



Laboratory

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SUMMARY OF KEY UPDATES (2022)

- Updated information on critical concentrations, clinical breakpoints, broth microdilution test ranges, and how the World Health Organizations (WHO) and others are reviewing and reconsidering these test characteristics and criteria
- New table of molecular and growth-based drug susceptibility tests available at 5 reference laboratories in the United States (U.S.)
- New table detailing molecular assays not available in the U.S.
- Updated information about specific mutations that confer resistance and the sensitivity and specificity of testing based on the WHO mutation catalog and other sources
- New section focused on Xpert MTB/RIF assay including cycle threshold (Ct) values, fluorescence curves, and interpretation examples for each major category of result
- Updated and expanded table of Xpert MTB/RIF performance on nonrespiratory specimens
- Updated information about the use of whole genome sequencing (WGS) in the National Tuberculosis Molecular Surveillance Center for identifying chains of transmission

The role of the laboratory is critical in the diagnosis of tuberculosis (TB), and even more so for drug-resistant (DR) TB.

Definitive diagnosis of DR-TB requires that *Mycobacterium tuberculosis* complex (MTBC) be isolated, drug susceptibility testing (DST) be completed, and results be conveyed to the clinician. Prompt turnaround time for laboratory results is of paramount importance in rapid diagnosis and appropriate treatment, infection control, and public health management of DR-TB.

Molecular technology enables much more rapid diagnosis of drug resistance. However, new technologies generate new questions, and the best way to interpret molecular resistance results is still evolving. New algorithms regarding the use of growth-based methods (historically considered the gold standard), especially for first-line drugs, are being evaluated given expanding access to methods like WGS. Despite increasing knowledge and experience with molecular methods, **conventional growth-based DST remains crucial.** Note: Growth-based DST is complex and various methods are used. Differences in methodology, critical concentra-

tions and inoculum preparation may generate discrepant results, making the interpretation of growth-based DST results difficult. These challenging laboratory results can have significant implications for treatment and often necessitate expert consultation.

***M. tuberculosis* complex (MTBC) includes several species.
All can cause clinical tuberculosis disease.
M. tuberculosis is by far the most common.**

- *M. tuberculosis* (>95% of cases)
- *M. bovis** (1-2% in U.S. overall; up to 5% in certain regions of U.S.)
- Other species (<1% of cases)**
M. africanum, *M. canettii*, *M. caprae*, *M. pinnipedii*,
M. microti, *M. mungi*, *M. orygis*, *M. suricattae*

* Excludes *M. bovis* Bacillus Calmette–Guérin (BCG)

** Based on unpublished genotyping data from California, 2004-2022

General information on TB laboratory work

Several types of laboratories perform diagnostic mycobacteriology testing, including hospital-based laboratories, local and state public health laboratories, and commercial laboratories. Laboratories may choose to provide different levels of services and different methods for the services they offer. See **Table 1** for a list of mycobacteriology laboratory services and **Figure 1** for the general workflow of mycobacteriology laboratories. Services and protocols may vary based on the setting where the specimen is collected (outpatient vs. hospital), type of specimen (sputum vs. cerebrospinal fluid [CSF]), and third-party payer source. A single specimen can pass through several different laboratories to complete testing.

Case managers and treating physicians should understand the laboratory practices of the facilities processing their patients' specimens.

TABLE 1. **Mycobacteriology laboratory services**

Test	Expected turnaround time (TAT) (from specimen receipt at laboratory; batched testing and variable scheduling may increase TAT)	Comments
Acid-fast bacilli (AFB) smear	1 day	Fluorochrome staining is more sensitive than carbol-fuchsin acid fast staining (Ziehl-Neelsen [ZN] or Kinyoun methods). A specimen that is smear-negative by ZN may be smear-positive by a fluorochrome method. Centers for Disease Control and Prevention (CDC) recommends fluorochrome staining.
Molecular testing for detection of <i>M. tuberculosis</i> complex and drug resistance	0-2 days by Xpert MTB/RIF 1-2 days for Hain/line probe assay, quantitative polymerase chain reaction (qPCR), pyrosequencing (PSQ), Sanger. 3-7 days by targeted next generation sequencing (tNGS) 4-7 days by WGS from pure culture	Commercial and in-house developed tests available. Xpert MTB/RIF is U.S. Food and Drug Administration- (FDA-) cleared for testing sputa; widely available. Other commercial assays are available and recommended outside the U.S. See Table 6 for more information.
Mycobacterial culture	Cultures usually turn positive in 2-3 weeks. A preliminary report of culture-positivity may be issued before culture identification is attempted. 6-8 weeks to report negative.	When a culture takes 5-6 weeks to turn positive, discuss with laboratory; consider possibility of cross-contamination.
Identification of positive cultures	3-4 weeks by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or sequencing for identification of <i>M. tuberculosis</i> complex and most nontuberculous mycobacteria (NTM). Some laboratories have validated Xpert MTB/RIF for identification of <i>M. tuberculosis</i> . TAT for identification may be longer depending on laboratory methods used, laboratory batching, and whether the culture is mixed or contaminated.	Testing time by MALDI-TOF MS is less than 2 hours. Batching may increase turnaround time. MALDI-TOF MS and 16S sequencing are commonly used for identification of NTM. Some NTM may not be differentiated to species level by these methods. AccuProbe tests are no longer available.
Growth-based DST	1-2 weeks after setting up DST by broth systems (Mycobacteria Growth Indicator Tube [MGIT] or VersaTrek) 3-4 weeks after setting up DST by solid media (agar proportion method) and 1-3 weeks by microdilution method (Sensititre).	DST cannot be performed on mixed or contaminated cultures. Laboratories usually perform DST in batches; this causes further delay of TAT.

TABLE CONTINUES >

Test	Expected turnaround time (TAT) (from specimen receipt at laboratory; batched testing and variable scheduling may increase TAT)	Comments
Genotyping / WGS	WGS: 7 days after received at sequencing lab WGS results: Whole-genome multilocus sequence typing (wgMLST): 14 days Whole-genome single nucleotide polymorphism (wgSNP) comparison: variable	WGS has replaced conventional genotyping (mycobacterial interspersed repetitive units [MIRU] and spoligotyping). wgMLST is routinely performed. wgSNP is performed as part of outbreak or cluster investigation.
Interferon gamma release assays (IGRA)	1-2 days	Usually performed by clinical laboratory (not mycobacteriology laboratory).

Communication between clinician and laboratory

The optimal laboratory diagnosis of TB begins with a close relationship and open dialogue between the healthcare provider, TB control, and the TB laboratory.

Obtain the following information from the laboratory to ensure proper utilization of the test. Laboratories will benefit from having optimal samples to test.

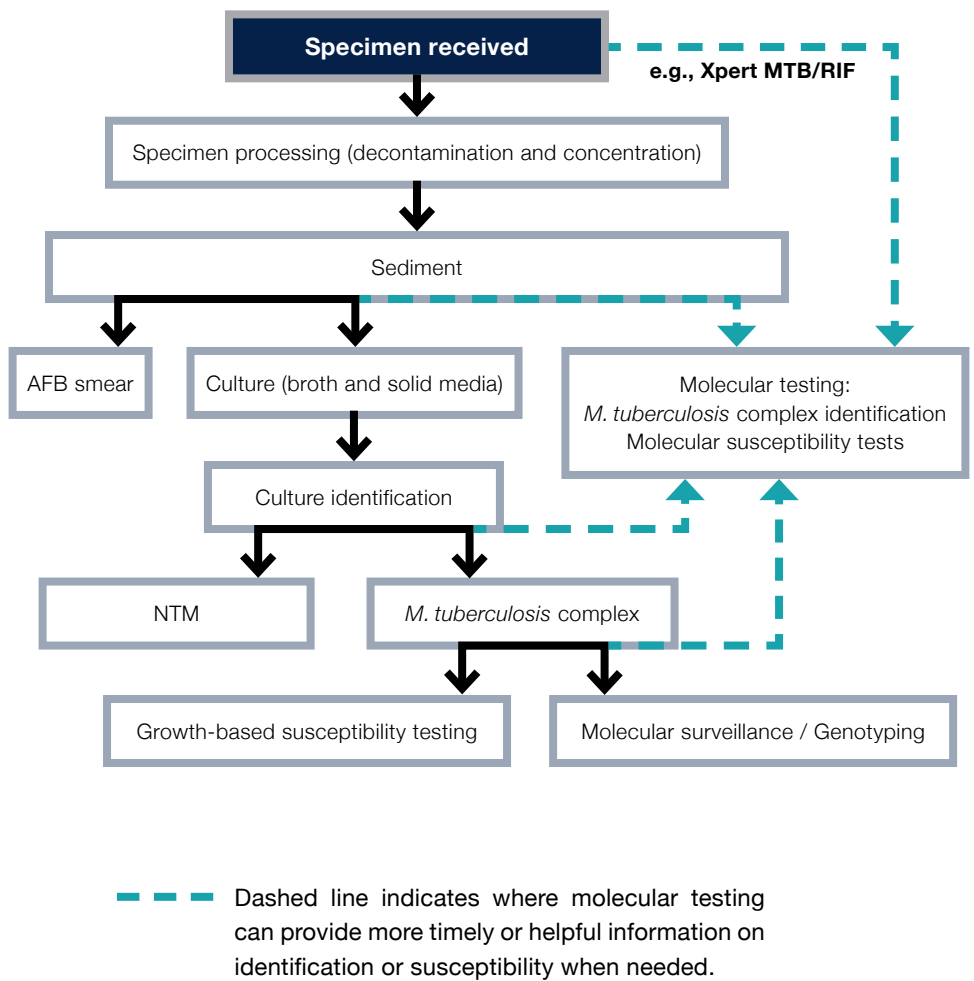
- **Test availability, performance characteristics, and limitations**
 - Is molecular detection of drug resistance available? By what method?
 - Is growth-based DST available? By what method?
 - Can the test be performed on a smear-negative specimen? What is the sensitivity and specificity?
 - Can the test be performed on an extrapulmonary specimen? What specimen types are accepted?
 - Is preapproval required?
- **Specimen requirements**
 - Acceptance and rejection criteria
 - Specimen volume: laboratories usually welcome more volume than the minimal amount
 - Transit and shipping conditions
- **Testing schedules**
 - Laboratories often batch the performance of tests. It is good to know the days of the week and the cutoff times. Make sure the specimen arrives before the cutoff time for best turnaround time and to avoid specimen aging.
- **Turnaround times and availability of reports**
 - Knowing the date and time the report will be available allows the clinician to plan the timing of consultation with experts or seeing the patient.

In some situations, the following information may help the laboratory to maximize its contribution:

- Diagnostic versus follow-up specimen
- Date when anti-TB treatment was started and drug regimen
- Is drug resistance suspected?

As laboratory technologies advance, laboratories may need to inform clinicians about new tests that are available for implementation. As clinical practices evolve, clinicians may need to inform laboratories about tests that are no longer necessary to perform and about tests they hope laboratories can offer. Additionally, clinicians and laboratories may wish to work together on diagnostic algorithms. One example is the use of nucleic acid amplification tests (NAAT) for rapid identification of *M. tuberculosis* complex and molecular testing for drug resistance. Such communication can optimize scarce resources and maximize the laboratory’s contribution to patient care.

FIGURE 1. **Mycobacteriology laboratory workflow**



How should specimens be collected?

FOR ALL SPECIMENS:

- Contact your laboratory for specific instructions
- Collect into sterile container
- Do not use preservatives
- Follow proper collection procedures and obtain an adequate volume to enhance recovery of organisms
- Keep refrigerated and send for processing within 24 hours if possible to reduce overgrowth of other microorganisms, especially for non-sterile specimens

RESPIRATORY SPECIMENS:

Preferably 3 specimens collected at least 8 hours apart and at least 1 of which is an early morning expectorated specimen or induced (some programs prefer all specimens to be induced)

- **Note:** CDC and WHO guidelines differ; internationally, 2 specimens are recommended at least 1 hour apart. Incremental benefit of the third specimen is relatively small and may be even less if NAAT is used.

