

## XII. LABORATORY ASPECTS OF TB

### MODULE OUTLINE

1. Standards of Public Health Practice
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    - b. QuantiFERON<sup>®</sup>-TB Gold In Tube (QFT-GIT) Testing
    - c. Therapeutic Drug Monitoring (TDM)—“Drug Levels”
  5. Tools
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### 1. STANDARDS OF PUBLIC HEALTH PRACTICE

- XII-1. A GeneXpert test is performed by the Tennessee Department of Health, Division of Laboratory Services on all initial AFB-positive sputum specimens.
- XII-2. Drug susceptibility testing (DST) is performed by the Tennessee Department of Health, Division of Laboratory Services on each initial culture positive for *Mycobacterium tuberculosis* (M. tb) complex.
- XII-3. DST is repeated for patients on treatment who remain culture-positive for M. tb after three (3) months from the initial positive culture.
- XII-4. One (1) isolate (if pure) from each culture-positive patient is sent by the Tennessee Department of Health, Division of Laboratory Services to a CDC-contracted lab for genotyping.
- XII-5. Laboratory specimens and requisitions are submitted in accordance with Clinical Laboratory Improvement Amendments (CLIA) standards and policies of the Tennessee Department of Health, Division of Laboratory Services.

### 2. BACTERIOLOGIC TESTS FOR TB

**Table XII-1** is a quick guide to mycobacterial testing for TB.

**Table XII-1: Quick Guide to Mycobacterial Testing**

<b>Test</b>	<b>Fluorochrome Smear</b>	<b>Mycobacteria Culture</b>	<b>GeneXpert® MTB/RIF</b>	<b>AccuProbe®</b>	<b>INNO-LiPA Mycobacteria v2</b>	<b>MGIT 960 Drug Susceptibility</b>
<b>Nickname</b>	Smear	“Trek bottle” or “Solid media”	GeneXpert	Probe	LineProbe	MGIT
<b>Purpose</b>	Quick indicator of new or persistent infection	Cultivation of viable Mycobacteria	Detection of M.tbc and presence of gene mutations associated with rifampin resistance	Identify M.tbc, or MAC	Mycobacteria species identification from culture	Determine drug susceptibility to first-line drugs for treatment of M.tbc
<b>Expected Results</b>	Negative for AFB	No Mycobacteria isolated	M.tbc: Not Detected rpoB mutation: Not Detected rpoB mutation: Not Indicated	Negative for Mycobacterium tuberculosis complex (ex.)	No Mycobacteria isolated	Sensitive
<b>Actionable Results</b>	<1, 1-10, 10+ (may be 1+, 2+, 3+, 4+ or “positive” or “negative” from outside labs)	Mycobacterium complex (ex.)	M.tbc: Detected rpoB mutation: Detected	Positive for Mycobacterium avium complex (ex.)	Mycobacterium avium complex (ex.)	Resistant
<b>Turn-around time (TAT) from receipt in lab</b>	24 hours	Up to 6 weeks	24 hours	Requires culture growth—targeted TAT is 14-21 days	Requires culture growth—targeted TAT is 14-21 days	Requires culture growth—targeted TAT is 21-28 days

Test	Fluorochrome Smear	Mycobacteria Culture	GeneXpert® MTB/RIF	AccuProbe®	INNO-LiPA Mycobacteria v2	MGIT 960 Drug Susceptibility
<b>Limitations</b>	Viable and non-viable organisms will fluoresce	M.tbc is a slow grower and may take weeks to produce a positive culture	Sputum specimens only. Only detects the presence of rpoB mutation not whether the isolate will express true rifampin resistance. Detects organism DNA whether it is viable or nonviable	Testing capabilities at TDHLS is limited to two (2) organisms	Does not identify all species of mycobacteria (will see report of MOTT)	Interpretation determined by critical concentration. PZA very sensitive to changes in pH

The GeneXpert® MTB/RIF test is one type of nucleic acid amplification test (NAAT). A GeneXpert test is performed by the Tennessee Department of Health, Division of Laboratory Services on all initial AFB-positive sputum specimens (Standards of Public Health Practice XII-1). Table XII-2 provides a list of NAATs and tests that may be commonly confused as a NAAT.

**Table XII-2: List of Nucleic Acid Amplification Tests (NAATs) and Tests Commonly Confused with NAATs**

Test	AccuProbe®	Cepheid GeneXpert® MTB/RIF	Amplified <i>Mycobacteria tuberculosis</i> (MTD®) Direct	GenoType MTBDRplus®	Laboratory Developed Test (LDT)
Company	Gen-Probe, Inc.	Cepheid	Gen-Probe, Inc.	Hain	NA
Nucleic Acid Amplification?	No	Yes	Yes	Yes	Yes
Identification MTBC from culture isolates	Yes	No	No	Not usually	Sometimes
Detection of MTBC in clinical specimens	No	Yes	Yes	Yes	Yes
Detection of mutations associated with drug resistance	No	RIF	No	RIF, INH	RIF, others depending on platform
Genes associated with drug resistance detected	None	<i>rpoB</i>	None	<i>katG, inhA, rpoB</i>	<i>rpoB</i> , others depending on platform
Mechanism	No amplification; DNA probe hybridizes to a specific ribosomal RNA target	Real time polymerase chain reaction (PCR) and molecular beacon technology to simultaneously amplify and detect the <i>rpoB</i> gene	Transcription Mediated Amplification (TMA) to amplify the ribosomal RNA target followed by DNA probe hybridization to detect the amplified target	Polymerase chain reaction (PCR) to amplify the genes followed by hybridization to specific probes on nitrocellulose strips (line probe assay)	Various methods including PCR, real-time PCR, "molecular beacons," DNA sequencing

