CHAPTER 4: LABORATORY TESTING FOR TUBERCULOSIS

INTRODUCTION

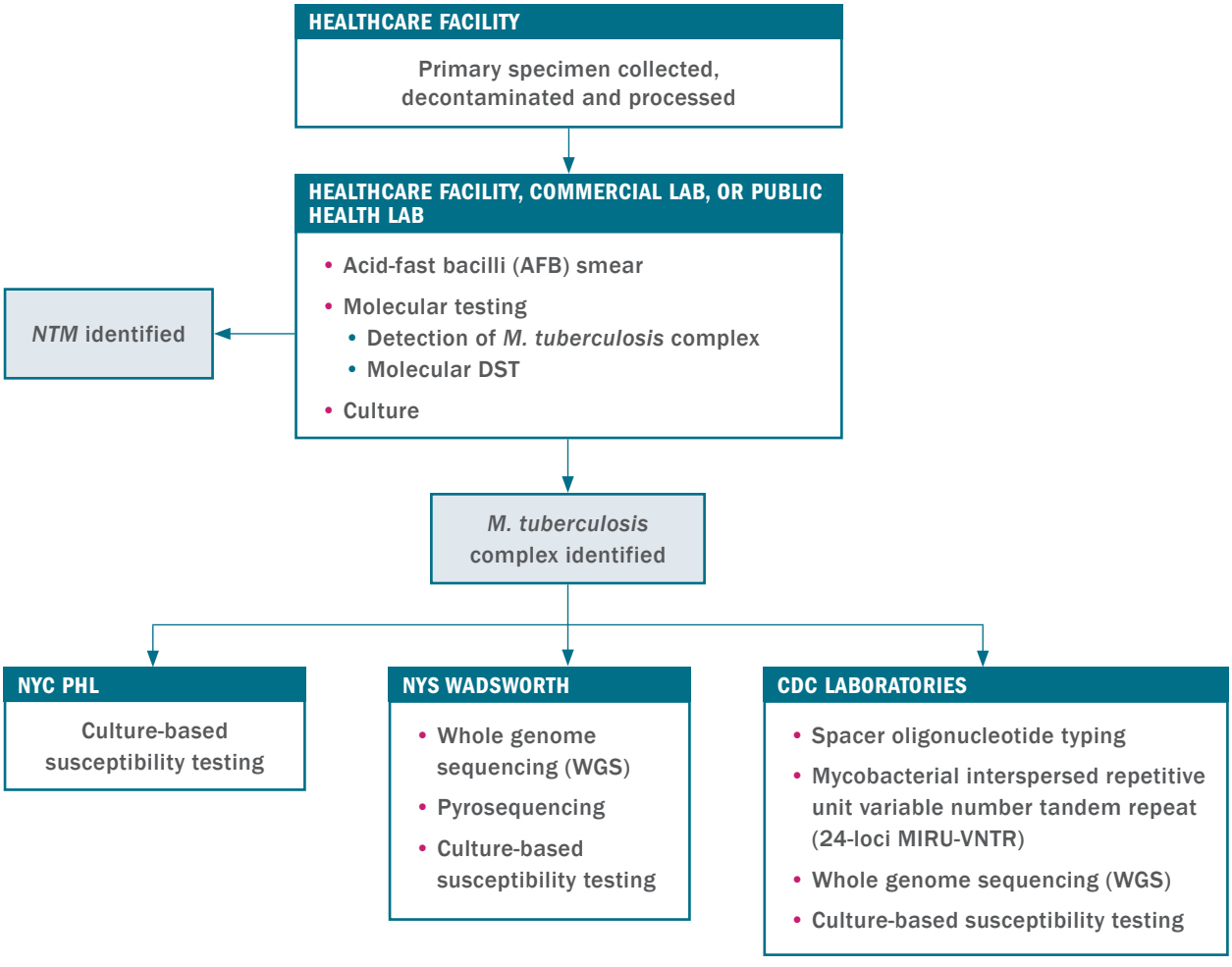
New York City (NYC) specimens undergo multiple types of laboratory testing for tuberculosis (TB). These tests include diagnostic tests for active TB disease, tests for drug susceptibility, tests that predict drug resistance, and genotyping. Prompt and accurate reporting of laboratory results supports appropriate diagnosis, treatment, infection control, surveillance, case management, and other clinical and public health activities.