Expectorated sputum

- Preferably early morning (before brushing teeth), consider rinsing mouth with sterile or bottled water to reduce risk of contamination with NTM
- Volume of 5mL ideal; should be >2mL

Sputum induced with nebulized hypertonic (3-10%) saline

- Note on the requisition form and label specimen as induced because these are more likely to be watery in appearance and could be mistakenly considered unacceptable by the laboratory

Bronchoscopy: lavage, brushings, biopsies

- Induced sputum has **equivalent or better yield** for diagnosis compared to bronchoscopy specimens
- Bronchoscopy can target specific areas of the lung
- Can obtain specimens from persons unable to produce sputum specimen by other methods
- Post-bronchoscopy sputum collection may have higher yield than sputum collected at other times

Gastric aspirate (for more information on how to perform gastric aspirate, see **Chapter 6, Pediatrics**)

- Used for diagnosing pulmonary TB in young children; can also be useful in frail elderly who are unable to produce sputum
- Yield highest in the youngest children
- Early morning collection after nothing by mouth (NPO) overnight
- Mycobacteria die rapidly in gastric lavage fluid, which needs to be neutralized with sodium carbonate to pH of 7.0, especially if specimen will not be processed immediately (ideally at the bedside, otherwise should be within 4 hours to preserve viability)
- Add 100 mg of sodium carbonate to 5-10 mL specimen; refrigerate

EXTRAPULMONARY SPECIMENS:

Urine

- Collect multiple early morning midstream specimens (not a 24-hour urine collection)
- Each specimen 10-40 mL, a larger volume can be split among 50mL conical tubes
- Do not pool specimens or use preservatives

Stool

- Not routinely performed, contact your laboratory to determine whether stool accepted
- Recovery of AFB is not high due to overgrowth of other bacteria
- Collect at least 1 gram
- No transport media needed
- Refrigerate if transport time >1 hour; do not freeze

Pleural fluid, peritoneal fluid, pericardial fluid, joint aspirate

- At least 10 mL specimen preferable
- No swabs
- Bloody specimens can be put in sodium polyanethol sulfonate (SPS) yellow-top tube

Blood

- Collect in patients with suspected mycobacteremia (e.g., sepsis, immunocompromised)
- Special blood culture media for AFB are commercially available
- Collect 5-10mL in tubes containing either SPS, heparin, or citrate
- Blood collected in ethylenediamine tetraacetic acid (EDTA) or in conventional blood culture bottles and coagulated blood are not acceptable

Cerebrospinal fluid

- Minimum of 2-3mL, but 5-10mL preferable

Tissue biopsy

- Any tissue specimen, not formalin-fixed, can be cultured for mycobacteria
- Submit in sterile cup kept moist with small amount of sterile saline
- Placement in formalin or other fixative eliminates ability to culture and perform growth-based DST. (It is possible to extract nucleic acid from formalin-fixed specimens for molecular testing, but this is only available at CDC Pathology Branch (Email: pathology@cdc.gov) and a few select laboratories—see section: **Molecular methods on fixed specimens**)
- Communication with operating room staff will increase the likelihood that a specimen will be submitted without formalin

Adapted from: *A Clinician's Guide to the TB Laboratory*, Heartland National Tuberculosis Center.

Microscopy, culture identification, and growth-based DST

AFB smear

CDC recommends using fluorochrome staining methods for AFB smear microscopy. Fluorochrome staining is more sensitive than staining methods using the basic fuchsin dyes, e.g., Ziehl-Neelsen. Stains are typically done on specimens decontaminated and concentrated with N-acetyl-l-cysteine-sodium hydroxide (NALC-NaOH). AFB smear results should be reported within 24 hours of receipt of specimens. Varied semi-quantitative reporting systems are in use: rare, few, moderate, numerous; 1+ to 3+ (WHO); and 1+ to 4+ (CDC). It is estimated that the detection limit for smear positivity is 5,000 to 10,000 AFB per mL of sputum. AFB smear is not *M. tuberculosis* complex-specific. NTM are stained positive as well. If fluorochrome staining methods are used, rapid growers are stained poorly or negative. *Nocardia*, *Rhodococcus*, *Legionella*, *Cryptosporidium*, *Isospora*, *Cyclospora*, *Actinomyces* and *Microsporidia* may also show various degrees of acid-fastness.

AFB culture

Culture is performed in liquid or solid media, but liquid media produce results more quickly. Most laboratories employ at least one liquid and one solid medium to maximize the ability to grow a wider range of AFB. The most commonly used system in the U.S. is the automated Bactec Mycobacteria Growth Indicator Tube (MGIT) 960 (Becton Dickinson, Sparks, MD) which uses liquid media and an incubation protocol lasting 42 days (6 weeks). Most positive cultures can be reported positive before 4 weeks in liquid and 5 weeks in solid media. Many laboratories will report final negative results at 6 weeks, but some laboratories may choose to issue final reports at 8 weeks. Some laboratories also issue a preliminary negative result after 3 or 4 weeks of incubation.

Culture identification

When a culture turns positive, a smear is made and stained to confirm presence of AFB. Most laboratories will issue a preliminary positive AFB culture report prior to species identification. AFB cellular morphology may indicate presence of *M. tuberculosis*, NTM, or contamination with other organisms. This information can be used to guide the most appropriate method for species identification. Mycobacteria can be identified by MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry), DNA sequencing (16S rRNA, *rpoB*, *hsp65*, ITS, etc.), or laboratory developed or validated polymerase chain reaction (PCR) assays including Xpert MTB/RIF. Other tests are available outside the U.S. Commercial AccuProbe tests are no longer available as of December 2022. Traditional tests using phenotypic observation for growth rates, pigmentation, optimal growth temperature and selected biochemical tests may assist identification, but dependence on these methods has diminished significantly.

If a mixed culture (*M. tuberculosis* complex and NTM) is suspected, rapid identification of *M. tuberculosis* complex and detection of drug resistance by molecular methods should be pursued. If *M. tuberculosis* complex is identified, a pure culture should be obtained for growth-based DST (see section: **Confirmation of drug-resistant results**). Isolation of *M. tuberculosis* complex from a mixed culture may take several weeks and sometimes may not be successful.

M. bovis, including BCG, is naturally pyrazinamide- (PZA) resistant; speciation within *M. tuberculosis* complex can be important particularly in regions where the prevalence of *M. bovis* is high or when mono-PZA-resistance is detected. Sequencing of *pncA*, to identify the characteristic mutation His57Asp, can differentiate *M. bovis* from *M. tuberculosis* complex-not-*M. bovis*.

Growth-based DST

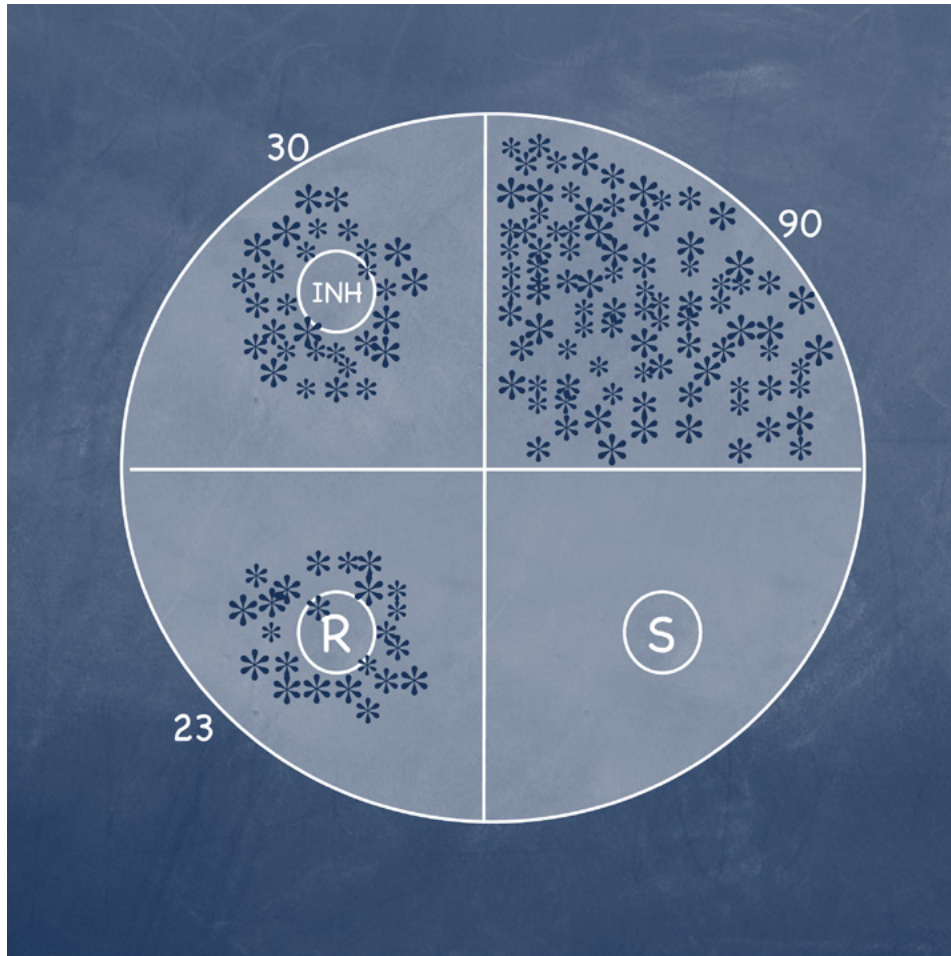
Growth-based DST is also referred to as phenotypic, conventional, or culture-based DST. Unlike molecular resistance testing, growth-based DST must be set up on a pure culture.

Several methods for performing growth-based DST are in use. In general, these methods have good concordance. However, while managing DR-TB cases, clinicians are likely to encounter growth-based DST results from multiple methods and laboratories. Various DST methods are validated to yield “equivalent” results, but discordant results may occur, and they are challenging to interpret. The two most common methods used in the U.S. are performed on solid media by the agar proportion method or in liquid broth systems. They are outlined below, along with features of each test that are important for clinicians to understand.

Solid media—agar proportion method

- The agar proportion method using Middlebrook 7H10 or 7H11 agar is the reference standard for DST in the U.S.
- A standardized cell suspension is prepared from a pure isolate and inoculated onto each quadrant of an agar plate. Each quadrant contains a specific drug at its critical concentration or no drug as a control. Plates are incubated for at least 21 days before colony counts are taken.
- The isolate is considered resistant if the number of colonies in the drug quadrant is > 1% of that in the control quadrant. An example of determining the results using the agar-proportion method is demonstrated in **Figure 2**.
- PZA is difficult to study using solid medium due to the requirements of testing at an acidic pH, causing many isolates to fail to grow. PZA growth-based DST typically is performed using liquid media.
- The critical concentrations used with 7H10 and 7H11 may be different.
- The Lowenstein-Jensen (LJ) proportion method is not used in the U.S. because it is more prone to contamination, but it is inexpensive and frequently used in low-resource settings.

FIGURE 2. **Agar proportion method for DST**



Quadrant plate

Standardized inoculum of growth of *M. tuberculosis* complex prepared from liquid or solid media has been inoculated into each of the 4 quadrants with the following results:

- **Control quadrant:** 90 colonies
- **Isoniazid (INH) quad:** 30 colonies
- **Rifampin (RIF) quad:** 23 colonies
- **Streptomycin (SM) quad:** 0 colonies
- Isoniazid 30/90 = 33% resistant
- Rifampin 23/90 = 25% resistant
- Streptomycin 0/90 = susceptible

This is an MDR-TB isolate.

Liquid media

MGIT 960 (Becton Dickinson, Sparks, MD)

- Growth-based DST using the MGIT 960 system is a modified proportion method and most frequently used in the U.S.
- FDA approved for testing RIF, INH, ethambutol (EMB), PZA, and SM.
- Results are available in about 1 week (4-14 days) after the test is set up.
- Some second-line drugs have also been validated for testing using MGIT 960 but are not FDA approved for this purpose in the U.S.
- The method is based on the fluorescence produced from reduced oxygen in the MGIT medium due to microbial growth. The fluorescence generated is then converted to “growth units” (GU). In general, higher GU indicates more growth.
- When the GU of the growth control reaches 400 within 4-14 days, the DST is valid for the system to interpret. If a drug-containing MGIT tube yields GU < 100, the organism is interpreted as susceptible to the drug; if GU is ≥ 100, the organism is resistant.

VersaTREK (ThermoFisher Scientific, Cleveland, OH)

- FDA approved for testing first-line drugs (RIF, INH, EMB, PZA).
- Results are available in about 1 week after test set-up (3-13 days). Resistant results may be interpreted as early as 3 days of incubation while susceptible results require a minimum of 6 days.
- The method is based on detection of pressure changes (oxygen consumption due to microbial growth) within the headspace above the broth medium in a sealed bottle.