<b>Test</b>	<b>AccuProbe®</b>	<b>Cepheid GeneXpert® MTB/RIF</b>	<b>Amplified <i>Mycobacteria tuberculosis</i> (MTD®) Direct</b>	<b>GenoType MTBDRplus®</b>	<b>Laboratory Developed Test (LDT)</b>
FDA approved	Yes (cleared)	Yes	Yes	No	No
Turnaround time from specimen receipt in lab	Requires growth in culture	24-48 hours	24-48 hours	24-48 hours	24-48 hours
Nickname	Probe, DNA probe	GeneXpert, Xpert	MTD, "Direct test," often mistakenly called "probe"	The "Hain test" Line Probe Assay (LPA)	"Home brew" molecular beacons, PCR
How reported— Positive results	Ex: Mycobacterium tuberculosis	Detected	"Detected," "Positive," "Positive for M. tuberculosis complex rRNA"	"point mutation detected"	
How reported— Negative results	No Mycobacteria isolated	Not Detected Not Indicated	"Not Detected"	"no point mutation detected"	
RVCT #	18	21	21	21	

The following are quick reference guides for laboratory tests performed by the Tennessee Department of Health, Division of Laboratory Services:

<b>Acid-Fast Stain (AFB smear/Fluorochrome/Smear Microscopy)</b>			
How does it work?	<p>Mycobacteria are able to form stable complexes with certain stains. Phenol in the primary stain allows the stain to penetrate into the cell wall. The cell wall mycolic acids retain this primary stain even after washing with acid-alcohol. The resistance to de-colorization with acid-alcohol is what causes mycobacteria to be called “acid-fast.”</p> <ul style="list-style-type: none"> <li>• A drop of processed sputum is spread on a microscope slide and placed on a slide warmer for heat fixation.</li> <li>• The slide is then moved to a staining rack and Auramine O stain is applied to the slide for 15 minutes.</li> <li>• The slide is washed with acid-alcohol for 2 minutes.</li> <li>• Acridine orange, a counterstain, is applied to the slide for 2 minutes.</li> <li>• The slide is then rinsed with distilled water, allowed to air dry and examined using a fluorescent microscope. Mycobacterium appears as green fluorescing bacilli against a red-orange background.</li> </ul>		
When is this test run?	Monday – Friday. Hospital and private lab testing may vary		
How long before results are ready?	Results are usually available within 24 hours of receipt of the specimen in the lab.		
How are the results classified?	<u># of Bacteria Seen</u>	<u>Interpretation</u>	
	State Lab Results	No fluorescing bacteria seen	No AFB found NEGATIVE
		<4 fluorescing bacteria per field (at least 12 rods per slide must be seen)	<1 POSITIVE
		4-36 fluorescing bacteria per field	1-10 POSITIVE
		>36 fluorescing bacteria per field	10+ POSITIVE
	Outside Lab Results	No fluorescing bacteria seen	No AFB found NEGATIVE
		1-3 fluorescing bacteria seen on entire slide	+/- or Rare AFB seen
		4-36 fluorescing bacteria per 100 fields	1+ POSITIVE
		4-36 fluorescing bacteria seen per 10 fields	2+ POSITIVE
		4-36 fluorescing bacteria seen per field	3+ POSITIVE
>36 fluorescing bacteria per field		4+ POSITIVE	
What do the results mean?	<p>The higher the number, the higher the bacterial load.  The higher the number, the more infectious the patient is to others.  Begin treatment with four (4) drugs until TB is confirmed or ruled out.  Any patient with a positive AFB smear needs to be on respiratory isolation</p>		
Are other tests needed?	Yes. A culture confirming or ruling out <i>M. tuberculosis</i> complex is needed. If positive for <i>M. tuberculosis</i> , drug sensitivity testing is needed.		

<b>Acid-Fast Stain (AFB smear/Fluorochrome/Smear Microscopy) (CONTINUED)</b>	
When would this test be used?	To quickly determine if TB is a possibility To determine the degree of infectiousness.
How would this test be ordered?	If sending a specimen to the Tennessee Department of Health, Division of Laboratory Services, a specimen requisition is completed indicating this test. If testing is done by an outside lab, an ordering physician will indicate this test to be performed.
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

<b>7H11 Agar Plate</b>	
How does it work?	7H11 agar is a transparent agar-based media for the isolation and colony morphology of mycobacterium. Oleic acid, albumin, and pancreatic digest of casein are the key ingredients which aid in the growth of the tubercle bacilli.
When would this media be used?	When a broth culture exhibits growth, the laboratory uses this media to obtain growth of the mycobacterium on solid media. The laboratory also inoculates every processed sample directly on to a 7H11 plate.
How long before growth is obtained?	Visible growth can occur in as few as 3-5 days with rapid-growing mycobacterium. With <i>M. tuberculosis</i> , and other slow-growing bacteria, it can take up to 4 weeks before growth is obtained.
How are the results classified?	<ul style="list-style-type: none"> <li>• Positive for growth</li> <li>• Negative for growth</li> <li>• Contaminated</li> </ul>
What do the results mean?	When growth is observed on the agar media, the laboratory technician determines if the growth is a mycobacterium or if it is some other organism. If the growth is a mycobacterium species, identification procedures are started. If the growth proves to be an organism other than a mycobacterium, then the plate is considered to be contaminated and no further studies are performed. If no growth is seen on the agar, it is reported as negative.
Are other tested needed?	The Tennessee Department of Health, Division of Laboratory Services will initiate identification procedures if the growth is a mycobacterium species.
How would this test be ordered?	Not applicable
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