GENERAL INFORMATION ABOUT LABORATORY TESTS FOR NEW YORK CITY SPECIMENS

Laboratory services and protocols vary depending on laboratory capacity and the facility where a specimen is collected. Specimens often pass through several laboratories in order to complete all required testing. (See *Figure 4.1:*

Mycobacteriology Laboratory Workflow for New York City Specimens.) The NYC Health Department Public Health Laboratory (PHL) performs diagnostic mycobacterial testing for all patients evaluated in a NYC Health Department TB clinic and provides laboratory testing services for NYC healthcare facilities and commercial laboratories. Diagnostic mycobacterial testing is also performed by hospital and commercial laboratories, and additional specialized testing—including susceptibility testing, drug resistance testing, and genotyping—is performed by the New York State (NYS) Department of Health Wadsworth Center (Wadsworth Center) and the Centers for Disease Control and Prevention (CDC). (See *Table 4.1: Laboratory Tests for Tuberculosis Disease Diagnosis, Drug Susceptibility, and Genotyping in New York City*.) The Bureau of TB Control (BTBC) collaborates with clinicians, laboratory partners, and others to facilitate testing and transfer of specimens between local healthcare facilities, commercial laboratories, NYC PHL, NYS Wadsworth, CDC, and contracted CDC laboratories. Specimen collection, handling, and transfer conform to established guidelines and are critical to the prompt and accurate diagnosis and treatment of TB.

FIGURE 4.1: Mycobacteriology laboratory workflow for New York City specimens



Abbreviations Used: CDC=Centers for Disease Control and Prevention; DST=drug-susceptibility test; *M. tuberculosis=mycobacterium tuberculosis*; NTM=nontuberculous mycobacterium; NYC PHL=New York City Department of Health and Mental Hygiene Public Health Laboratory; NYS Wadsworth=New York State Department of Health Wadsworth Center

TABLE 4.1: Laboratory tests for tuberculosis disease diagnosis, drug susceptibility, and genotyping in New York City

TEST TYPE	LABORATORIES	TURNAROUND TIME[1]	NOTES
AFB smear	• NYC PHL • Commercial • Hospital • NYS Wadsworth	Within 30 hours	Specimen types: respiratory, body fluids, tissue Factors influencing sensitivity: • Staining method (fluorochrome technique has a higher sensitivity than carbol fuchsin based techniques) • Experience of the microscopist
NAA tests	• NYC PHL • Commercial • Hospital • NYS Wadsworth	Within 2 to 5 days (some labs do not perform daily)	• Commercial, FDA-approved and non-FDA laboratory-developed tests available • High sensitivity and specificity for testing smear-positive respiratory specimens • Smear-negative respiratory or non-pulmonary specimens can have reduced sensitivity and specificity
Mycobacterial culture	• NYC PHL • Commercial • Hospital • NYS Wadsworth	Mycobacterial growth detection within 1 to 8 weeks Mycobacterial identification by DNA probe within 2 to 3 days of identifying growth	• Many labs finalize cultures at 6 weeks; NYC PHL finalizes cultures after 8 weeks • Reference labs use both liquid and solid culture media • If DNA probe for TB and MAC is negative, specimen might be sent to reference lab for identification
Phenotypic DST	• NYC PHL • Commercial • Hospital • NYS Wadsworth	Reported within 17-30 days from the date of identification of *M. tuberculosis*	• If the isolate has resistance to first-line drugs (except for PZA) by broth-based methods, the isolate is tested by the agar-proportion method for first- and second-line drugs • If susceptibility testing is unsuccessful, mutation analysis can be performed by pyrosequencing or Sanger Sequencing
Molecular DST • GeneXpert MTB/RIF assay • Pyrosequencing • Sanger sequencing • WGS	• NYC PHL • Commercial • Hospital • NYS Wadsworth • CDC	GeneXpert within 24 to 48 hours Pyrosequencing, Sanger sequencing, and WGS within 1 to 2 weeks	• Detection of RIF mutations requires confirmatory testing by sequencing • **GeneXpert:** Highly sensitive for mutations associated with RIF resistance • **Pyrosequencing, Sanger sequencing, and WGS:** Detect mutations associated with resistance to numerous anti-TB drugs
Genotyping • WGS • Spoligotyping • MIRU-VNTR	• NYS Wadsworth • CDC	3 days to 2 weeks	Performed by WGS, spacer oligonucleotide typing (spoligotyping), and mycobacterial interspersed repetitive unit-variable number tandem repeat analysis (MIRU-VNTR) for epidemiologic purposes

1. From time of specimen receipt

Abbreviations Used: AFB=acid-fast bacilli; CDC=Centers for Disease Control and Prevention; DNA=deoxyribonucleic acid; DST=drug-susceptibility test; FDA=Food and Drug Administration; INH=isoniazid; *M. tuberculosis*=*Mycobacterium tuberculosis*; MAC=Mycobacterium avium complex; NAA=nucleic acid amplification; NYC PHL=New York City Public Health Laboratory; NYS=New York State; PZA=pyrazinamide; RIF=rifampin; WGS=whole genome sequencing

Any laboratory conducting mycobacteriology testing for NYS residents must obtain and maintain certification from NYS's Clinical Laboratory Evaluation Program (CLEP).