Sensititre (ThermoFisher Scientific, Cleveland, OH)

- The method uses a 96-well broth microdilution plate [MYCOTB AST plate] to test both first- and second-line drugs. It is intended for research use only and it does not include PZA. It provides minimum inhibitory concentration (MIC) results for each of the 12 drugs tested (see **Table 2**). Alternate plates that include testing for other drugs such as bedaquiline (BDQ), linezolid (LZD), clofazimine (CFZ) and delamanid (DLM) have been used. WHO has proposed an alternate methodology and broth microdilution plate configuration that is more consistent with current treatment recommendations.
- Test is set up from cell suspension using growth obtained from solid media, which may delay DST set-up due to slower growth on solid media. Results are available within 7-21 days after the test is set up.
- *M. tuberculosis* complex has been traditionally tested using a single critical concentration of a drug to provide the categorical interpretation (susceptible or resistant). The usefulness of MIC results for clinical management of TB patients requires further investigation but is likely to be helpful in reassessing the optimal critical concentration and clinical breakpoints. See section: **MIC—when to order and how to interpret**.

Confirmation of results showing resistance

Growth-based DST results from liquid DST systems are verified by microbiologists. Laboratories treat drug-resistance results seriously to avoid reporting false resistance. The verification steps may include:

- Rule out the presence of non-*AFB* bacteria or NTM in the culture from which DST was set up and from the resulting DST plates or broth tubes.
- Check for concordance with the molecular drug testing results if available or perform molecular testing to confirm resistance.
- When molecular testing is not available for certain drugs, best practice recommendations indicate retesting by the growth-based DST to confirm resistance. Before retesting, the culture must be checked thoroughly to ensure purity. Retesting may be performed with the same method or a different method according to the laboratory's policy. Alternatively, the laboratory may choose to refer to a reference laboratory.
- **When the patient does not have risk factors for drug resistance, the treating physician should communicate with the public health program and the laboratory to confirm resistance results, ensure that the risk of contamination or a mixed culture has been ruled out, and discuss any other sources of a possibly erroneous result. If available, testing on a different specimen or isolate may be helpful.**

CLINICAL SCENARIO

➤ A patient who has never lived outside the U.S. has a first episode of culture-positive TB that is reported to have resistance to INH, RIF, and PZA.

The physician is surprised by this result and confirms lack of risk factors for drug resistance. The patient has clinically improved after 4 weeks of first-line TB treatment. The clinician calls the laboratory to confirm the results. A smear of the growth from the drug-containing MGIT reveals mixed morphology. Molecular testing shows *M. tuberculosis* complex but no mutations indicating drug resistance. Further testing indicates presence of NTM and *M. tuberculosis* complex in the DST cultures.

The patient continues to do well on first-line TB treatment.

Critical concentration, minimum inhibitory concentration (MIC), and what they mean

Critical concentrations

DST in the mycobacteriology laboratory has been typically performed using a single drug concentration—the **critical concentration**, which provides categorical interpretation (susceptible or resistant).

- The **critical concentration** has been defined as the concentration of drug that inhibits 99% of wild-type *M. tuberculosis* complex strains that have not been exposed to the drug but does not appreciably suppress the growth of strains that are resistant to the drug (based on clinical treatment failure).

A **critical concentration is not a minimum inhibitory concentration (MIC)**; however, the MIC of microorganisms susceptible at a critical concentration have an $MIC \leq$ critical concentration and those resistant have $MIC >$ critical concentration.

See section: ***MIC—when to order and how to interpret.***

- The critical concentration used for an individual drug may differ based on the method of growth-based DST (see **Table 2**). Although critical concentrations are validated to provide equivalent results across methods, it is difficult to achieve 100% equivalency and some discordance has been encountered.
- Discordance can also be encountered within the same method, especially when the MIC of a strain is close to the critical concentration. The reproducibility of testing in these strains tends to be poor.
- High- and low-level resistance:
 - Some drugs, such as INH, are routinely tested at more than one concentration. Some experts use these results to select a higher dose of the drug when it tests resistant at the lower concentration and susceptible at the higher concentration. The higher dose may achieve *in vivo* concentrations sufficiently high to overcome resistance at the lower concentration.
- **Table 2** shows the critical concentrations for commonly used methods for growth-based DST. It also shows the normal peak concentration in serum for standard doses of anti-mycobacterial drugs. The clinical relevance of the relationship between *in vitro* susceptibility at a given critical concentration and the normal peak concentration can involve complex pharmacodynamics including the mechanism of action of the drug, the penetration of the drug at the site of infection, whether mycobacteria are in an active or dormant state, and the patient's metabolism of the drug.

Critical concentrations and the data and methods used to set them have recently been revisited by WHO and others. Several changes have been recommended to update and standardize methods used to determine critical concentrations and clinical cut-points. Notably, this has resulted in the recommendation to lower the RIF critical concentration by one dilution in MGIT and 7H10 media from 1.0 to 0.5 in an effort reduce discordant results between phenotypic and molecular tests particularly for mutations that confer borderline resistance (see **Table 2**).

Summary of recommended changes to interpretive criteria for phenotypic DSTs:

- Evaluate critical concentrations against the epidemiological cutoff values (ECOFFs) which are set at the upper end of the MIC distribution of phenotypically wild-type isolates.
- Consider establishing a range of MICs that account for uncertainty based on technical limitations of phenotypic testing. This range, called an area of technical uncertainty (ATU), may also be helpful because some mechanisms of resistance may have MIC distributions that are borderline and can overlap the ECOFF. These overlapping distributions result in discordant results between phenotypic tests and between phenotypic and molecular tests and can cause considerable confusion.
- Consider pharmacokinetic and pharmacodynamic data to account for when increased dosing of drugs might overcome apparent resistance.

Figure 3 shows hypothetical distribution of MIC for susceptible organisms (S in green), organisms with borderline resistance mechanisms (yellow), and high-level resistance (HLR) mechanisms in red. Also shown is how an ECOFF may be determined, equated to a critical concentration (CC), and determination of an ATU.

FIGURE 3. **Hypothetical MIC distributions**

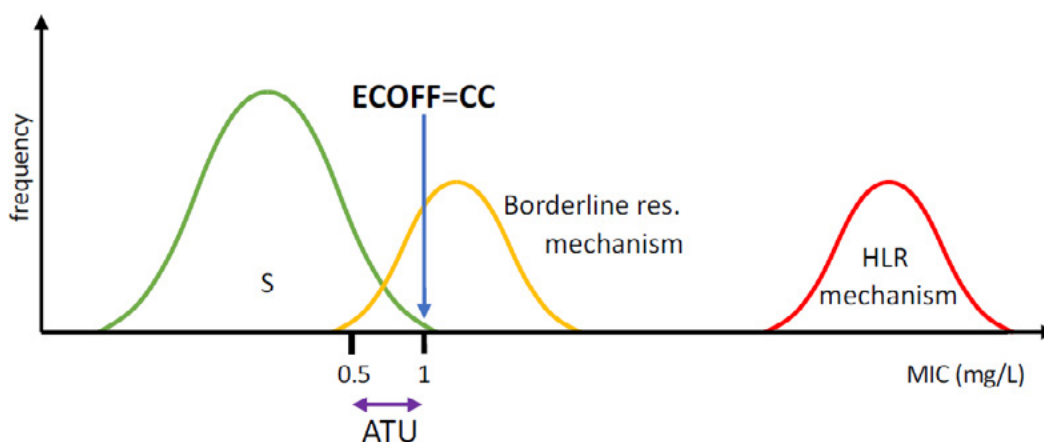


Figure reproduced from: WHO, Optimized broth microdilution plate methodology for drug susceptibility testing of *Mycobacterium tuberculosis* complex. Geneva: World Health Organization; 2022. License: CC BY-NC-SA 3.0 IGO.

TABLE 2. **Critical concentrations of antimycobacterial agents by broth systems or agar proportion methods**

Drug	Normal peak concentration in serum with standard doses* (mcg/mL)	MGIT 960 low/high (mcg/mL)	VersaTREK low/high (mcg/mL)	Agar 7H10 low/high (mcg/mL)	Agar 7H11 low/high (mcg/mL)	Sensititre 7H9 Broth Microdilution U.S. / WHO proposed (mcg/mL, range of concentrations tested****)
FIRST-LINE DRUGS						
Isoniazid (INH)	3-6	0.1 / 0.4	0.1 / 0.4	0.2 / 1	0.2 / 1	0.03-4 / 0.016-2
Rifampin (RIF)	8-24	0.5†	1	0.5†	1	0.12-16 / 0.016-2
Rifabutin (RFB)	0.3-0.9	0.5	NA	0.5	0.5	0.12-16 / NA
Pyrazinamide (PZA)	20-60	100	300	NR	NR	NA
Ethambutol (EMB)	2-6	5	5 / 8	5 / 10	7.5	0.5-32 / 0.125-8
WHO GROUP A DRUGS						
Levofloxacin (LFX)	8-12	1.5	NA	1	NA	NA / 0.06-8
Moxifloxacin (MXF)	3-5	0.25	NA	0.5	0.5	0.06-8 / NA
Bedaquiline (BDQ)	1.3-3.3b	1	NA	NA	0.25	NA / 0.008-1
Linezolid (LZD)	12-26	1	NA	1	NA	NA / 0.06-4
WHO GROUP B DRUGS						
Cycloserine (CS)	20-35	NR	NR	NR	NR**	2-256 / 1-64
Clofazimine (CFZ)	0.5-2.0	1	NA	NA	NR**	NA / 0.008-1
OTHER ORAL DRUGS						
Pretomanid (PA)	2.3-4.3	NA	NA	NA	NA	NA / 0.016-2
Ethionamide (ETA)	1-5	5	NA	5	10	0.3-40 / 0.125-16
Para-aminosalicylate (PAS)	20-60	NA	NA	2	8	0.5-64 / NA
INJECTABLE AGENTS						
Streptomycin (SM)	35-45	1 / 4	NA	2 / 10	2 / 10	0.25-32 / NA
Capreomycin (CM)	35-45	2.5 or 3	NA	10	10	NA
Amikacin (AK)	35-45	1 or 1.5	NA	4	NA	0.12-16 / NA
Kanamycin (KM)	35-45	2.5	NA	5	6	0.6-40 / 0.125-16

NR: not recommended. **NA:** not available. MGIT is a trademark of Becton Dickinson and Company. VersaTREK and Sensititre are trademarks of ThermoFisher Scientific.

* Serum drug concentrations are provided for comparison with the critical concentration, not as a substitute for therapeutic drug monitoring.

** Some laboratories do offer this testing. See Table 3.

*** Source: Thermo Scientific Product brochure for MYCOTB plate available in the U.S. Alternate ranges proposed by WHO in 2022 included where available.

† Revised by WHO in 2020 from 1.0 mcg/mL. Other entities and laboratories may still be using 1.0 mcg/mL.

‡ Expected BDQ concentrations are higher at 2 weeks after the initial loading dose (2.8-3.3 mcg/mL) and gradually decline to 1.3 mcg/mL by 24 weeks. See **Chapter 5, Medication Fact Sheets**, for more detail.

MIC—when to order and how to interpret

MIC testing differs from testing using a critical concentration in that the organism is tested at a series of drug concentrations, usually a series of two-fold dilutions, and the result is the lowest concentration that inhibits growth of the bacteria. Although in most cases testing using the critical concentration is sufficient, there are situations described below with certain drugs where MIC can be helpful in guiding management of difficult cases. There is limited availability of MIC testing in U.S. laboratories. Categorical interpretations (susceptible or resistant) for MIC results for INH, RIF and EMB for *M. tuberculosis* complex have been recommended by the Clinical and Laboratory Standards Institute (CLSI).

Situations in which MICs may be useful for clinical management:

- **LZD management**

Many experts now manage LZD using therapeutic drug monitoring (TDM) to limit side effects. To ensure that peak concentrations are high enough, some experts recommend targeting a 2-hour peak concentration that is 4- to 16-times higher than the LZD MIC. (See section: **Therapeutic drug monitoring** in this chapter, and **Chapter 4, Treatment**).

