenital TB were known to have active TB at the time of delivery. A significant percentage of pregnant women with pulmonary tuberculosis are unaware of their disease and may have few, if any, symptoms.

Often, diagnosis in the newborn leads to the retrospective diagnosis of active disease in the mother. Women who have only pulmonary TB are not likely to infect the fetus, but may infect their infant after delivery. If neonatal or congenital TB is suspected and the mother has a normal CXR, evaluation for gynecological or other forms of extrapulmonary TB should be performed in the mother.

Neonatal TB symptoms typically are nonspecific and may overlap with those of other congenitally or neonatally acquired infections. The diagnosis of congenital TB should be considered in infants in whom pulmonary symptoms do not respond to empiric antibiotic therapy or who have evidence of sepsis or fungemia that is unresponsive for treatment.

Evaluating Neonates for Tuberculosis

If the mother had untreated or very recently diagnosed TB, the newborn should be assessed for signs of congenital TB. Initial assessment should include:

- Medical evaluation
- TST and CXR (TST is usually negative in newborn infants with congenitally or perinatally acquired infection)
- Three gastric aspirates on 3 consecutive days

- Lumbar puncture if there is a high clinical suspicion for active TB
- In pregnant woman, regardless of TB treatment status during pregnancy, examination of the placenta microscopically for granuloma, staining for AFB and sending for AFB culture

In infants suspected of having congenital TB, begin treatment with isoniazid, rifamycin, pyrazinamide and an injectable agent (amikacin is recommended, but streptomycin or kanamycin can be used).

- Add corticosteroids if the neonate has meningitis.
- If no granulomas are found in placenta and the infant does not have clinical evidence of active tuberculosis, administer isoniazid for 3 months or until mother is culture-negative.

About 50% of children born to mothers with active untreated disease will develop TB in their first year of life if treatment for LTBI is not given to the baby.

At age 4-6 months:

- Repeat TST if negative at initial assessment.
- If positive, re-evaluate for active disease and treat with isoniazid for a total of 9 months, once active disease has been excluded.

Bacille Calmette-Guérin Vaccination

If the mother (or primary care taker or other family member) is suspected of being non-compliant with TB treatment or is infectious and has MDRTB, Bacille Calmette-Guérin (BCG) vaccination may be considered to protect the infant (Appendix I-G, p. 227), who should also be separated from the mother until the mother's disease activity is determined.

BCG should be considered in the newborn with a negative TST if he/she:

- Is going to be exposed continually to an untreated mother or caretaker and cannot be separated from the mother/caretaker
- Cannot be given long-term treatment with isoniazid for LTBI

- Is going to be continually exposed to a mother with MDRTB and cannot be separated from the mother

If the mother has completed treatment for active TB during pregnancy and there is no evidence of active disease at the time of birth, there is

minimal risk to the infant and no need for specific therapy or separation from the mother. The child should have a TST at birth and at age 6 months, but needs no treatment or further evaluation unless the TST is positive.

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