In addition to diagnostic testing, the NYC Health Code mandates that laboratories either perform drug-susceptibility tests (DSTs) or submit an isolate of the initial culture from any positive *Mycobacterium tuberculosis* (*M. tuberculosis*) specimen to a laboratory that performs DST. (See *Chapter 17: Laws Governing Tuberculosis Care in New York City*.) Laboratories must also submit a portion of isolate to the NYC PHL for genotyping.

TESTS FOR TUBERCULOSIS DISEASE

ACID-FAST BACILLI SMEAR

Specimens collected for mycobacterial testing are typically submitted for both acid-fast bacilli (AFB) smear and culture. Specimen from non-sterile sources (e.g., sputum) require processing by digestion and decontamination of the specimen. The processed specimen is used to prepare a smear that is stained for AFB. AFB smear results are reported within 24-30 hours of specimen receipt.

The smear is important, both clinically and epidemiologically. Typically, AFB smear is the first test result obtained. A positive test result may increase the clinical suspicion of active TB disease. AFB smear prepared from sputum are also used to assess a patient's infectiousness. When positive, the results are reported on a scale which reflects the semi-quantitative estimate of the number of bacilli excreted. (See *Table 4.2: Quantitative Scale for Acid-Fast Bacilli Smears by Stain Used*.)

TABLE 4.2: Quantitative scale for acid-fast bacilli 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	*Suspicious; recommend resubmission of new specimen*
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, Centers for Disease Control and Prevention, & Infectious Diseases Society of America. (2000). Diagnostic standards and classification of tuberculosis in adults and children. American Journal of Respiratory and Critical Care Medicine, 161, 1376-1395

Abbreviations Used: AFB=acid-fast bacilli

Additional diagnostic studies must be performed to confirm a TB diagnosis among patients with a positive AFB smear result since a positive AFB smear does not differentiate between *M. tuberculosis* and nontuberculous mycobacteria (NTM), certain actinomycetes, and other biological species. Sensitivity of a sputum smear is 50 to 80% among patients with pulmonary TB disease. At least 500 to 10,000 bacilli per milliliter (ml) of specimen must be present to detect bacteria on stained smears. In contrast, only 10 to 100 organisms are needed for a positive culture.

NUCLEIC ACID AMPLIFICATION TESTS

Although mycobacterial culture remains the gold standard for the diagnosis of TB disease, culture confirmation of TB may take several weeks or longer from the day of specimen collection. Nucleic amplification assay (NAA) tests identify DNA unique to *M. tuberculosis* complex in raw or processed clinical samples within a few hours. These tests can rapidly detect *M. tuberculosis* DNA with high sensitivity and specificity for respiratory AFB smear-positive specimens, but have lower sensitivity on AFB smear-negative specimens. These tests are not Food and Drug Administration (FDA)-approved for extrapulmonary specimens; however, some laboratories may have validated these tests for extrapulmonary specimens, and they can be used to test patients where there is a high clinical suspicion of TB.

There are various commercial tests currently available for use by hospital, public health, and reference labs.

- **GeneXpert MTB/RIF assay:** Approved by the FDA in August 2013 and used by many hospitals and commercial laboratories, this assay detects DNA of *M. tuberculosis* complex and genetic mutations associated with resistance to rifampin (RIF) in unprocessed sputum and concentrated sputum sediments. The assay is an NAA test using a disposable cartridge in conjunction with the GeneXpert system to extract, amplify, and detect *M. tuberculosis* complex DNA and mutations associated with RIF resistance. As many RIF-resistant isolates are also resistant to isoniazid (INH), RIF resistance can be used as a marker for multidrug-resistant TB (MDR-TB).

AFB SMEAR-POSITIVE RESPIRATORY SPECIMENS: In AFB smear-positive respiratory specimens, the positive predictive value of the NAA test is > 95%. Therefore, if the NAA is positive, presume the patient has TB disease and begin anti-TB therapy, pending culture results. If the NAA is negative, clinical judgment will need to determine whether to begin anti-TB therapy. These patients are likely to have an infection with NTM, especially if a second NAA from an AFB smear-positive specimen also tests negative for TB. For these patients, it may be appropriate to delay anti-TB therapy and contact investigation until culture results are available.

AFB SMEAR-NEGATIVE RESPIRATORY SPECIMENS: In AFB smear-negative respiratory specimens where TB is highly suspected, the diagnosis of TB is not completely excluded by a negative NAA test, as current NAA tests are only 50 to 80% sensitive in detecting TB. A decision about treatment must be based on epidemiological factors, clinical assessment, radiological, and other diagnostic tests while cultures are being finalized. Additional NAA testing may be warranted.