- **Resistance to fluoroquinolone**

When fluoroquinolone resistance is found by critical concentration or by molecular testing, an MIC—usually for moxifloxacin (MFX)—can help inform whether a dose increase may benefit the patient. Although there is minimal published evidence to support this approach, some MDR-TB experts would consider using “high-dose” MFX at 600mg or 800mg daily for patients with MFX MIC of 1 or 2 mcg/mL when other options are limited. Consider QT prolongation if other QT-prolonging agents are used.

- **Resistance to injectables**

In cases with extensive resistance, obtaining an MIC to an injectable medication to which there is resistance at the critical concentration may help determine whether an increased dose is likely to benefit the patient. High peak levels can be achieved with high intermittent dosing (e.g., 25 mg/kg 2-3x per week) and some MDR-TB experts would use this dosing regimen if it could achieve a peak that is 4- to 16-times higher than the MIC.

- **BDQ**

BDQ is sometimes tested by determining an MIC. Although clinical break-points and critical concentrations have been proposed or established, MIC results may provide more information while knowledge is accumulating about the correlation between genetic mutations, phenotypic resistance, and clinical outcomes. See **Table 3, Phenotypic and molecular drug susceptibility testing available at select referral laboratories** for specific laboratory information.

TABLE 3. **Phenotypic and molecular drug susceptibility testing available at select referral laboratories, At-a-Glance.** See online version for full details

Drug	California Department of Public Health		Centers for Disease Control and Prevention		National Jewish Health		Florida Health		New York State Dept of Health (Wadsworth Center)	
	Molecular	Phenotypic	Molecular	Phenotypic	Molecular	Phenotypic	Molecular	Phenotypic	Molecular	Phenotypic
INH	PSQ	MGIT	tNGS	Agar	LPA	Agar, MGIT	LPA, Sanger	Sensititre	PSQ, WGS	Agar, MGIT
RIF	PSQ	MGIT	tNGS	Agar	LPA	Agar, MGIT	LPA, Sanger	Sensititre	PSQ, WGS	Agar, MGIT
RFB	PSQ	MGIT		Agar		MGIT	LPA, Sanger	Sensititre		Agar
RPT								Sensititre		
EMB		MGIT	tNGS	Agar		Agar, MGIT	Sanger	Sensititre	WGS	Agar, MGIT
PZA		MGIT	tNGS	MGIT		MGIT	Sanger	Sensititre	WGS	MGIT
LFX	PSQ	MGIT	tNGS	Agar, Sensititre	LPA	MGIT	LPA, Sanger	Sensititre	PSQ, WGS	Agar
MFX	PSQ	MGIT	tNGS	Agar, Sensititre	LPA	MGIT	LPA, Sanger	Sensititre	PSQ, WGS	Agar, MGIT
LZD			tNGS	Agar, Sensititre		MGIT	Sanger	Sensititre		MGIT
BDQ			tNGS	Sensititre			Sanger	Sensititre		MGIT
DLM										
CFZ			tNGS	Sensititre		Agar	Sanger	Sensititre		MGIT
CS						Agar		Sensititre		Agar
PAS				Agar		Agar		Sensititre		Agar
SM		MGIT		Agar	LPA	Agar, MGIT		Sensititre	WGS	Agar
AK	PSQ	MGIT	tNGS	Agar	LPA	Agar, MGIT	LPA	Sensititre	WGS	Agar
KM		MGIT	tNGS	Agar	LPA	Agar, MGIT	LPA		WGS	
CM		MGIT	tNGS	Agar	LPA	Agar, MGIT	LPA	Sensititre	WGS	
ETA		MGIT		Agar		Agar, MGIT	LPA		WGS	Agar
Pa				MGIT				MGIT		
MPPM/CLAV*								Sensititre		

See online table for full details. Some drugs or methods may be available only upon special request or for surveillance purposes only. CDC tests using broth microdilution and tNGS expected to be available by the second quarter of 2023. Table compiled by Shereen Katrak, MD, MPH, Pennan Barry, MD, MPH, and Rebecca Wang, California Department of Public Health.

Molecular: PSQ = Pyrosequencing tNGS = Targeted next generation sequencing LPA = Line probe assay (GenoType assays, Hain Lifescience)

Sanger = Sanger sequencing **WGS** = Whole genome sequencing

Phenotypic: **MGIT** = Bactec MGIT 960 (Becton Dickinson) **Agar** = Agar proportion **Sensititre** = Sensititre broth microdilution (ThermoFisher Scientific)

Molecular methods for detection of *M. tuberculosis* complex DNA and drug resistance mutations

Molecular assays able to be performed directly on clinical specimens prior to growth in culture have significantly shortened turnaround time from weeks to hours for detection of *M. tuberculosis* complex and drug resistance. These tests, often referred to as nucleic acid amplification tests (NAATs) are **recommended by CDC for routine use in patients for whom a diagnosis of TB is being considered.**

It is important for clinicians who are interpreting molecular tests for drug resistance to know the advantages and limitations of the tests. There are two major types of molecular tests described below: sequencing and nonsequencing (or probe-based tests, such as Xpert MTB/RIF and Hain Line-probe assay). The chief distinction is that probe-based tests can only determine that there is a mutation present in the gene; they generally cannot identify specific mutations unless a probe is designed to specifically detect a single mutation (see section: ***Line-probe assay***). In contrast, tests that employ sequencing identify and report specific mutations. Therefore, silent mutations can be differentiated from mutations conferring drug resistance (see section: ***Difficulties interpreting results from molecular tests – Types of mutations***).

In the U.S., **CDC and the Association of Public Health Laboratories (APHL) recommend confirming a resistant result from a nonspecific probe-based test with a sequencing test.** Furthermore, results with mutation identification provided by sequencing methods enable laboratories to study the correlation between specific mutations and the levels of drug resistance (e.g., MIC or low- vs high-level resistance in INH) using the growth-based DST.

Indications for use of molecular assays for drug resistance are found in **Chapter 2, *Diagnosis*.**

Genes associated with drug resistance

Table 4 provides a summary of genes associated with drug resistance and the predominant mutations found in clinical isolates.

- Although major genes associated with drug resistance have been identified, the understanding of drug resistance at the genetic level remains variable and incomplete. Therefore, 100% sensitivity for detecting all drug resistance is not currently achievable.
- Specificity for resistance detection by molecular methods for certain drugs is not 100% (using growth-based DST as the gold standard).

rpoB codon numbering change

Historically *rpoB* codon numbers were assigned according to *Escherichia coli* numbering. However, in the era of WGS, this numbering system can cause confusion. Scientific literature and laboratory reports are now switching from the older *E. coli* numbering to *M. tuberculosis* numbering. To convert from *E. coli* to *M. tuberculosis* codon numbers for most codons including all within the RIF resistance determining region (RRDR), subtract **81**. For example, the most common mutation found in MDR-TB is **450Leu** (*M. tuberculosis* numbering) which is denoted by **531Leu** in *E. coli* numbering. An exception to this rule is the mutation at *E. coli* codon 146 which translated to codon 170 in *M. tuberculosis*.* All *rpoB* codon numbers in this guide are *M. tuberculosis* numbers. Codon numbers for other genes and loci have historically used *M. tuberculosis* numbers and so there is no change in numbering of other loci.

*For more information see: <https://www.aplh.org/aboutAPHL/publications/Documents/ID-2019Apr-MTBC-DST-RIF-White-Paper.pdf>

TABLE 4. **Genes and mutations associated with drug resistance in *M. tuberculosis***

Antimicrobial agent	Gene	Sensitivity	Specificity	Selected mutations* and comments	
Isoniazid (INH)	<i>katG</i>	91.2	98.4	315Thr(ACC)	Most frequent mutation, associated with high-level INH resistance. Some other mutations in codon 315: Thr(ACA), Asn(AAC), Ile(ATC), Thr(ACT), Gly(GGC) Hain MTBDR <i>plus</i> identifies 315Thr(ACC) and 315Thr(ACA)
	<i>inhA promoter</i>			-15T	Often associated with low-level INH resistance and ethionamide (ETA) resistance. Some other mutations: -8A, -8C, -8G, -9T, -16G, -17T Hain MTBDR <i>plus</i> identifies -15T, -16G, -8A, -8C
	<i>fabG1</i>			203Leu(CTA)	Acts with its adjacent region as a promoter to upregulate the expression of <i>inhA</i> .
	<i>ahpC promoter</i>			-54A	Associated with INH resistance. May be a compensatory mutation. Some other mutations: -48T, -51T, -52A, -52T
Rifampin (RIF)	<i>rpoB</i>	93.8	98.2	450Leu(TTG)	Most frequent mutation seen with MDR TB. Associated with RIF and RFB resistance. Detectable by Hain MTBDR <i>plus</i> , mutation identity reported Detectable by Probe E of Xpert MTB/RIF, mutation identity not reported
				445Tyr(TAC) 445Asp(GAC)	Associated with RIF and RFB resistance. Detectable by Hain MTBDR <i>plus</i> , mutation identified Detectable by Probe D of Xpert MTB/RIF, mutation identity not reported**
				435Val(GTC)	Often associated with RIF resistance but retains RFB susceptibility Detectable by HAIN, mutation identity reported Detectable by Probe B of Xpert MTB/RIF, mutation identity not reported**
				Silent mutation: 433Phe(TTT)	Most frequent silent mutation. Not associated with RIF resistance Detectable by Hain MTBDR <i>plus</i> , missing WT3, mutation identity not reported** Detectable by Probe B of Xpert MTB/RIF, mutation identity not reported; incorrectly reported as "RIF resistance detected"
				Borderline resistance mutations**	430Pro(CCG), 435Tyr(TAC), 445Asn(AAC), 445Leu(CTC), 445Ser(AGC), 452Pro(CCG), 491Phe(TTC) is outside the <i>rpoB</i> core region and is not interrogated by many assays (including Xpert MTB/RIF and Hain MTBDR <i>plus</i>) and will be missed.

TABLE CONTINUES >

* See **Figure 10** for information on understanding how mutations can be named and reported. Table 4 gives the codon number for the location of the mutation, the resulting amino acid change using the three letter abbreviation, and the nucleic acid code for the mutation in parentheses, e.g., 450Leu(TTG). For mutations in noncoding regions, the number given is the nucleotide position number followed by the variant nucleotide base, e.g., 1401G.

** For further explanation, see section: **Difficulties interpreting results of molecular tests**. Borderline resistance mutations (previously often referred to as "disputed" mutations) are mutations in the *rpoB* gene that are associated with variable susceptibility results in growth-based DST assays but are considered to confer resistance by WHO and to have clinical significance. MIC testing may be warranted.

Sensitivity and specificity are from the 2021 WHO Mutation catalog for the categories Associated with R and Associated with R – Interim. These categories may include more or different mutations and loci than interrogated in some current molecular assays.