<b>Gen-Probe AccuProbe DNA Probe Test</b>	
How does it work?	The Gen-probe identification test is a rapid DNA probe test which uses nucleic hybridization for the identification of certain mycobacterium. Gen-Probe manufactures four Accuprobe kits to test for the following organisms: <i>M. tuberculosis</i> complex, <i>M. avium</i> complex, <i>M. gordonae</i> , and <i>M. kansasii</i> .
When is the test run?	The Tennessee Department of Health, Division of Laboratory Services typically runs this test on Tuesdays and Thursdays.
How long before results are ready?	Test results are available after 2:00pm CT on testing days.
How are the results classified?	Positive, negative, or indeterminate for the target organism.
What do the results mean?	Positive: The isolate is identified as one of the target organisms Negative: The isolate is not one of the target organisms Indeterminate: The test is inconclusive and must be repeated
Are other tests needed?	If the isolate is identified as <i>M. tuberculosis</i> , and if this is the first time the patient has had a positive MTB culture, then drug susceptibility testing (DST) will be done. This is automatically done by the Tennessee Department of Health, Division of Laboratory Services.
When would this test be used?	This test is used to identify growth on solid media or growth in liquid media as <i>M. tuberculosis</i> complex and <i>M. avium</i> complex.
How would this test be ordered?	This test is automatically performed on specimens received at the Tennessee Department of Health, Division of Laboratory Services.
Is this result considered a nucleic acid amplification test (NAAT) or a culture?	This result is a culture result and should be recorded as the culture result on either Question #18 or Question #20 on the RVCT.
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

<b>Trek Bottles</b>	
How does it work?	<p>The MGIT tube is intended for the detection and recovery of mycobacteria using BACTEC 960 equipment. The tubes contain 7ml of modified Middlebrook 7H9 broth and are flushed with 10% CO<sub>2</sub>.</p> <p>A fluorescent compound is embedded in silicone on the bottom of the round bottom MGIT tubes which later allows the fluorescence to be detected in the presence of actively growing organisms.</p> <p>Tubes are monitored by the BACTEC 960 every 60 minutes for increasing fluorescence. Analysis of the fluorescence is used to determine if the tube is positive for growth.</p>
When would this media be used?	VersaTREK detects mycobacterial growth by automatically monitoring (every 24 minutes) the rate of oxygen consumption within the headspace of the culture bottle. A positive growth response is reported via a visible and audible positive signal by the system. VersaTREK combines a liquid culture medium (VersaTREK Myco Broth), a growth supplement (MycoGS), and, for potentially contaminated species, an antibiotic supplement (MycoAS for MycoPVNA), with a detection system that automatically incubates and continuously monitors culture bottles inoculated with specimens possibly containing mycobacteria.
How long before growth is obtained?	For mycobacteria—1-6 weeks Negative (no growth)—TREK bottles are held for 6 weeks before reporting as negative
How are the results classified?	Positive for growth Negative for growth Contaminated
What do the results mean?	Positive: growth noted, identification procedures are started Negative: no growth Contaminated: growth other than mycobacteria is present
Are other tests needed?	The Tennessee Department of Health, Division of Laboratory Services will initiate identification testing if the TREK bottle is positive. Drug susceptibility testing will be ordered by the lab when indicated.
How would this test be ordered?	Not applicable
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

<b>Cepheid GeneXpert MTB/RIF Assay</b>	
How does it work?	<p>The Cepheid GeneXpert MTB/RIF Assay is a diagnostic test that can identify <i>Mycobacterium tuberculosis</i> (MTB) in clinical specimens from respiratory sources. The GeneXpert purifies, concentrates, amplifies (by real-time PCR), and identifies targeted nucleic acid sequences in the TB genome. Currently, the Tennessee Department of Health, Division of Laboratory services only runs the GeneXpert MTB/RIF Assay on sputum specimens.</p> <p>The GeneXpert MTB/RIF Assay does not take the place of a culture. A negative MTB/RIF Assay does not exclude the possibility of isolating <i>M. tuberculosis</i> from culture.</p> <p>This test also detects the presence of the rpoB gene mutations which is indicative of Rifampin resistance.</p>
When is the test run?	The Tennessee Department of Health, Division of Laboratory Services runs this test on the same day that a “new” positive smear is reported. <b><u>GeneXpert testing is not performed on AFB smear-negative sputum specimens. If the TB physician feels strongly that a patient has TB despite the lack of acid-fast bacilli present in the sputum specimen and would like a GeneXpert run, the request MUST be made through Central Office.</u></b>
How long before results are ready?	Results are usually ready by 7:30am CT the morning following smear reporting.
How are the results classified?	<p>MTB DNA Detected: <i>M. tuberculosis</i> is detected</p> <p>MTB DNA Not Detected: <i>M. tuberculosis</i> is not detected</p> <p>MTB DNA Indeterminate: Equivocal results. Test must be repeated</p> <p>rpoB mutation Detected: Detection of the gene mutation associated with Rifampin resistance</p> <p>rpoB mutation Not Indicated: No detection of the gene mutation associated with Rifampin resistance</p>
Are other tests needed?	A culture for <i>M. tuberculosis</i> must be performed.
When would this test be used?	CDC Updated Guidelines for the Use of NAAT state: Nucleic acid amplification testing (NAAT) should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management of TB control activities.
How would this test be ordered?	The Tennessee Department of Health, Division of Laboratory Services automatically runs this test on all newly AFB smear-positive sputum samples. GeneXpert MTB/RIF Assay testing is not performed on subsequent AFB smear-positive samples for the same patient.
Is this result considered a nucleic acid amplification test (NAAT) or a culture?	This result is a nucleic acid amplification test (NAAT) result and should be recorded on Question #21 on the RVCT.
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