NAA tests 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, most NAA tests should be used only for initial diagnosis for patients on TB medication less than one week, and not for follow-up evaluation of patients. The Xpert MTB/RIF should not be performed on specimens from patients where anti-TB medication has been initiated.

For patients with a positive NAA test but negative culture, the treating provider must determine if the patient has TB based on epidemiologic risk factors and/or clinical response to treatment.

NAA tests must be interpreted within the context of a patient's signs and symptoms and should always be performed in conjunction with the AFB smear and culture.

MYCOBACTERIOLOGY CULTURE

Despite advances in molecular detection of *M. tuberculosis* complex, growth of the organism through a culture remains the gold standard for diagnosis. Culture is able to detect as few as 10 bacteria/ml of a specimen, and is necessary for species identification. Pure culture growth of *M. tuberculosis* is necessary to perform phenotypic drug-susceptibility tests (DSTs), whole genome sequencing (WGS), certain molecular DSTs, and genotyping methods.

Specialized culture media are used for mycobacterium. Mycobacteriology laboratories use both liquid media and solid media to culture mycobacteria. Using multiple types of culture media increases diagnostic yield. Liquid media systems allow for rapid growth-detection of mycobacterial within one to three weeks compared with solid media (three to eight weeks' growth). The laboratory will usually issue a negative culture report after six to eight weeks of incubation. NYC PHL incubates cultures for up to eight weeks before reporting them as No Growth or Negative for Mycobacterium species.

When mycobacterial growth is observed in culture, the culture growth may be tested with a DNA probe (e.g., Hologic [formerly Gen Probe] Accuprobe®) to identify *M. tuberculosis* complex or other NTM. Additional testing on solid media may be performed if the DNA probes fail to identify a mycobacterial species. Currently, the NYC PHL primarily uses High Performance Liquid Chromatography (HPLC) by the Mycobacterial Identification (MIDI) system to analyze the mycobacterial culture to identify *M. tuberculosis* complex and many other NTM's. MIDI HPLC is validated for isolates grown on solid medium only. NYS Wadsworth performs multiple laboratory-developed, real-time PCR assays that can identify *M. tuberculosis* complex, species within the *M. tuberculosis* complex, *M. avium* complex, and *M. abscessus* on primary specimens and isolates.

TESTING FOR DRUG SUSCEPTIBILITY AND PREDICTION OF DRUG RESISTANCE

To formulate an effective anti-TB regimen, DST results are needed on initial isolates from all patients. An anti-TB regimen is constructed based on the susceptibility results. Drug susceptibility testing is performed by culture-based (phenotypic) and molecular methods.

CULTURE-BASED SUSCEPTIBILITY TESTING

All initial *M. tuberculosis* complex isolates are tested using culture-based susceptibility. Culture-based susceptibility testing is the gold standard and is performed at PHL regardless of the molecular results. NYS Wadsworth performs WGS only on *M. tuberculosis* isolates, unless WGS predicts resistance. Predicted resistance by WGS is confirmed by culture-based methods at NYS Wadsworth.

DST is routinely performed on the initial positive culture and can be performed in liquid (Mycobacterial Growth Indicator Tube [MGIT] 960; Becton Dickinson) or on solid media (agar proportion method, also known as the conventional method). *M. tuberculosis* complex isolates are routinely tested for susceptibility to first-line drugs: INH, RIF, pyrazinamide (PZA), and ethambutol (EMB). Streptomycin (SM) may also be tested depending on the laboratory. First-line DST results are usually reported within 17-30 days after culture growth has been identified as *M. tuberculosis* complex. Additional testing of first-line and second-line drugs is performed when resistance is observed to any first-line drugs with the exception of PZA (see *Testing of Susceptibility to Pyrazinamide* section). In NYC, when the healthcare facility laboratory cannot perform DSTs, the isolate is sent to a public health laboratory, usually NYC PHL or NYS Wadsworth.

The agar proportion method is performed to confirm resistance on first-line drugs detected in liquid media as well as to look for resistance to second-line drugs. On request, NYS will perform additional liquid-based testing (MGIT) on moxifloxacin (MFX), linezolid (LZD), bedaquiline (BDQ) and clofazimine (CFZ) if first-line resistance is seen on INH and/or RIF. The agar proportion method allows for the calculation of the proportion of organisms that is resistant to a given drug at a specified concentration. This method uses Middlebrook 7H10 agar plates or 7H11 agar plates.