Ala: alanine, **Arg:** arginine, **Asn:** asparagine, **Asp:** aspartic acid, **Cys:** cysteine, **Gln:** glutamine, **Glu:** glutamic acid, **Gly:** glycine, **His:** histidine, **Ile:** isoleucine, **Leu:** leucine, **Lys:** lysine, **Met:** methionine, **Phe:** phenylalanine, **Pro:** proline, **Ser:** serine, **Thr:** threonine, **Trp:** tryptophan, **Tyr:** tyrosine, **Val:** valine

Antimicrobial agent	Gene	Sensitivity	Specificity	Selected mutations* and comments	
Ethambutol (EMB)	<i>embB</i>	86.7	93.3	306Val(GTG)	<p>Most frequent mutation associated with EMB resistance. Detectable by HAIN MTBDRs/ v1, mutation identity reported.</p> <p>Some other mutations in codon 306: Leu(CTG), Ile(ATA), Thr(ACG), Ile(ATT), Ile(ATC), Leu(TTG). Variable susceptibility results in growth-based DST associated with these mutations have often been encountered.</p>
Pyrazinamide (PZA)	<i>pncA</i>	72.3	98.8	No predominant mutations 57Asp(GAC) is found in <i>M. bovis</i>	<p>Widely distributed throughout the gene and the promoter. Not all mutations are associated with PZA resistance. Sensitivity reported here is likely an underestimate related at least in part to propensity of phenotypic testing to be falsely resistant.</p> <p>The report by Miotto et al and supplemental tables list specific mutations and can be a useful reference when interpreting <i>pncA</i> results. https://doi.org/10.1128/mBio.01819-14</p>
Fluoroquinolones Moxifloxacin (MXF) Levofloxacin (LFX)	<i>gyrA</i>	87.7 (MXF)	91.6 (MXF)	94Gly(GGC)	<p>Most frequent mutation, usually MXF MIC > 1. Detectable by Hain MTBDRs/ v1 and v2, mutation identity reported</p> <p>Some other mutations in codon 94: Tyr(TAC), His(CAC), GCC(Ala), AAC(Asn)</p>
				90Val(GTG)	<p>A frequent mutation. MXF MIC ≤ 1. MXF may still contribute to therapy.</p> <p>Detectable by Hain MTBDRs/ v1 and v2, mutation identity reported</p>
				91Pro(CCG) 88Cys(TGC) 88Ala(GCC)	Other mutations conferring FQ resistance.
	<i>gyrB</i>				Included in Hain MTBDRs/ v2
Amikacin (AK)	<i>rrs</i>	77.3	99.0	1401G	<p>Most common mutation; associated with AK-resistance. Detectable by Hain MTBDRs/ v1 and v2, mutation identity reported</p>
				1402T	AK often tests susceptible by growth-based DST.
				1484T	Associated with AK-resistance Detectable by Hain MTBDRs/ v1 and v2, mutation identity reported
	<i>eis</i>			-14T	<p>Associated with AK resistance.</p> <p>Detectable by Hain MTBDRs/ v2, mutation identity reported</p>

TABLE CONTINUES >

Antimicrobial agent	Gene	Sensitivity	Specificity	Selected mutations* and comments	
Capreomycin (CM)	<i>rrs</i>	69.4	98.3	1401G	Most common mutation; usually associated with CM-resistance. Detectable by Hain MTBDRs/ v1 and v2, mutation identity reported
				1402T	Associated with CM-resistance.
				1484T	Associated with CM-resistance. Detectable by Hain MTBDRs/ v1 and v2, mutation identity reported
	<i>tlyA</i>	No predominant mutations	Mutations are widely distributed throughout the gene. Some mutations are highly associated with CM resistance: GGG196GAG, GC insertion at nucleotide 202, GT insertion at nucleotide 755		
Kanamycin (KM)	<i>rrs</i>	73.2	98.4	1401G	Most common mutation; associated with KM resistance. Detectable by Hain MTBDRs/ v1 and v2, mutation identity reported
				1402T	Associated with KM-resistance.
				1484T	Associated with KM-resistance. Detectable by Hain MTBDRs/ v1 and v2, mutation identity reported
	<i>eis</i>	-10A	Highly associated with KM-resistance. Detectable by Hain MTBDRs/ v2. Some other mutations: -14T, -37T		
Bedaquiline (BDQ)	<i>Rv0678</i> <i>atpE</i> <i>pepQ</i> <i>Rv1979c</i>				Knowledge about mutations that confer BDQ resistance is evolving. Resistance may require mutations in multiple loci. <i>Rv0678</i> is involved in efflux and requires a functional efflux pump to confer resistance. Mutations appear to be common in both resistant and susceptible isolates. Cross resistance with clofazimine. <i>atpE</i> mutations are at the BDQ target site and associated with high level resistance. The role of <i>pepQ</i> and <i>Rv1979c</i> mutations is not clear.
Linezolid (LZD)	<i>rpIC</i>	38.2	99.8	154Arg/460C	Most common mutation in published reports Listed sensitivity likely underestimate as it only accounts for <i>rpIC</i> 154Arg
	<i>rrl</i>			2814T 2270T	

Table adapted with modification from: Lin SY, Desmond EP. Molecular diagnosis of tuberculosis and drug resistance. *Clin Lab Med.* 2014;34(2):297-314.

Molecular tests for drug resistance

There are several types of molecular tests for drug resistance detection. These tests have varying methodologies, advantages, and availability. See **Table 5** for comparison of current molecular tests and more detail in the text that follows.

TABLE 5. **Current molecular methods for detection of drug resistance in *M. tuberculosis*** See Table 3 for availability of these tests

	Cepheid Xpert MTB/RIF	Hain GenoType MTBDRplus & MTBDRsl	Pyrosequencing ^a (Laboratory-developed, non-commercial tests)	Sanger sequencing ^a (Laboratory-developed, non-commercial tests)	Targeted next generation sequencing (Laboratory-developed, non-commercial tests)	Whole genome sequencing (WGS) (Laboratory-developed, non-commercial tests)
Methodology	Real-time PCR Molecular beacon probes	PCR Line probes	PCR Pyrosequencing	PCR Sanger sequencing	PCR NGS	WGS
Specimen types	Clinical specimen Concentrated specimen ^b	Concentrated specimen ^b Culture	Concentrated specimen ^b Culture	Concentrated specimen ^b Culture	Concentrated specimen Culture	pure culture
Testing time	2.5 h	6-7 h	5-6 h	1-2 days	3-7 days	5-6 days
Drugs tested	RIF	RIF, INH (MTBDRplus) FQ, AK, CM, KM, EMB (MTBDRsl v1) FQ, AK, CM, KM (MTBDRsl v2)	INH, RIF, FQ, AK, CM, KM Other drugs possible	INH, RIF, EMB, FQ, AK, CM, KM, PZA Other drugs possible	INH, RIF, EMB, FQ, AK, CM, KM, PZA Other drugs possible	INH, RIF, EMB, FQ, AK, CM, KM, PZA Other drugs possible
Results	Mutation detected or not detected No sequences provided	Mutation detected or not detected Sequences of a few frequent mutations are provided	Sequences provided	Sequences provided	Sequences provided	Sequences provided
Methodology limitations	Difficult to detect heteroresistance Presence of silent mutations causes false interpretation of resistance	Difficult to detect heteroresistance Presence of silent mutations causes false interpretation of resistance	Heteroresistance can be detected; sensitivity not well characterized Not suitable for detecting mutations spread throughout a gene (e.g., <i>prnA</i>)	Heteroresistance can be detected; sensitivity not well characterized	Sensitivity for testing smear-negative sediments likely to be low.	Need pure culture

TABLE CONTINUES >

	Cepheid Xpert MTB/RIF	Hain GenoType MTBDRplus & MTBDRs/	Pyrosequencing ^a (Laboratory-developed, non-commercial tests)	Sanger sequencing ^a (Laboratory-developed, non-commercial tests)	Targeted next generation sequencing (Laboratory-developed, non-commercial tests)	Whole genome sequencing (WGS) (Laboratory-developed, non-commercial tests)
Advantages	<p>Little hands-on time</p> <p>Easy to perform</p> <p>Easy to implement</p> <p>Point-of-care capability</p> <p>FDA cleared; widely available in clinical and public health laboratories</p>	<p>Low equipment costs</p>	<p>Allows correlation between specific mutations and MICs</p> <p>Fairly wide applicability</p> <p>Possible to detect mixed population</p>	<p>Allows correlation between specific mutations and MICs</p> <p>Wide applicability</p> <p>Possible to detect mixed population</p>	<p>Excellent for heteroresistance detection</p> <p>Allows correlation between specific mutations and MICs</p>	<p>Heteroresistance can be detected but much less sensitive than tNGS</p> <p>Allows correlation between specific mutations and MICs</p>

a. Assays using pyrosequencing or Sanger sequencing technologies are laboratory-developed tests. The performance characteristics may vary. Laboratories may validate their own assays for testing specimens from nonrespiratory specimens. Check with your laboratory.

b. Clinical specimens concentrated by NALC-NaOH are suitable. Smear-negative specimens may be tested, but the sensitivity is lower than that for smear-positive specimens.

c. See **Table 6** for other WHO recommended assays not available in the U.S.

Table adapted with modification from Lin SY, Desmond EP. Molecular diagnosis of tuberculosis and drug resistance. *Clin Lab Med*. 2014;34(2):297-314

TABLE 6. **Molecular tests for diagnosis and detection of drug resistance in *M. tuberculosis* complex that are not currently authorized or available in the U.S.**

Test	Manufacturer	Test type	MTBC targets	Comments
			Resistance targets	
Xpert MTB/RIF (Ultra)	Cepheid	Cartridge-based real time PCR and melt curve	IS6110 <i>rpoB</i>	Increased sensitivity compared with Xpert MTB/RIF. Correctly reports the common silent mutation, 514Phe (TTT), as susceptible.
Xpert MTB/XDR	Cepheid	Cartridge-based multiplexed real time PCR	<i>inhA, katG, fabG1, ahpC, gyrA, gyrB, rrs, eis</i>	Reflex test to identify INH, FQ, amikacin resistance. Similar analytic sensitivity to Xpert MTB/RIF but less than Xpert MTB/RIF Ultra. Requires upgrade to 10-color GeneXpert platform
Truenat MTB Truenat MTB Plus Truenat MTB-RIF Dx	Molbio Diagnostics	Chip-based real-time micro-PCR Battery operated	<i>nrdB</i> <i>nrdz</i> , IS6110 <i>rpoB</i>	MTB or MTB Plus used to identify Mtb. MTB-RIF Dx is reflex test for RIF-resistance
RealTime MTB test RealTime MTB RIF/INH	Abbott Molecular	Automated NAAT on high throughput platform	<i>Pab</i> , IS6110	MTB test to identify Mtb. MTB RIF/INH is reflex test to identify INH or RIF resistance
BD MAX™ MDR-TB	Becton Dickinson	Automated multiplexed real-time PCR on high throughput platform	IS6110, IS1081 <i>rpoB, inhA, katG</i> (codon 315)	Simultaneously identifies Mtb, INH, and RIF resistance
FluoroType® MTB FluoroType MTBDR	Hain Lifescience	Automated NAAT on high throughput platform	<i>rpoB</i> <i>rpoB, inhA, katG</i>	MTB test to identify Mtb. MTB RIF/INH is reflex test to identify INH or RIF resistance
cobas® MTB assay cobas® MTB-RIF/INH assay	Roche Diagnostics	Automated NAAT on high throughput platform	16S rRNA, <i>esxJ, esxK, esxM, esxP, esxW</i> <i>rpoB, inhA, katG</i>	MTB test to identify Mtb. MTB RIF/INH is reflex test to identify INH or RIF resistance

TABLE CONTINUES >

Test	Manufacturer	Test type	MTBC targets	Comments
			Resistance targets	
TB-LAMP Loopamp™ Mycobacterium tuberculosis complex detection kit	Eiken Chemical Company	Loop-mediated isothermal amplification (LAMP) Manual assay that requires less than 1 hour to perform and can be read with the naked eye under UV light.	6 primers bind to 8 locations	Replaces smear for patients with signs and symptoms consistent with TB. Requires little infrastructure.
AlereLAM Alere Determine TB LAM Ag	Alere	Lateral flow urine lipoarabinomannan antigen assay (LAM)		Recommended in people with HIV and: <ul style="list-style-type: none"> • advanced HIV (CD4<100) • who are seriously ill • who have signs and symptoms of TB or are hospitalized and CD4<200
GenoType® MTBDRplus (version 2) GenoType MTBDRsl assay versions 1.0 and 2.0	Hain Lifescience	Line probe assay based on reverse-hybridization DNA strip technology	<i>rpoB, katG, inhA</i> (MTBDRplus) <i>gyrA, rrs, embB</i> (MTBDRsl v1) <i>gyrA, gyrB, rrs, eis</i> (MTBDRsl v2)	Available in some laboratories in the U.S. For smear positive specimens or cultured isolates.
Genoscholar™ NTM+MDRTB II Genoscholar™ PZA-TB	Nipro	Line probe assay based on reverse-hybridization DNA strip technology	<i>rpoB, katG, inhA</i> (NTM+MDRTB II) <i>pncA</i> (PZA-TB)	NTM+MDRTB II also identifies <i>M. avium</i> , <i>M. intracellulare</i> , <i>M. kansasii</i> PZA-TB assay recommended on culture isolates
Deeplex® Myc-TB	Genoscreen	Targeted NGS	<i>hsp65</i> <i>rpoB, ahpC, fabG1, katG, inhA, pncA, embB, gyrA, gyrB, rrs, eis, tlyA, gidB, rrs, rpsL, ethA, rv0678, rrl, rplC</i>	Also identifies NTM species
Ion AmpliSeq™ TB Panel	Thermo Fisher Scientific	Targeted NGS	<i>embB, eis, gyrA, inhA, katG, pncA, rpoB, rpsL</i>	

Compiled from: WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021. License: CC BY-NC-SA 3.0 IGO.