<b>MTB Drug Susceptibility Testing (DST)</b>	
How does it work?	<i>M. tuberculosis</i> isolates are tested for sensitivity/resistance to isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB) and streptomycin using the BACTEC MGIT susceptibility method. If resistance is detected in any of the drugs, the sensitivity testing is repeated. All isolates showing any resistance are submitted to CDC for confirmation.
When is this test run?	DST is performed by the Tennessee Department of Health, Division of Laboratory Services on each initial culture positive for <i>Mycobacterium tuberculosis</i> ( <b>Standard of Public Health Practice</b> ). The Tennessee Department of Health, Division of Laboratory Services sets up sensitivity testing on Wednesdays and Fridays. Results are read when the quality control (QC) is completed for all of the drugs being tested on the machine.  The Tennessee Department of Health, Division of Laboratory Services will automatically repeat DST testing on all isolates that are culture-positive after three (3) months from the initial positive culture ( <b>Standard of Public Health Practice</b> ).
How long before results are ready?	Results are ready 7-14 days after the actual susceptibility testing is started. Isolates must meet certain QC criteria before susceptibility testing can be performed. This can add up to seven (7) days to the turnaround time for the susceptibility results. The Tennessee Department of Health, Division of Laboratory Services reports any preliminary and final resistance by phone to the region or metro.
How are the results classified?	Sensitive Resistant Contaminated
What do the results mean?	Sensitive: the drug can be used to treat the MTB isolate Resistant: the drug has little or no effect on the MTB isolate Contaminated: the susceptibility is contaminated with an organism other than MTB and will have to be repeated.
Are other tests needed?	No, unless multi-drug resistance (MDR) is demonstrated. If MDR is demonstrated, the isolate is sent to CDC for an expanded panel of drug testing.
When would this test be used?	This test would be used: <ul style="list-style-type: none"> <li>• To determine the susceptibility of an MTB isolate to INH, RIF, PZA, EMB and streptomycin</li> <li>• When a patient is not responding to treatment and a physician feels that susceptibility testing should be repeated</li> <li>• Every three (3) months for as long as the patient's culture remains positive for MTB</li> </ul>
How would this test be ordered?	The Tennessee Department of Health, Division of Laboratory Services automatically performs DST on the first culture-positive isolate for MTB.
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

<b>Human Immunodeficiency Virus (HIV) Testing</b>	
What is this test?	This test is an immunoassay kit for the simultaneous qualitative detection of Human Immunodeficiency Virus (HIV) p24 antigen and antibodies to HIV Type 1 (HIV-1 groups M and O) and HIV Type 2 (HIV-2) in human serum or plasma.
Why is this test run?	To determine if a patient is infected with Human Immunodeficiency virus (HIV).
When is this test run?	The Tennessee Department of Health, Division of Laboratory Services runs this test Monday-Friday. Hospital and private lab testing may vary.
How long before results are ready?	The turnaround time for results from the Tennessee Department of Health, Division of Laboratory Services is within one week from receipt of the sample in the lab. The turnaround time for hospital and private labs may vary.
How are the results classified?	Negative Positive Unsatisfactory
What is the confirmatory process?	If a result is initially positive after the initial testing, the sample is run 2 more times. If two out of the three total runs is positive, then Multispot testing are done. If the Multispot test is positive, then the confirmation result is reported as positive. If the Multispot testing is negative, then a nucleic acid amplification test (NAAT) is performed.
What do the results mean?	Negative: patient is not infected with HIV Positive: patient is infected with HIV Unsatisfactory: test should be repeated
How is this test ordered?	This order for this test is denoted on the lab requisition and/or by lab order entry (LOE).
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

<b>QuantiFERON®-Gold In-Tube (QFT-GIT) Testing</b>	
How does it work?	QFT-GIT is an <i>in vitro</i> diagnostic test using a peptide cocktail stimulating ESAT-6, CFP-10 and TB7.7 (p4) proteins to stimulate cells in heparinized whole blood. Detection of interferon- $\gamma$ (IFN- $\gamma$ ) by Enzyme-Linked Immunosorbent Assay (ELISA) is used to identify <i>in vitro</i> response to these peptide antigens that are associated with <i>Mycobacterium tuberculosis</i> infection.
When is this test run?	The Tennessee Department of Health, Division of Laboratory Services runs this test Monday-Friday depending on the volume of samples received.
How long before results are ready?	Generally results are available within 72 hours of receipt in lab.
How are the results classified?	Positive Negative Indeterminate Unsatisfactory
What do the results mean?	Positive: <i>M. tuberculosis</i> infection is likely Negative: <i>M. tuberculosis</i> infection is unlikely but cannot be excluded, especially when illness is consistent with TB disease and likelihood of progression to TB disease is increased Indeterminate: results cannot be interpreted and testing should be repeated Unsatisfactory:
Are other tests needed?	Depending on the results, additional testing to rule out TB disease may be needed.
When would this test be used?	QFT-GIT testing may be used in place of (but not in addition to) a tuberculin skin test (TST) in all situations in which the CDC recommends tuberculin skin testing as an aid in diagnosing <i>M. tuberculosis</i> infection. QFT should only be used to test individuals who are dispositioned as “high-risk” for TB infection and/or the progression of TB infection to active TB disease as indicated through administration of the “TB Risk Assessment Tool” (TB RAT). Exclusion criteria: <ul style="list-style-type: none"> <li>• Children &lt;5 years of age</li> <li>• Any child, regardless of age, requiring TB testing as part of the EPSDT exam</li> <li>• Any person determined to be “low-risk” through the TB RAT</li> <li>• Health care workers (HCWs) and other individuals required by an employer or educational institution to have a baseline or serial testing TB test (e.g., annually) as a condition of employment or enrollment. QFT should not be used for any employment/admission testing or serial testing</li> <li>• Persons requesting a TB test for employment purposes (excludes eligible health department employees that have risk factor(s) for TB exposure or progression).</li> <li>• County jail inmates and jail employees in jurisdictions in which targeted TB testing is financially supported by the TTBEF.</li> </ul>
How is this test ordered?	Testing is ordered by completing a lab order entry (LOE) and completing the QFT requisition.
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

The following are quick reference guides for laboratory tests that may be performed by other labs outside of the Tennessee Department of Health.