- Countable colonies (50 to 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 provider should request that the laboratory perform additional susceptibility testing if the patient continues to either have culture-positive sputum after two to three months of adequate treatment or develops new positive cultures after a period of negative cultures (i.e., patient has culture converted). (See *Table 4.3: Drug Concentrations for Various Methods Used by New York City Reference Laboratories for Mycobacterium Tuberculosis Complex Antimicrobial Susceptibility Testing*.)

TABLE 4.3: Drug concentrations[1] for various methods used by New York City reference laboratories for *Mycobacterium tuberculosis* complex antimicrobial susceptibility testing

	DRUG	BROTH-BASED SYSTEM[2]				SOLID MEDIA AGAR PROPORTION METHODS[2]		
		Bactec MGIT 960				Middlebrook 7H10 Agar		Middlebrook 7H11 Agar
		NYC PHL	NYS	NJH	NJH (Single drug MIC)	NYC PHL	NYS	NJH
FIRST LINE DRUGS	INH	0.1[3]	0.1[3]	0.1[3]	0.025, 0.05, 0.1, 0.2, 0.4, 0.8	0.2	0.2	0.2
	INH (high)	Not Tested	0.4	0.4	N/A	1.0, 5.0	1.0	1.0
	RIF[4]	1.0[3]	1.0[3]	0.5	0.5, 1.0, 2.0	1.0	1.0	1.0
	PZA	100.0	100.0	50, 100, 200, 400	50, 100, 200[3], 400[3]	-	-	-
	EMB	5.0	5.0	5.0	2.5, 5.0, 10.0	5.0	5.0, 10.0	7.5
OTHER DRUGS	AK	-	-	-	1.0, 2.0, 4.0, 8.0	-	1.0, 2.0, 4.0	6.0
	BDQ	-	1.0	-	-	-	-	-
	CM	-	-	-	2.0, 4.0, 8.0	10.0	10.0	10.0
	CPFX[5]	-	-	-	1.0, 2.0, 4.0	2.0	-	-
	CFZ	-	0.5	-	N/A	-	-	0.12, 0.25, 0.5
	CS	-	-	-	N/A	30.0	30.0	60.0
	ETA	-	-	-	1.0, 2.0, 4.0, 8.0	5.0	5.0	10.0
	KM	-	-	-	2.0, 4.0, 8.0	6.0	5.0	6.0
	LFX[5]	-	-	-	0.5, 1.0, 2.0	-	-	-
	LZD	-	1.0	-	0.5, 1.0, 2.0, 4.0, 8.0	-	-	-
	MFX[5]	-	-	-	0.25, 0.5, 1.0, 2.0	-	-	-
	OFX[5]	-	2.0	-	1.0, 2.0, 4.0	-	1.0, 2.0, 4.0	-
	PAS	-	-	-	N/A	2.0, 10.0	10.0	8.0
	RBT	-	-	-	0.12, 0.25, 0.5, 1.0	0.5	0.5, 1.0, 2.0	-
	SM	-	1.0	-	1.0, 2.0, 4.0, 8.0	2.0	2.0	2.0
	SM (high)	4.0	-	-	N/A	10.0	10.0	4.0

1. Concentration in mcg/mL. 2. Phenotypic susceptibility testing: broth-based assay or agar-based assay; any drug resistance found for either method usually means the drug should not be used in the treatment regimen. 3. Critical concentration of the drug in this medium is the MIC 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. tuberculosis* strain. 4. RIF is the class agent for RPT. Results for RIF reflect RPT susceptibility. 5. FQN testing – each laboratory generally tests 1 member of the class.

Abbreviations Used: AK=amikacin; BDQ=bedaquiline; CFZ=clofazimine; CM=capreomycin; CPFX=ciprofloxacin; CS=cycloserine; EMB=ethambutol; ETA; ethionamide; FQN=fluoroquinolones; INH=isoniazid; KM=kanamycin; LFX=levofloxacin; LZD=linezolid; *M. tuberculosis=Mycobacterium tuberculosis*; mcg/mL=micrograms per milliliter; MFX=moxifloxacin; MGIT=Mycobacterial Growth Indicator Tube; MIC=minimal inhibitory concentration; N/A=Not applicable; NJH=Denver National Jewish Health Advanced Diagnostic Laboratories; NYC PHL=New York City Public Health Laboratory; NYS=New York State; OFX=ofloxacin; PAS=para-aminosalicylic acid; PZA=pyrazinamide; RBT=rifabutin; RIF=rifampin; SM=streptomycin

Table created in consultation with NJH, NYC PHL, and NYS

On a case-by-case basis, DST may be requested for second-line drugs (e.g., on an isolate without known first-line drug resistance) when a second-line drug (i.e., FQN) is being considered to treat a patient.