Association of Public Health Laboratories. Issues in Mycobacterium tuberculosis Complex Drug Susceptibility Testing: Rifampin. May 2022

Available at: <https://www.aphl.org/aboutAPHL/publications/Documents/ID-2022-MTBC-DST-Rifampin.pdf> Accessed May 8, 2022.

Additional information gathered from manufacturer websites.

Probe-based tests

Molecular beacon assay

The most widely available and used probe-based test in the U.S. is the Xpert MTB/RIF assay which is based on molecular beacon technology. The following two sections provide general information about the test and result categories.

Overview of the assay

- FDA cleared for testing **smear-positive or negative sputum** specimens, raw or concentrated (sediments). Some labs have validated for other specimen types.
- Detects **MTBC** and **RIF-resistance (RIF-R)**

Realtime PCR and Ct values

FIGURE 4.

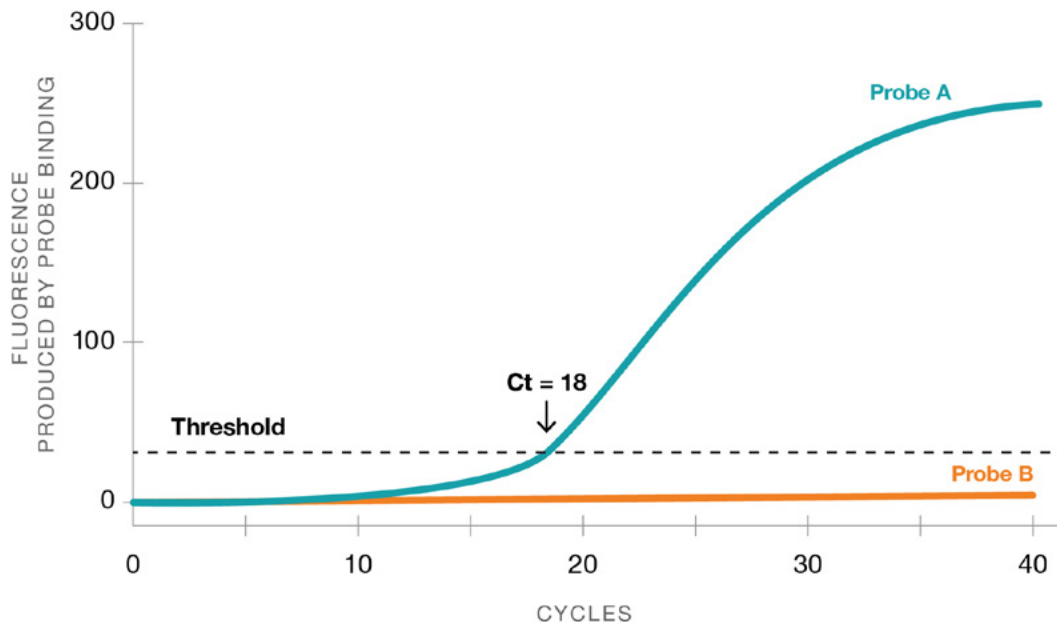


Figure 4 shows fluorescence curves for two probes run on an example molecular beacon assay.

- Probe A produced signals, indicating the sample had sequence matching the probe sequence.
- Cycle threshold (Ct) is the cycle number at which the fluorescence signal crosses the threshold level.
- Probe A had signals crossing the threshold at cycle 18, so the Ct is 18 indicating the sample contains relative high amounts of the targeted DNA. Lower Ct values indicate higher amounts of DNA in the sample.
- Probe B had no signal. No signal could mean the targeted DNA was not present or below the detection limit in the sample, the targeted DNA in the sample contains a mutation preventing probe binding, or there was an inhibitor in the sample. Note that Xpert MTB/RIF contains an internal control (SPC) to detect inhibitors.

Methodology

The assay uses **nested Real-time PCR** and five **molecular beacon probes**.

- Molecular target: 81-bp RIF-R determining region (**RRDR**) in the *rpoB* **core region**
- **Principles:** All 5 beacons with wildtype MTBC sequences generate signals when hybridizing to wildtype MTBC sequence in samples. If a sample has a mutation within a probe's detecting range, the probe will not generate a signal. Because the core principle of the assay is that **presence of any mutation in RRDR confers RIF-R, failure in producing signals from any probe is interpreted RIF-R.**
- The assay detects presence or absence of mutations. Due to its inability to provide the mutation identity, the assay is **unable to differentiate silent mutations from mutations conferring RIF-R.**

Test performance

Identification of MTBC:

- Specificity: 98%
- Sensitivity: **Smear-positive** specimens: 98%; **Smear-negative** specimens: 67%
- Sensitivity increases with multiple samples: 59.3% (1 sample); 71.4% (2 samples); Specificity: 99.2%

Detection of RIF-R:

- Sensitivity 96%, Specificity 98%

Interpretation criteria

Two essential criteria [See package insert for complete criteria]

[Xpert MTB-RIF ENGLISH Package Insert 301-1404 Rev G \(cepheid.com\)](#)

- MTBC is detected when at least two probes yield valid signals.
 - **Note: Xpert MTB/RIF does not have a specific probe for detection of MTBC**
- RIF-R is detected when $\Delta Ct_{max} > 4$.
 - $\Delta Ct_{max} = \text{Highest Ct} - \text{lowest Ct}$
 - Ct is the value of PCR cycle crossing the threshold. (**Figure 4**)

Testing a second specimen

Testing a concentrated specimen (sediment) is better than a raw specimen.

- Obtaining a good quality sputum (3-5 mL first morning sputum) may increase chances for valid results.

Confirmation by sequencing

The Xpert test kit package insert states that if MTBC and RIF-R are detected, results **must be confirmed** by a reference laboratory. Specimens **should also be tested for the genetic mutations associated with resistance to other drugs.**

Confirming Xpert MTB/RIF results of a smear-negative sample by sequencing is challenging due to lower sensitivity by sequencing methods, especially when the Xpert MTB/RIF Ct values are higher than 28.

Testing positive cultures

Retesting and confirmation may take more than one week to complete. If unable to obtain valid results, rather than testing more smear-negative samples, waiting for cultures to grow and to test by a sequencing method can be a sensible option because MTBC cultures usually turn positive in about 2 weeks.

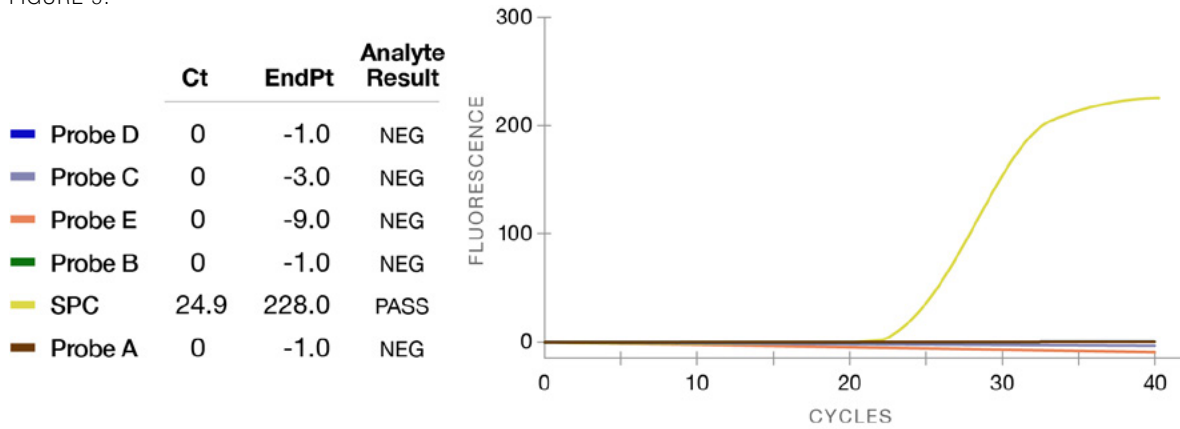
Other specimen types

Some labs have validated Xpert MTB/RIF for other specimen types. Discuss with your laboratory what is available. See performance information in **Table 6** and **Chapter 6: Pediatrics.**

RESULT CATEGORIES

MTBC not detected, RIF-R not detected

FIGURE 5.



Result explanation (Fig 5):

All five probes yield no signal, so MTBC was not detected in the specimen.

SPC (specimen processing control) yields signal meaning no inhibition; the test is valid.

Possible reasons for result:

- MTBC is not present in the specimen
- MTBC is present but below the detection limit of the assay

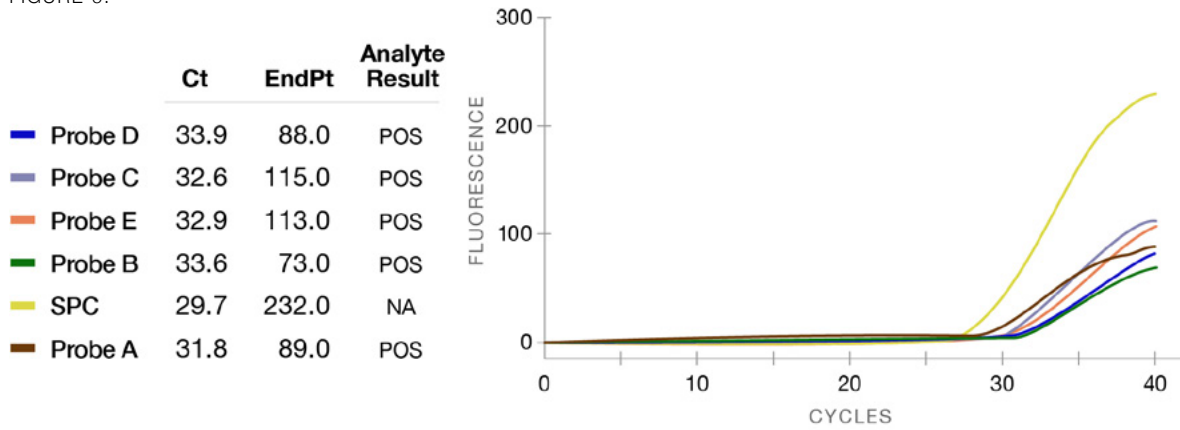
Possible clinical responses or conclusions:

- If the specimen was smear-negative, consider testing another specimen if there is suspicion for TB.
- If the specimen was smear-positive (> 1+), the patient is likely infected with NTM. Retesting may not be necessary.
- If mixed MTBC and NTM infection is possible or smear was low positive, consider testing another specimen.

RESULT CATEGORIES

MTBC detected, RIF-R not detected

FIGURE 6.



Result explanation (Fig 6):

- All five probes yield signals indicating MTBC was detected in the specimen.
- Ct >30 indicates a low amount of MTBC DNA
- No mutations are detected because the $\Delta Ct \text{ max} \leq 4$ ($33.9 - 31.8 = 2.1$)

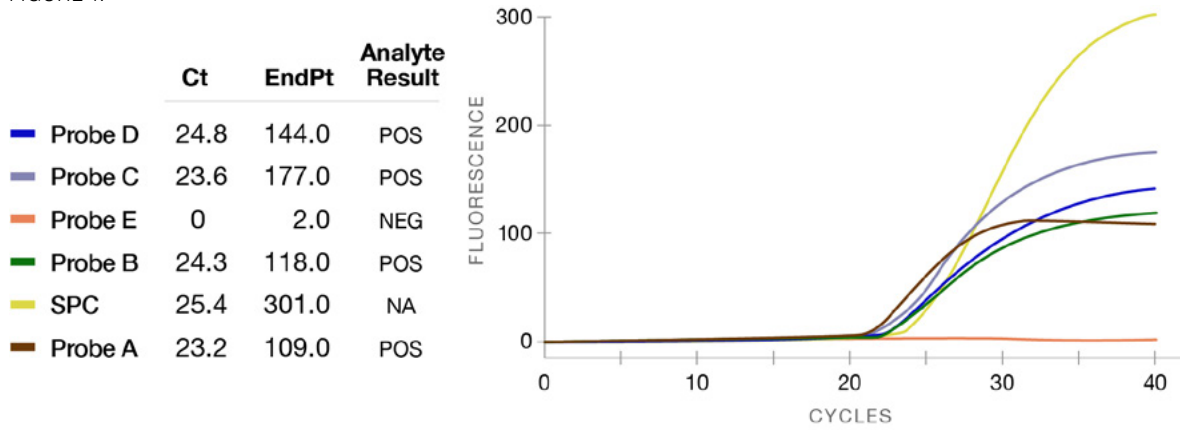
Possible clinical responses or conclusions:

- Most frequently obtained result and is reliable; no need to test another specimen

RESULT CATEGORIES

MTBC detected, RIF-R detected

FIGURE 7.