<b>Amplified Mycobacteria Tuberculosis Direct Test (MTD)</b>	
How does it work?	The Amplified <i>Mycobacterium tuberculosis</i> Direct Test (MTD) is a target-amplified nucleic acid probe test for the detection of <i>M. tuberculosis</i> complex rRNA in concentrated specimen sediments prepared from sputum, bronchial specimens (BAL or bronchial aspirates), or tracheal aspirates.
When is this test?	The Tennessee Department of Health, Division of Laboratory Services does not run this test. Outside laboratory schedules may vary.
How long before results are ready?	Results are usually available the same day the test is run.
How are the results classified?	Positive Negative Indeterminate
What do the results mean?	Positive: <i>M. tuberculosis</i> complex rRNA is detected Negative: <i>M. tuberculosis</i> complex rRNA is not detected Indeterminate: equivocal results. Test must be repeated
Are other tests needed?	A culture for <i>M. tuberculosis</i> must be performed
When would this test be used?	CDC Updated Guidelines for the Use of NAAT state: Nucleic acid amplification testing (NAAT) should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management of TB control activities.
How would this test be ordered?	Private providers would order this test.
Is this result considered a nucleic acid amplification test (NAAT) or a culture?	This result is a nucleic acid amplification test (NAAT) result and should be recorded on Question #21 on the RVCT.
How much would this test cost?	The fee for this test may vary depending on the lab performing the test.

<b>Polymerase Chain Reaction</b>	
How does it work?	<p>PCR is a sensitive laboratory technique that is used to detect and repeatedly copy small amounts of DNA or RNA. Detection is based on multiplication not of whole bacilli, as in a culture, but of the genetic material, chromosomal DNA or ribosomal RNA. Provided all ingredients are present in the reaction tube, this will only take place when the target genetic sequences to which the added primers can bind are found in the sample. Specificity of the test will thus depend on the use of the correct primers, using sequences typical for MTB or MTB complex. In principle, from one target sequence of one bacillus, the reaction can produce millions of copies and thus yield a positive result.</p> <p>In practice, however, sensitivity is of the same order as that of a culture or about 100 bacilli due to the limited amount of sample that can be used and sometimes also due to the interference of reaction-inhibiting substances. Extremely careful technique is needed. Carry-over of DNA is even more likely to happen than of whole bacteria so that early studies found up to 50% of positive results to be false. PCR tests do not differentiate live organisms from dead ones.</p>
When is the test run?	The Tennessee Department of Health, Division of Laboratory Services does not run this test. Outside laboratory schedules may vary.
How long before results are ready?	Results are usually reading within 24 hours.
How are the results classified?	<p>MTB detected</p> <p>MTB not detected</p>
What do the results mean?	<p>MTB detected: genetic material for MTB complex is detected</p> <p>MTB not detected: genetic material for MTB complex is not detected</p>
Are other tests needed?	A final culture for <i>M. tuberculosis</i> complex is needed to make a final diagnosis.
When would this test be used?	Hospitals and private labs often do PCRs to test for MTB.
How would this test be ordered?	Private providers would order this test.
How would this test be documented on the RVCT?	There is no place on the RVCT to record PCR results.
How much would this test cost?	The fee for this test may vary depending on the lab performing the test.

<b>High Performance Liquid Chromatography (HPLC)</b>	
How does it work?	HPLC is used to identify mycobacteria by analysis of mycolic acids. Mycobacteria contain large amounts of mycolic acids in their cell walls. The type and amount of mycolic acids vary among the species of mycobacteria. HPLC testing generates chromatographs based on the mycolic acids that are present in the test organism. By comparing these graphs with known reference chromatograph patterns, the identification of the organism can be determined.
When is this test run?	The Tennessee Department of Health, Division of Laboratory Services does not run this test. Outside laboratory schedules may vary.
When are the results ready?	The availability of the results depends on the outside lab performing the test.
How are the results classified?	HPLC identifies the name of the mycobacterium being tested.
What do the results mean?	Results indicate which mycobacterium has grown.
Are other tests needed?	If the organism is identified as MTB and if this is the first time that MTB has been isolated from the patient, drug susceptibility testing needs to be performed.
When would this test be used?	This test would be used to identify an isolate suspected of being mycobacteria.
How is this test ordered?	Private providers would order this test.
How would this test be documented on the RVCT?	This result is a culture result and should be recorded as the culture result on either Question #18 or Question #20 on the RVCT.
How much would this test cost?	The fee for this test may vary depending on the lab performing the test.

<b>GenoType MTBDRplus® “Hain Test”</b>	
How does it work?	<p>The GenoType MTBDRplus® (Hain Test) is a rapid screening test for multidrug-resistant TB (MDR-TB). The test used a polymerase chain reaction (PCR) to amplify the genes followed by hybridization to specific probes on nitrocellulose strips (line probe assay).</p> <p>This test is used to detect resistance to isoniazid (INH) and rifampin (RIF) by detecting mutations in the following genes associated with resistance: <i>katG</i>, <i>inhA</i>, <i>rpoB</i></p>
When is this test run?	This test is run when multidrug resistance is suspected. The Tennessee Department of Health, Division of Laboratory Services does not run this test. Currently, the Florida Department of Health State Laboratory runs this test on any samples sent from Tennessee.
When are the results ready?	With results available within 1-2 days, estimated turnaround time (TAT) from specimen collection to reporting results is <7 days.
How are the results classified?	<p>Point mutation detected</p> <p>No point mutation detected</p>
What do the results mean?	<p>Point mutation detected: detection of the gene(s) associated with resistance is detected</p> <p>No point mutation detected: detection of the gene(s) associated with resistance is not detected</p>
Are other tests needed?	Final drug susceptibility testing (DST) results are needed to confirm results.
When would this test be used?	This test would be used when a provider suspects the possibility of drug resistance.
How is this test ordered?	The Tennessee State TB Control Officer needs to approve the test being ordered through the “Prior Authorization ” process.
How much does this test cost?	The cost of the test would be covered by the TTBEF.