For MDR-TB specimens, BTBC selectively requests phenotypic DST for MFX, LZD, CFZ, and BDQ from NYS Wadsworth, and additional drugs for minimum inhibitory concentration DST, from Denver National Jewish Health Advanced Diagnostic Laboratories (NJH).

TESTING OF SUSCEPTIBILITY TO PYRAZINAMIDE

PZA testing is different from that of other first-line drugs, as testing must be measured at a lower pH than the other first-line drugs (pH 6.0 has been chosen for testing PZA in liquid and solid media). Testing for susceptibility to PZA is difficult and results for a single patient may vary between different specimens or results for a single specimen may be discrepant between different laboratories. The specimen can also be tested by molecular methods, such as WGS or Sanger sequencing, which may detect a mutation in the pncA gene that is associated with PZA resistance.

If an isolate shows resistance to PZA, especially if the isolate is resistant to PZA alone, the species of *M. tuberculosis* complex need to be confirmed, because *M. bovis* and *M. bovis*-bacille Calmette-Guérin (BCG) are naturally PZA-resistant, whereas the majority of *M. tuberculosis* isolates are PZA-susceptible. This is especially important if the laboratory identifies isolates only to the level of the *M. tuberculosis* complex.

MOLECULAR METHODS TO DETECT MUTATIONS ASSOCIATED WITH DRUG RESISTANCE

Molecular tests use DNA-based methods to detect *M. tuberculosis* complex and mutations associated with clinical drug resistance. These tests are also referred to as “mutation analysis,” “molecular DST,” or “tests to detect molecular or genetic markers of drug resistance.” They are variations of NAA tests/PCR methods and decrease the time required for identification of *M. tuberculosis* complex and detection of drug-resistance from weeks to days. These tests allow for earlier detection of RIF resistance and initiation of appropriate therapy. This is an area of active investigation and molecular methods are constantly being refined and improved. (See *Table 4.4: Molecular Methods to Detect Drug Resistance by Method* and *Table 4.5: Molecular Methods to Detect Drug Resistance by Drug and Gene Target*.)

GENEXPERT MTB/RIF

GeneXpert MTB/RIF assay: Approved by the FDA in August 2013 for use in hospital, commercial, and public health laboratories, this assay detects DNA of *M. tuberculosis* complex and genetic mutations associated with resistance to RIF in unprocessed sputum and concentrated sputum sediments. The assay is an NAA test using a disposable cartridge in conjunction with the GeneXpert Instrument system. As many RIF-resistant isolates are also resistant to INH, RIF resistance can be used as a marker for MDR-TB.

TABLE 4.4: Molecular methods to detect drug resistance mutations by method

TEST TYPE	DETECTS	SPECIMEN TYPES FOR INTENDED USE
GeneXpert MTB/RIF	*M. tuberculosis* mutations associated with RIF resistance	• AFB smear-positive or smear-negative primary sputum samples or concentrated sediments prepared from induced or expectorated sputa
Pyrosequencing	Mutations associated with resistance to INH, RIF, and FQNs	• Performed on primary specimens and on mixed cultures when other methods are unsuccessful or susceptibility cannot be obtained
Sanger sequencing	Mutations associated with first- and second-line drugs	• Performed on primary specimens and on mixed cultures when other methods are unsuccessful or susceptibility cannot be obtained
WGS	• Speciation of *M. tuberculosis* complex • Mutations associated with first- and second-line drugs	• Requires pure culture growth of *M. tuberculosis*

Abbreviations Used: AFB=acid-fast bacilli; BAL=bronchoalveolar lavages; FQN=fluoroquinolone; INH=isoniazid; *M. tuberculosis*=*Mycobacterium tuberculosis*; RIF= rifampin; WGS=whole genome sequencing

PYROSEQUENCING

Pyrosequencing (PSQ) is a real-time sequencing method that analyzes short reads of DNA in order to detect mutations associated with drug resistance within a specific gene. PSQ is performed to detect mutations associated with resistance to INH, RIF, and FQNs. However, PSQ is not used for detecting pncA gene mutations that may be associated with PZA resistance due to the size of the pncA gene. Therefore, pncA sequencing is primarily determined by Sanger sequencing or whole genome sequencing. Pyrosequencing can be performed on primary specimens and on mixed cultures when phenotypic susceptibility tests are unsuccessful or cannot be obtained.

SANGER SEQUENCING

Sanger sequencing is capable of sequencing long reads of DNA in order to detect mutations associated with first- and second-line drug resistance within a specific gene. However, unlike PSQ, Sanger sequencing is able to detect mutations spread throughout the gene associated with resistance to first- and second-line drugs. Sanger sequencing can be performed on primary specimens and on mixed cultures when phenotypic susceptibility testing is unsuccessful or cannot be obtained.