Result explanation (Fig 7):

- Four probes yield signals so MTBC is detected in the specimen
- Ct range is 23-25 indicating a moderate amount of MTBC DNA
- Probe E has no signal (Ct=0)
- A mutation is detected because $\Delta Ct \text{ max} > 4$ ($24.8 - 0 = 24.8$)

Reason for result:

- A mutation is present in the segment of *rpoB* covered by probe E. Probe E detects the most common mutation conferring RIF resistance, S450L.

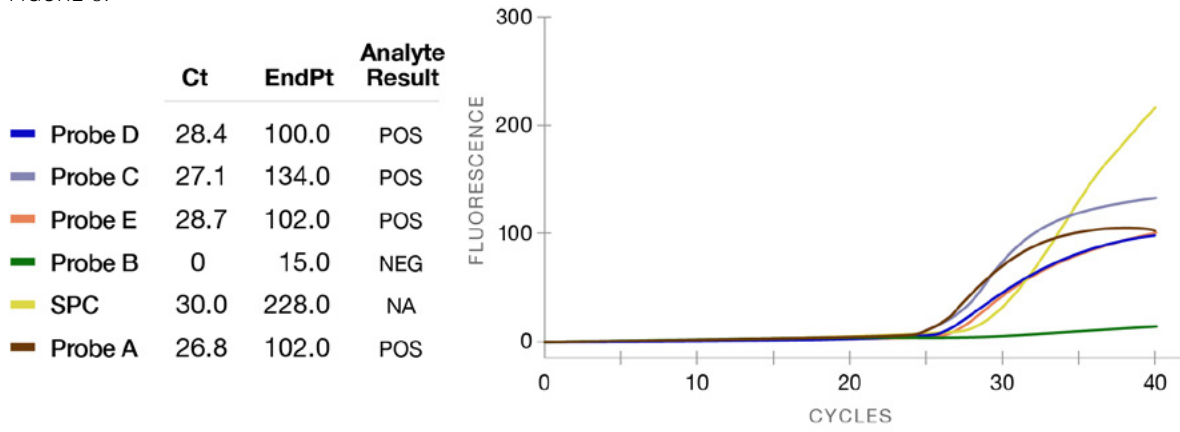
Possible clinical responses or conclusions:

- RIF-R result should be **confirmed by a sequencing method.**
- It is advisable to **obtain reports issued from Xpert computer** such as shown in the figures. The detailed information in the report may help decipher results.

RESULT CATEGORIES

MTBC detected, RIF-R detected

FIGURE 8.



Result explanation (Fig 8):

- Four probes yield signals so MTBC is detected in the specimen
- Ct range is 26-29 indicating a low to moderate amount of MTBC DNA
- Probe B has no signal (Ct=0)
- A mutation is detected because $\Delta Ct \text{ max} > 4$ ($28.7 - 0 = 28.7$)

Possible reason for result:

- A mutation is present in the segment of *rpoB* covered by probe B.

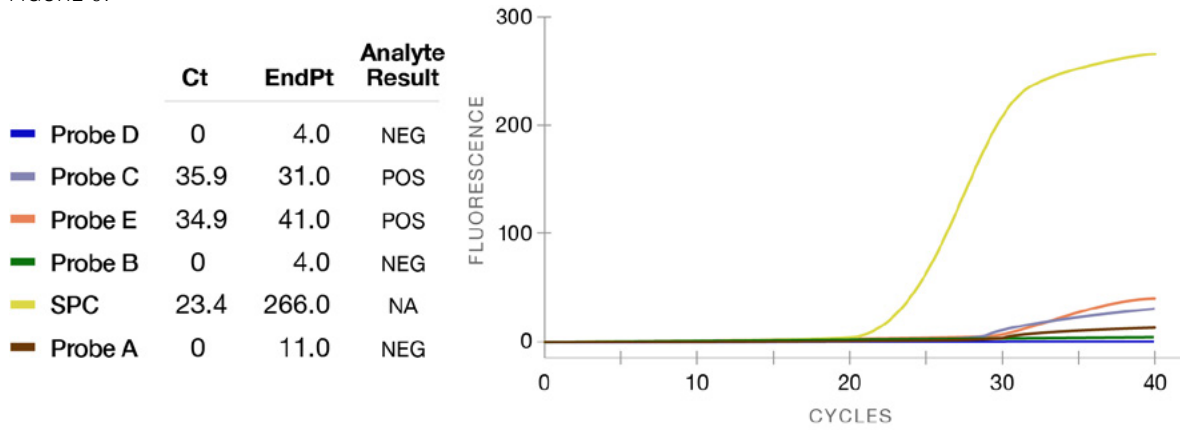
Possible clinical responses or conclusions:

- RIF-R result should be **confirmed by a sequencing method**.
- It is advisable to **obtain reports issued from Xpert computer** such as shown in the figures. The detailed information in the report may help decipher results.
- **Caution** should be taken when RIF-R is detected by **probe B**.
 - Probe B detects several mutations conferring RIF-R and **F433F** (TTC > TTT), the **most frequently encountered silent mutation**.
 - This silent mutation was present in 25% (111/437) of samples with a mutation in the RRDR and 74% (111/151) of samples with a mutation in the region covered by probe B. [Unpublished analysis among 5,193 samples submitted for pyrosequencing at California Department of Public Health Microbial Disease Laboratory] Published analysis by CDC reported 19% of 64 samples testing RIF-R on Xpert had this silent mutation.

RESULT CATEGORIES

Doubtful results: possible faulty detection of MTBC and RIF-R

FIGURE 9.



Result explanation (Fig 9):

- Only two probes yield signals. Assay interpretation criteria concludes MTBC is detected in the specimen
- Ct values are >34 indicating very low amount of DNA

Possible reasons for result:

- MTBC with at least 3 *rpoB* mutations in segments covered by three different probes
- False positive MTBC detection

Possible clinical responses or conclusions:

- This result is likely to occur with testing **smear-negative** specimens which yield no or very low signals, and especially when RIF-R is detected by 3 probes.
- RIF-R due to 3 mutations in RRDR is very rare.
- Of 5193 specimens sequenced at CDPH, only 2 specimens had 3 mutations. None were detectable by 3 probes; mutations in one specimen were detectable by probe D and those in the other specimen by probes A and B.
- Some cultures have grown NTM in this scenario

For further information, see section: **Difficulties interpreting results from molecular tests.**

TABLE 7. Interpretation of line-probe assay

Line-probe band pattern	Interpretation/comments
All wild-type bands are present with absence of all mutant bands.	<ul style="list-style-type: none"> No mutations are present within the targeted DNA segment; this suggests susceptibility to the drug.
Missing at least one wild-type band and presence of one of the mutant bands.	<ul style="list-style-type: none"> A specific mutation is present and the mutation identity is reported. Drug resistance is predicted.
Missing at least one wild-type band but none of the mutant bands are present.	<ul style="list-style-type: none"> A mutation is present but not one of the frequent mutations; the mutation identity is not reported. It is likely to be associated with drug resistance, but silent mutations or other mutations not conferring resistance cannot be ruled out. Need to confirm by a sequence-based method.
All wild-type bands are present and one of the mutant bands is also present.	<ul style="list-style-type: none"> Possibly a mixed population or a mixed infection with two different strains, a wild-type strain and a drug-resistant strain. The variable intensity of the band may add difficulties in interpretation for this scenario. It is advisable to repeat the test or to confirm by a sequence-based method, or to defer the interpretation to culture-based drug susceptibility testing results.

(Table adapted with modification from Lin SY, Desmond EP. Molecular diagnosis of tuberculosis and drug resistance. *Clin Lab Med.* 2014;34(2):297-314.)

Sequence-based tests

A sequence-based test not only detects presence or absence of mutations, but also reports the identity of a mutation. This allows a user to identify if a mutation is a silent mutation not associated with drug resistance, or a mutation that confers *in vitro* resistance. With increased use of sequence-based tests and MIC testing, the usefulness of MIC data with associated mutations has gained significant importance in providing critical information to clinicians treating drug resistant TB. Accumulating MIC data enables greater confidence in predicting the level of drug resistance weeks before growth-based DST can be performed.

- Pyrosequencing (PSQ)** is a real-time sequencing method that sequences a short stretch of nucleotides and is capable of detecting any mutation within the targeted length with the mutation identified. It is not suitable for detecting mutations which are widely spread throughout the gene, such as PZA-resistance associated *pncA* mutations. A well-designed PSQ assay is sensitive enough to detect mutations from smear-positive concentrated specimens.
- Sanger sequencing** is the gold standard of sequencing, using dye-terminator technology. It is capable of sequencing hundreds of nucleotides. CDC's MDDR service provides sequencing that detects mutations associated with resistance to INH, RIF, EMB, PZA, AK, CM, KM, and fluoroquinolones by Sanger sequencing and it also uses PSQ to screen resistance to INH and RIF when drug resistance status is unknown. The service has a short turnaround time (1-2 days).

- **Targeted next generation sequencing (tNGS)** and **whole genome sequencing (WGS)** can provide the same information as PSQ and Sanger sequencing but on a much wider range and larger number of genes. Along with increasing knowledge correlating mutations and resistance, WGS is enabling development of more sophisticated and accurate resistance predictions. WGS is becoming more available in clinical and reference laboratories. In some settings and laboratories WGS results have obviated the need for growth-based DSTs for isolates without mutations. WGS requires software to handle enormous amounts of data and has a longer turnaround time (5-7 days) than other sequencing methods. At present, WGS requires higher concentrations of DNA extracted from cultures.
- tNGS uses PCR to amplify segments of DNA of targeted genes. The analytic sensitivity may be similar to or slightly lower than PSQ and Sanger sequencing methods. tNGS is excellent in detecting heteroresistance with much higher sensitivity than WGS, Sanger, or PSQ methods.

Choice of molecular tests

- If a sequence-based method is available locally, it is the method of choice.
- If Xpert MTB/RIF is readily available, it can be used for detection of *M. tuberculosis* complex and RIF-resistance. When a mutation is detected, it must be confirmed by a sequence-based method.
- If INH-resistance is suspected, use a method which can at least detect the most common INH-associated mutations in *katG* and *inhA*.
- If RIF-resistance is detected, MDR-TB is likely and the specimen should be tested for mutations associated with resistance to other drugs.

Difficulties interpreting results from molecular tests

Molecular testing is enabling much more rapid diagnosis of likely drug resistance, yet with new technologies come new questions. Difficulties interpreting results may arise from the way tests are reported, clinicians' lack of familiarity with these results, and — most importantly — from evolving knowledge regarding the clinical implications of specific mutations. Among the more challenging situations for the clinician is when molecular and growth-based test results are discordant.

- **Discordance between molecular and growth-based test results** may occur and can be confusing. Examples of this are:
 - Isolates with certain mutations in *rpoB* may test susceptible for RIF by growth-based methods. These mutations are referred to as **borderline resistance mutations** by WHO (See **Table 4**; these have previously been called “disputed” mutations). Laboratories performing sequence-based assays should be able to identify these mutations in test reports. However, reporting parameters and language may vary.
 - Several clinical case series including one from California have been published reporting **poor treatment outcomes for patients with these mutations** when treated with standard first-line therapy. In a 2013 study evaluating samples from two countries with a high burden of DR-TB, borderline RIF-resistance mutations in *rpoB* were responsible for over 10% of rifampin resistance among first-line failure and relapse cases.

- The recognition of discordance between molecular and growth-based tests associated with these mutations has triggered review of rifampin critical concentration in liquid media (see section: **Critical concentration, minimum inhibitory concentration (MIC), and what they mean**). WHO and other expert groups recommend that **isolates with these borderline RIF-resistance mutations be considered and managed clinically as RIF-resistant regardless of growth-based test results**.
- **Silent and neutral mutations** (defined in **Types of mutations**) can be additional causes for discordance between molecular and growth-based test results and can be identified through sequencing as sources of false-positive molecular resistance results.

Types of mutations

Silent mutations: Alteration in DNA sequence but no resulting amino acid change, and thus, not associated with drug resistance.* Also called synonymous mutations.