<b>Therapeutic Drug Monitoring (TDM) “Drug Levels”</b>	
How does it work?	Therapeutic drug monitoring measures the concentration of TB medications in the bloodstream at certain intervals after receiving a dose of medication. This test may help distinguish between malabsorption and delayed absorption.
When is this test run?	This test is run when TB patients are at risk for malabsorption or showing a slow response to TB therapy.
When are the results ready?	Results are available 1-2 weeks after the sample is received in the lab.
How are the results classified?	Results for specific drug concentrations are reported in mcg/ml.
What do the results mean?	Results are accompanied by narratives and additional comments that explain normal ranges. These lab results should be reviewed by a physician and dosage adjustments made.
Are other tests needed?	Repeated drug levels may be necessary.
When would this test be used?	This test would be used when patient is not responding to therapy or has risk factors that would cause malabsorption of the TB medication.
How is this test ordered?	The Tennessee State TB Control Officer needs to approve the test being ordered through the “Prior Authorization ” process.
How much does this test cost?	The cost of the test would be covered by the TTBEF.

<b>Genotyping</b>	
How does it work?	<p>TB genotyping uses two polymerase chain reaction (PCR) methods on all <i>M. tuberculosis</i> isolates: spacer oligonucleotide typing (spoligotyping) and variable-number tandem repeat of mycobacterial interspersed repetitive units (VNTR-MIRU).</p> <p>Spoligotyping is a hybridization assay that detects variability in the direct repeat (DR) region in the DNA of <i>M. tuberculosis</i>. The DR region consists of multiple copies of a conserved 36-base-pair sequence (the direct repeats) separated by multiple unique spacer sequences (the standard spoligotyping assay uses 43). Different <i>M. tuberculosis</i> strains have various complements of the 43 spacers, and these different complements form the basis of the assay. The standard spoligotyping assay is performed by using a membrane. In this format, each of the 43 spacers produces either a dark band or no band.</p> <p>Variable number of tandem repeat (VNTR) typing is based on analysis of DNA segments containing “tandem repeated” sequences in which the number of copies of the repeated sequence varies among strains. The method relies on PCR amplification and calculation of the number of repeats on the basis of size of the amplified product. MIRUs are a class of tandem repeated sequences. Like spoligotyping, this typing methods yields results in a standardized code that can be easily analyzed and communicated between laboratories and TB programs. A total of 41 MIRU loci have been reported; however, most laboratories target only 12 loci (MIRU). Newer versions of the method at the National TB Genotyping Service (NTGS) laboratories now include 24 loci (MIRU2).</p>
When is this test run?	This test is run whenever isolates (that are culture-positive for MTB) are sent to the genotyping lab.
When are the results ready?	Results are usually available within 10 working days of receipt of the isolates in the genotyping lab.
How are the results classified?	<p>Spoligotype, MIRU and MIRU2 results are reported.</p> <p>Spoligotype + MIRU = PCR Type</p> <p>Spoligotype + MIRU + MIRU2 = GenType</p>
When would this test be used?	Genotyping can be used to confirm epidemiologic linkages (known or unknown); in source case investigations for pediatric TB cases, and to confirm false-positive cultures.
How is this test ordered?	One (1) isolate (if pure) from each culture-positive patient is sent by the Tennessee Department of Health, Division of Laboratory Services to a CDC-contracted lab for genotyping ( <b>Standard of Public Health Practice</b> ).
How much does this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

<b>T-SPOT.TB<sup>®</sup> Test</b>	
How does it work?	The T-SPOT.TB test enumerates the response of the effector T cells that have been sensitized to <i>Mycobacterium tuberculosis</i> . Interferon-gamma is captured and presented as spots from T cells sensitized to TB infection. Results are interpreted by subtracting the spot count in the negative (NIL) control from the spot count in the other panels.
When is the test run?	The Tennessee Department of Health, Division of Laboratory Services does not perform this test. Oxford Diagnostic Laboratories are the only labs that perform this test.
When are the results ready?	Results are ready in 36 hours from the receipt of the sample in the lab.
How are the results classified?	Positive Negative Borderline Invalid
What do the results mean?	Positive: >8 spots Negative: <4 spots Borderline: 5, 6, or 7 spots (test should be redone) Invalid: test should be redone
When would this test be used?	T-SPOT.TB <sup>®</sup> testing may be used in place of (but not in addition to) a tuberculin skin test (TST) in all situations in which the CDC recommends tuberculin skin testing as an aid in diagnosing <i>M. tuberculosis</i> infection.
How is this test ordered?	Private providers would order this test.
How much does this test cost?	The fee for this test may vary depending on pricing from the vendor.