WHOLE GENOME SEQUENCING

WGS utilizes the TB isolate genome to identify the genus and species, genetic mutations associated with drug resistance, spoligotype results, and single nucleotide polymorphisms (SNP) to characterize and compare TB isolates. WGS requires a pure culture for analysis because many factors interfere with direct-from-specimen analysis.

WGS identifies mutations associated with drug resistance within five to 10 days of specimen receipt at the lab. The interpretation of molecular assays that examine resistance-associated mutations must be done with an understanding of the limitations of the test results. Although the detection of mutations may indicate resistance to a particular drug, the lack of detection of mutations does not confirm drug-susceptibility. These methods do not test for all mutations that may be associated with drug resistance, some of which are known, and others are unknown.

DISCORDANT RESULTS

Discordant results for testing of drug resistance can occur. BTBC communicates with laboratories and the clinician whenever discrepant results are reported. In general, drug resistance found by any method usually means the drug should not be used in the treatment regimen or, if used, cannot be counted upon as an effective agent in the treatment regimen.

Clinicians who have concerns about discrepancies between DST results, molecular mutations, and clinical response should call the TB HOTLINE at 844-713-0559.

TABLE 4.5: Molecular methods to detect drug resistance by drug and gene target*

DRUG	GENE(S)	TEST TYPE				
		Xpert® MTB/RIF	Pyro-sequencing	Sanger sequencing	Whole genome sequencing	Research mutation only[1]
RIF	• rpoB	✔	✔	✔	✔	
INH	• inhA • katG		✔	✔	✔	
	• oxyR-ahpC PR • mabA-inhA PR • mabA				✔	
EMB	• embB			✔	✔	
	• embC-embA PR				✔	
PZA	• pncA				✔	
	• pncA PR			✔	✔	
ETA	• mabA • mabA-inhA PR • ethA				✔	
FQN	• gyrA		✔	✔	✔	
	• gyrB		✔	✔	✔	
SMN	• rrs • rpsL				✔	
KM	• rrs • eis PR			✔	✔	
AK	• rrs			✔	✔	
CM	• rrs			✔		
	• tlyA			✔		
BDQ	• atpe					✔

*There are currently no molecular tests validated for clofazimine or linezolid. This table reflects the mutations that are known at the time of publication.

1. Observed in laboratory-induced resistant strains

Abbreviations Used: AK=amikacin; BDQ=bedaquiline; CM=capreomycin; EMB=ethambutol; ETA=ethionamide; FQN=fluoroquinolone; INH=isoniazid; KM=kanamycin; PZA=pyrazinamide; PR=promoter region; RIF=rifampin; SM=streptomycin

GENOTYPING

Genotyping is a process by which genetic information is used to characterize *M. tuberculosis* strains. NYC conducts universal genotyping on at least one isolate from each culture-positive TB patient using multiple genotyping methods.

Genotyping is a useful tool for distinguishing between relapse and re-infection, supporting and refuting transmission between epidemiologically-linked persons, detecting or confirming outbreaks, investigating and identifying false-positive results (i.e., laboratory cross-contamination), and characterizing TB strains in a population.

Genotyping methods used in NYC include:

- **Whole genome sequencing (WGS):** WGS is currently used to generate spoligotyping results by NYS Wadsworth and CDC, and can provide greater resolution for examining genetic relatedness through analysis of high-quality single nucleotide polymorphisms (SNP), which enables further strain differentiation. The turnaround time for SNP analysis is very quick once WGS sequence data becomes available.
- **Spacer oligonucleotide typing (spoligotyping):** Spoligotyping is a PCR based method with a quick turnaround time. Spoligotyping identifies spacer sequences found in the direct repeat region in the *M. tuberculosis* chromosome. Spoligotyping detects the presence or absence of 43 spacers. The spacing pattern is then translated into a 15-digit octal code that is used to communicate results between laboratories and jurisdictions.
- **Mycobacterial interspersed repetitive unit–variable number tandem repeat (24-loci MIRU-VNTR):** VTNR-MIRU analysis is a PCR based method that determines the number of repeated sequences in 24 defined regions (loci) of the TB chromosome. Similar to spoligotyping, MIRU has a quick turnaround time and uses a standard nomenclature that makes results easy to compare across jurisdictions.
- **IS*6110* restriction fragment length polymorphism (RFLP):** This method measures the number and length of specific DNA fragments in the insertion sequence 6110, a genetic marker unique to members of the *M. tuberculosis* complex. An RFLP result comes in the form of an image with bands that show the pattern and copies of IS*6110*. The images are scanned and analyzed by a computer to compare RFLP results. RFLP is useful as a method for differentiating TB strains when greater than six bands are present. However, RFLP has a slow turnaround time, and results may be difficult to communicate between labs and jurisdictions because different labs do not use the same nomenclature. This method is rarely used in the U.S.