<b>Mycobacteria Growth Indicator Tubes (MGIT)</b>	
How does it work?	<p>The MGIT tube is intended for the detection and recovery of mycobacteria using BACTEC 960 equipment. The tubes contain 7ml of modified Middlebrook 7H9 broth and are flushed with 10% CO<sub>2</sub>.</p> <p>A fluorescent compound is embedded in silicone on the bottom of the round bottom MGIT tubes which later allows the fluorescence to be detected in the presence of actively growing organisms.</p> <p>Tubes are monitored by the BACTEC 960 every 60 minutes for increasing fluorescence. Analysis of the fluorescence is used to determine if the tube is positive for growth.</p>
When would this media be used?	MGIT tubes are inoculated each day that clinical specimens are received in the lab. After undergoing the decontamination and concentration process, all specimens received for routine culture are inoculated onto this media.
How long before growth is obtained?	<p>For mycobacteria—1-6 weeks</p> <p>Negative (no growth)—MGIT tubes are held for 6 weeks before reporting as negative</p>
How are the results classified?	<p>Positive for growth</p> <p>Negative for growth</p> <p>Contaminated</p>
What do the results mean?	<p>Positive: growth noted, identification procedures are started</p> <p>Negative: no growth</p> <p>Contaminated: growth other than mycobacteria is present</p>
Are other tests needed?	The Tennessee Department of Health, Division of Laboratory Services will initiate identification testing if the MGIT tube is positive. Drug susceptibility testing will be ordered by the lab when indicated.
How would this test be ordered?	Not applicable
How much would this test cost?	The Tennessee Department of Health, Division of Laboratory Services does not charge a fee to perform this test.

### **3. SPUTUM SPECIMENS**

#### **Sputum Specimen Collection**

##### Induced Sputum

##### Equipment and Supply List

- Aerosol nebulizer
- Disposable aerosol tubing
- Disposable mouthpiece
- 3% NaCl solution (ampule)
- Cup of water
- Sputum collection container
- Lab requisition
- Labeled mailing container
- Tissues
- Disposable gloves
- Surgical mask (for patient)
- N95 respirator (for healthcare worker)
- Disinfectant (tuberculocidal)

##### Procedure

1. Don personal protection equipment: disposable gloves and N95 respirator
2. Explain the procedure to the patient
  - a. Purpose of the procedure
  - b. Do not begin procedure until the nurse has left the room and closed the door
  - c. How to notify nurse if assistance is needed or when procedure is complete
  - d. Importance of staying in the room or booth until coughing has stopped
  - e. Importance of replacing surgical mask before leaving the room or booth (if infectious)
3. Collect supplies and turn on environmental controls (i.e., negative pressure, Isoport M400, HEPA filters, UVGI lights) and prepare equipment
  - a. Prepare the nebulizer
    - i. Fill medication chamber with one (1) ampule of 3% NaCl
    - ii. Attach mouthpiece and connect aerosol tubing to machine
    - iii. Test nebulizer to ensure that adequate mist is produced
  - b. Place sign on door warning persons not to enter
  - c. Label sputum container
4. Instruct patient in sputum induction
  - a. Rinse mouth and drink water prior to beginning procedure
  - b. Place lips tightly around mouthpiece and inhale mist with deep breaths
  - c. Cough vigorously if spontaneous coughing does not occur

- d. Open and expectorate sputum into the sputum container, securing lid tightly when done
- e. Continue attempts until five (5) ml of sputum has been obtained (show patient prior to induction how much is needed in the container)
- f. Where to place specimen container when finished
- g. Turn off nebulizer machine
- h. Do not leave the room until coughing has ceased or nurse returns
  - i. Close the door after leaving the room
5. Turn machine on and leave the room
6. Observe patient and monitor (if in booth or room with a window)
7. Ensure door is closed after procedure is finished
8. Place a sign on the door indicating when the room will be safe to enter
9. Prepare room for next patient or end of the day (wait the required time for room to clear of infectious airborne particles or wear N95 respirator when entering the room)
  - a. Remove and discard disposable items in regular trash container
  - b. Wipe equipment/surfaces with approved disinfectant between procedures and at the end of the day
  - c. Assure nebulizer and Isoport M400 are turned off

### Natural Sputum

If a natural sputum is collected at the health department, the specimen should be obtained in a negative pressure room. If a negative pressure room is unavailable, the specimen should be collected outdoors and away from people, windows and air intakes (**Refer to Tool XII-1 for instructions on collecting a natural sputum**).

**Refer to Tool XII-2 for instructions for collecting natural sputum in the home setting.**

### Sputum Shipping and Handling

**Tips for the Use of Mailing Container Kits Provided by Tennessee Department of Health, Division of Laboratory Services, to Package Category B Specimens for Shipping by US Postal Service**

1. Mailing container (double cans) consisting of three (3) parts (each part is labeled in **Figure XII-1**):
  - a. Primary receptacle containing the specimen (labeled as “1a” on **Figure XII-1**).
  - b. Secondary mailing container also called the “inner container” or “metal container” (labeled as “1b” on **Figure XII-1**).
  - c. Outer mailing container also called the “cardboard shipping container” (labeled as “1c” on **Figure XII-1**).
2. Package specimen into secondary mailing container (“inner container” or “metal container”).

3. Screw the lid with the black rubber gasket (labeled as “3” on **Figure XII-1**) onto the secondary container (“inner container” or “metal container”).
4. The secondary container (“inner container” or “metal container”) is pre-labeled with a Biohazard Label (labeled as “4” on **Figure XII-1**).
5. Tri-fold the requisition so that the patient information is on the outside and is easily visible to laboratory personnel.
6. Wrap the requisition around the secondary container (“inner container” or “metal container”).
7. Place the sealed secondary container (“inner container” or “metal container”) with the requisition wrapped around it inside the outer mailing container (“cardboard shipping container”) and secure the with the lid.
8. The outer mailing container (“cardboard shipping container”) should have the following labels on it:
  - a. UN3373 biological substance category B label (labeled as “8a” on **Figure XII-1**).  
**The Tennessee Department of Health, Division of Laboratory Services will no longer provide these labels on the outer mailing container. These labels should be purchased by the metro or region.**

**Figure XII-1: Sputum Specimen Container**



**Reference:**

1. United States Postal Service Domestic Mail Manual, 601 Mailability, 8.0 Hazardous Materials. 2012.  
<http://pe.usps.com/text/dmm300/601.htm#>

**4. COMPLETING LABORATORY REQUISITIONS**

Laboratory specimens and requisitions are submitted in accordance with Clinical Laboratory Improvement Amendments (CLIA) standards and policies of the Tennessee Department of Health, Division of Laboratory Services (**Standard of Public Health Practice XII-5**).

**Sputum Specimens and Other Specimens for Mycobacteria Smear and Culture**

The following items must be completed on the lab requisition (**Refer to Appendix P**):

1. Specimen Collection Information
  - a. Patient last name
  - b. Patient first name
  - c. Patient date of birth (DOB)

- d. Patient gender
  - e. Patient county of residence
  - 2. Submitter Information
    - a. Submitter name (name of local health department submitting the specimen)
- NOTE:** Please write the entire name of the local health department (or use local health department stamp). Do not use acronyms.
- b. Submitter address (may use local health department stamp)
  - 3. Specimen information
    - a. Date of collection
    - b. Specimen source
      - i. For sputum specimens, mark as “natural sputum” or “induced sputum”
  - 4. Test Requested
    - a. Mark “Mycobacteria Smear & Culture”

### **QuantiFERON® -TB Gold In-Tube (QFT-GIT) Testing**

In order for QFT-GIT samples to be tested, a two-step procedure is required:

1. Entering a lab order entry (LOE)
2. Complete the “QuantiFERON Test Request and Processing Log” (**Refer to Appendix Q**)

### **Therapeutic Drug Monitoring (TDM)—“Drug Levels”**

**Refer to Appendix H and Appendix I.**

## **5. TOOLS**

XII-1: Collection of Natural Sputum in the Home Setting

**Tool XII-1**  
**Collecting Natural Sputum**

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## Collection of Natural Sputum

Procedure	Key Points
<ul style="list-style-type: none"> <li>❖ Preparation:               <ul style="list-style-type: none"> <li>➤ Follow <b>Handling Specimen and Preparation for shipping</b> requirements for specimen labeling and completion of lab requisition.</li> <li>➤ If collecting sputum in clinic setting, place patient in sputum booth or negative pressure room and turn on environmental controls: HEPA filters, UVGI lights, negative pressure, Isoport M400, etc. <b>or</b> have patient collect sputum in designated area outside; away from doors, windows, and vents.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Sputum collection should begin with the initial patient visit and may start on any day of the week.</li> <li>• All sputum specimens must be obtained in a sputum booth, negative pressure room, or outside the building.</li> <li>• Sputum specimens are to be collected in the containers provided by the TN State Lab.</li> <li>• If collecting in sputum room/booth, place sign on door warning persons not to enter.</li> <li>• Employees must wear an N95 respirator when performing sputum collection at any site (health department or patient home).</li> </ul>
<ul style="list-style-type: none"> <li>❖ Explain the procedure to the patient:               <ul style="list-style-type: none"> <li>➤ Purpose of procedure.</li> <li>➤ Patients are given verbal instructions regarding correct sputum collection.</li> <li>➤ Importance of staying in the negative room or sputum booth or outside until coughing has stopped.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Patient should not begin to collect sputum until the nurse has left the area.</li> <li>• Importance of replacing the surgical mask before leaving the room/booth or re-entering the health department (if infectious).</li> </ul>
<ul style="list-style-type: none"> <li>❖ Instruct patient in collection of sputum:               <ul style="list-style-type: none"> <li>➤ A specimen of 5-10 ml volume is adequate; container should not be more than half full.</li> <li>➤ Instruct the patient where to place the specimen container when finished.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Rinse mouth well with water prior to specimen collection.</li> <li>• Sputum should be coughed from deep down in the chest.</li> <li>• Open the plastic specimen container and spit sputum (phlegm) into the tube, securing lid tightly when done.</li> </ul>
<ul style="list-style-type: none"> <li>❖ Prepare specimen for shipping to State lab:               <ul style="list-style-type: none"> <li>➤ Retrieve specimen and follow the <b>Handling Specimen and Preparation for Shipping</b> requirements.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>➤ If collected in sputum room or booth, adequate time must be allowed for removal of airborne contaminants before entering area.</li> </ul>
<ul style="list-style-type: none"> <li>❖ Prepare room for the next patient or the end of the day.</li> </ul>	<ul style="list-style-type: none"> <li>➤ Wipe surfaces with approved disinfectant after use and at the end of the day.</li> </ul>

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**Tool XII-2**  
**Collecting a Natural Sputum in the**  
**Home Setting**

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## **INSTRUCTIONS FOR HOME SPUTUM COLLECTION**

- 1) When the patient will be collecting specimens at home, give him/her a labeled container and written instructions for natural sputum collection. Label innermost, plastic specimen tube with 2 identifiers: patient name and date of birth before obtaining specimens and before giving container to patient for home collection. (Can use a PTMBIS label with patient identifiers)
- 2) Instruct patient to collect an early morning specimen before eating.
- 3) If the patient is unable to raise early morning sputum, suggest that he/she stand or sit in a steamy environment for 15 minutes first by running hot water in the shower. Also, drinking several glasses of water may rehydrate the patient and assist in raising sputum.
- 4) Instruct patient to collect specimen outdoors (weather permitting) or in a room with outside ventilation with windows and outside doors open. Unexposed individuals should not be in the room.
- 5) Do not remove the lab slip from the container.
- 6) Return the specimen container to the health department, unless arrangements are made for a health department representative to pick up the specimen at the patients home/work/school.
- 7) Notify the Health department nurse when the specimen has been collected.
- 8) Instruct the patient to return the specimen to the local health department after collection, or to call the nurse/DOT worker for pickup.
- 9) Instruct patient to collect specimen outdoors (weather permitting) or in a room with outside ventilation with windows and outside doors open. Unexposed individuals should not be in the room.