FALSE-POSITIVE INVESTIGATION

A false-positive TB laboratory test result occurs when the reported result has been reported in error, either due to a contamination of a clinical device, a clerical error, or laboratory cross-contamination during specimen processing. A false-positive specimen can lead to the misdiagnosis of TB disease and unnecessary treatment of a patient. It can also lead to unnecessary contact investigations and erroneously counting the patient in the surveillance system. Prompt identification and investigation of specimens suspected to have a false-positive result is important.

BTBC conducts active surveillance for potentially false-positive laboratory results. Patients with a single positive culture from an extrapulmonary specimen are not routinely investigated unless requested. Criteria for initiating a false-positive investigation include:

- Presence of a single *M. tuberculosis* positive respiratory culture in a patient with several culture-negative specimens.
- Clinical signs and symptoms of a patient are not compatible with the TB culture results
- New positive culture in a patient who previously culture converted
- Changes in drug-susceptibility pattern in a patient without suspicion of acquired drug resistance
- Suspected lab cross-contamination
- Matching genotypes among specimens processed together in a laboratory
- Discordant genotypes among culture-positive specimens from the same patient
- Rare strains in multiple patients within a short time period
- Presence of a TB lab strain (H37Rv)

Requests for a false-positive investigation come from BTBC providers and staff, non-BTBC providers, other TB programs, and laboratorians. BTBC works closely with laboratories and providers to investigate all potential false-positive specimens. The false-positive investigation is a stepwise process that requires review of the specimen collection and processing dates, examination of the genotype of specimens and characteristics of the patients involved, and identification of the source of confirmed contamination. When possible, it may be necessary to collect another specimen or test another isolate from the same patient and/or repeat testing of the original sample.

The outcome of a false-positive investigation is determined based on multiple factors. (See *Table 4.6: Outcomes, Definitions, Criteria and Clinical Decisions in False-Positive Investigations*.) It is important to communicate the results back to the treating providers, BTBC staff involved with the case management of the patient, surveillance teams, and the involved laboratories.

TABLE 4.6: Outcomes, definitions, criteria, and clinical decisions in false-positive investigations

OUTCOME	DEFINITION(S)	REASON(S)	CLINICAL DECISION*
Confirmed false-positive	• Specimen is contaminated or • Specimen belongs to another patient	• Cross contamination • Mislabeled specimens • Species misidentified • TB lab strain (H37Rv) identified • Contaminated medical devices	• Patient should usually not be treated for TB
Inconclusive	• Unable to determine if a contamination occurred or source of contamination	• Contamination source could not be identified • Unable to perform further TB testing (i.e., DST or genotype) • Additional specimen is not available	• Decision to treat the patient is made based on clinical factors
Unlikely	• Specimen has a true result or • Specimen is attributed to the correct patient	• Genotypes do not match • Unique genotype identified • Additional specimen confirms previous genotype results	• Patient should be treated for TB • If the physician does not feel TB is the correct diagnosis, must present other clinical data to support decision not to treat the patient

*If the physician suspects TB, the patient should be treated regardless of the false-positive investigation outcome.

Abbreviations Used: DST=drug-susceptibility tests; TB=tuberculosis

OTHER LABORATORY TESTING

Additional diagnostic testing of body fluids such as white blood cell (WBC) count, protein, glucose, and adenosine deaminase (ADA) may be necessary to support the clinical diagnosis of extrapulmonary TB. (See *Chapter 3: Diagnosis of Tuberculosis Disease in Adults*.)

As part of management and clinical monitoring of a patient with TB disease, additional laboratory examinations may be performed including: an HIV test, a complete blood count (CBC), a comprehensive metabolic panel (including assessment of kidney and liver function), thyroid function, and a pregnancy test for persons of childbearing age. Bloodwork results are assessed at the start of treatment and may be used to monitor therapy. (See *Chapter 8: Clinical Monitoring and Follow-Up for Tuberculosis Treatment*.)

SUMMARY

Appropriate and timely collection of specimens for laboratory testing is critical to ensure diagnostic test results are optimized to make clinical decisions. Use of molecular methods to identify TB and determine mutations for drug resistance allows for more timely initiation of appropriate TB treatment and decreased transmission of infectious TB. This is a rapidly changing area with both clinical and epidemiological implications. Continuous communication between laboratories, providers, and BTBC staff is vital to ensure the patient's treatment is based on all available diagnostic information.

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