- 433Phe(TTT) mutation in *rpoB* is the most common silent mutation. Information regarding this silent mutation contributing to false-positive rifampin resistance results when using Xpert MTB/RIF can be found in the section: **Probe-based assays**.

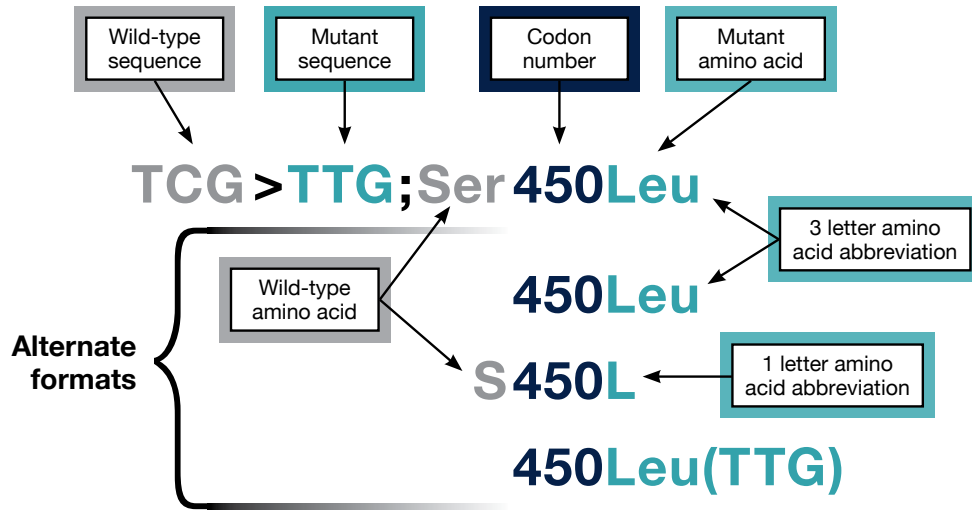
Missense mutations: Alteration in DNA sequence results in change in amino acid sequence. Also called nonsynonymous mutations.

- May confer different levels of resistance or no resistance.
- A missense mutation that has no effect on growth-based test results is also called a **neutral mutation**. Neutral mutations can be present in both drug-susceptible and drug-resistant strains.

* There are rare exceptions to this rule. The main example is the silent mutation in *fabG1*, Leu203Leu, which results in a “cryptic promoter” and INH resistance despite not resulting in an amino acid change.

Understanding sequence-based molecular test reports can be challenging. Results can be reported using various formats, abbreviations, and numbering systems. **Figure 11** shows variations of reporting formats based on the example of an *rpoB* mutation. All reports should indicate the location (codon number or nucleotide number) and the mutant sequence or amino acid detected. This information can be used to make additional conclusions about the likelihood and extent of resistance (see **Table 4: Genes and mutations associated with drug resistance**).

FIGURE 11. Guide to understanding sequence-based molecular test reports based on the example of an *rpoB* mutation.



- **Both growth-based susceptibility testing and molecular testing are important in constructing treatment regimens.** Growth-based testing still plays an integral role in providing crucial additional information and testing drugs for which molecular tests are not yet available.

CLINICAL SCENARIO

➤ Long-term elderly resident of the U.S. who was born in Mexico presents with 3 months of cough and cavitary lesion on chest radiograph.

He has not been treated for TB before and has no known contact with an MDR-TB case. Xpert MTB/RIF assay performed on AFB smear-positive sputum is reported as “MTB detected, RIF resistance detected.”

Confirmatory sequence-based testing is requested prior to starting an MDR-TB regimen because likelihood of MDR-TB is low given the patient’s history. **Sequencing assay reveals mutation at 433Phe(TTT), a silent mutation. Growth-based susceptibility testing confirms RIF susceptibility.**

The patient does well on standard first-line treatment.

Molecular tests on extrapulmonary specimens

Molecular tests for drug resistance can also be performed on non-respiratory specimens. However, no molecular assay is FDA-cleared for use on non-respiratory specimens, and assays therefore must be validated by individual laboratories. Many laboratories do not have the capability to validate or run molecular tests on extrapulmonary specimens. *Xpert MTB/RIF* performance for testing extrapulmonary specimens has been published (see **Table 8**).

TABLE 8. **Meta-analysis of the sensitivity and specificity of Xpert MTB/RIF in diagnosing extrapulmonary TB in adults compared against culture as a reference standard, by type of extrapulmonary specimen**

Specimen type	No. of studies No. of persons	pooled sensitivity (pooled 95% CrI*)	pooled specificity (pooled 95% CrI*)
Lymph node aspirate	14 studies 1588 persons	89 (82–93)	86 (78–92)
Lymph node biopsy	11 studies 786 persons	82 (73–89)	79 (59–91)
Cerebrospinal fluid	28 studies 3103 persons	70 (61–79)	97 (95–98)
Pleural fluid	24 studies 2926 persons	50 (39–61)	99 (97–100)
Peritoneal fluid	13 studies 619 persons	59 (42–76)	97 (95–99)
Urine	9 studies 943 persons	85 (71–93)	97 (91–99)
Synovial fluid	6 studies 471 persons	97 (92–99)	9 (67–99)
Pericardial fluid	5 studies 181 persons	60 (35–82)	88 (73–97)
Blood	1 study 74 persons	56 (21–86)	94 (84–98)

*CrI: credible interval; the CrI is the Bayesian equivalent of the confidence interval

Adapted from WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021. License: CC BY-NC-SA 3.0 IGO . Some data presented only in Web Annex 4, 2020.

WHO 2021 guidelines recommend Xpert MTB/RIF as the preferred initial diagnostic test for CSF for patients suspected to have TB meningitis, given the urgency of rapid diagnosis.

Molecular tests on formalin-fixed specimens

Rarely, only fixed specimens are obtained and no other specimens are available to confirm TB when AFB or other pathologic findings consistent with TB are seen. When laboratory confirmation of the diagnosis is important for patient care, specialized laboratories can extract DNA from fixed specimens for analysis. Extracted DNA can also sometimes be used to perform molecular tests for drug susceptibility.

- **CDC Infectious Diseases Pathology Branch** offers this service in addition to a wide range of testing for identifying other microbes. Requests should come primarily through public health laboratories; CDC approval is required before submission of specimens. (<http://www.cdc.gov/ncezid/dhcpp/idpb/specimen-submission/index.html>); email: Pathology@cdc.gov)
- **National Jewish Health Mycobacteriology Laboratory** offers *M. tuberculosis* complex and MDR-TB/XDR-TB testing on formalin-fixed specimens (FFPE). (<https://www.nationaljewish.org/for-professionals/diagnostic-testing/adx/tests/nucleic-acid-amplification-test-naat-clinical-specimen-only>)
- **University of Washington Medical Center Molecular Diagnosis Section** offers identification of *M. tuberculosis* complex and NTM from tissue specimens including fixed specimens. <http://depts.washington.edu/molmicdx/> email: molmicdx@uw.edu

Therapeutic drug monitoring (TDM)

When to order TDM

TDM is routinely used in several circumstances:

- **Aminoglycoside/CM** serum concentrations, especially in patients with **renal impairment**
- **CS** concentrations to minimize risk of **CNS toxicity** and to safely use optimal dose
- **LZD** concentrations to **minimize risk of hematologic and neurologic toxicity** (trough concentration) and ensure efficacy (peak concentration)
- **EMB** concentrations in patients with significant **renal impairment**

TDM is often considered for patients with:

- Known or suspected **malabsorption** (e.g., diabetes, HIV, gastrointestinal disorders)
- **Lack of expected clinical response** or **relapse** while on appropriate drugs and doses, administered by DOT
- **Few effective drugs** in their regimen, to optimize the effect of available drugs
- Potentially significant **drug-drug interactions** such as rifamycins and antiretrovirals
- **Obesity** or **very low body weight** to ensure appropriate dosing

Some experts measure serum drug concentrations in patients with immunosuppression, advanced age, or severe extrapulmonary TB to ensure optimal drug exposure in these potentially difficult to treat cases

Where to send a specimen for TDM

Most hospital and commercial laboratories perform AK serum concentrations. Only a few laboratories perform drug concentrations for other TB drugs.

Laboratories and contact information:

University of Florida

idpl.pharmacy.ufl.edu
peloquinlab@cop.ufl.edu
352-273-6710

National Jewish Health

njlabs.org
willeyr@njhealth.org or clinreflabs@njhealth.org
303-398-1422

How to send a specimen for TDM

Collecting and processing samples for TDM

- One milliliter of serum (about 2 mL of blood) is required per test. It is advisable to provide some excess serum in case there are technical problems.
- Specimens should be collected after at least 4-5 half-lives have elapsed since the initiation of the drug. In practice, approximately 1-2 weeks works well in most cases. A shorter time can be used for adjustments of dose or schedule.
- Random samples generally are not informative, including for aminoglycosides.
- Patients should come to clinic with their medications and should plan to be at the clinic for at least 2 hours and up to 6 hours if a second time point is ordered.
- See **Table 9** for timing of specimen collection. On the day of blood draws only, rifabutin (RFB) can be given 1 hour before the other TB drugs so that only 2 venipunctures are required.
- A second concentration after the peak (typically at 6 hours) may be obtained to evaluate for delayed absorption or to calculate a half-life to more accurately prescribe a drug dose and interval.
- Observe the taking or injection of the medications and record the dose, exact time, and date.
- Collect the blood by direct venipuncture into a plain red-top tube (timing as described by **Table 9**) and record the exact time of the blood collection.
- Label the tubes with the patient's name, date and time of collection, and the drug(s) to be assayed.
- For SM, note if the patient is also receiving ampicillin.
- After the blood clots, centrifuge the samples, harvest the serum into labeled polypropylene (or polyethylene) tubes, and freeze. The specimen should be stored frozen until ready for shipping; -70 degrees C is preferable, but at a minimum -20 degrees C.
- For detailed instructions for processing and submitting specimens for TDM, see:

University of Florida: idpl.pharmacy.ufl.edu

National Jewish Health: njlabs.org

TABLE 9. Suggested time for blood collection after an oral dose

Drug name	Hours after oral dose to “peak”	Time after dose for additional concentration if desired*
Bedaquiline**	5 hours	24 hours
Clofazimine	2-3 hours	6-7 hours
Cycloserine	2-3 hours	6-7 hours
Ethambutol	2-3 hours	6-7 hours
Ethionamide	2 hours	6 hours
Isoniazid	1-2 hours	4-6 hours
Levofloxacin	2 hours	6 hours
Linezolid**	2 hours	5-6 hours
Moxifloxacin	2 hours	6 hours
PAS	6 hours	
Pretomanid**	5 hours	24 hours
Pyrazinamide	2 hours	6 hours
Rifabutin	3-4 hours	7 hours
Rifampin	2 hours	6 hours
Rifapentine**	5-6 hours	6 hours

Drug name	Hours after completion of infusion/injection to “peak”	Time after dose for additional concentration if desired*
Amikacin	1.5-2 hours (IV)	6 hours (IV or IM)
Streptomycin	2 hours (IM)	

*An additional concentration may be obtained to evaluate for delayed absorption or to calculate a half-life to prescribe a drug dose and interval more accurately.

** trough concentrations drawn immediately before the dose is helpful for these drugs.

How to interpret results of TDM

See **Chapter 4, Treatment.**

National TB Molecular Surveillance

The Michigan Department of Community Health is under contract with CDC to provide sequencing/genotyping services to TB programs in the U.S. as the National TB Molecular Surveillance Center. **TB programs, through their state or county public health laboratories, should submit the initial isolate from each culture-positive TB patient to the molecular surveillance laboratory** in Michigan.

The goal of TB molecular surveillance is to reduce the burden of TB by identifying where transmission is currently occurring and interrupting it. CDC-supported molecular surveillance services are offered at no cost to TB programs. This surveillance service is not intended to provide information on resistance mutations for clinical management.

WGS provides much greater discriminatory power than conventional genotyping methods (MIRU analysis and spoligotyping). As of March 2018, the National TB Molecular Surveillance Center began performing WGS on all isolates as part of a planned transition away from conventional methods. Both WGS and conventional genotyping were performed in parallel until June 2022.

WGS results are provided to TB programs through TB Genotyping Information Management System (TB GIMS). Programs that perform their own WGS that meets specific quality and methodologic criteria may be able to submit electronic sequence files to CDC.

CDC provides two analyses of WGS data for molecular surveillance:

- **Whole-genome multilocus sequence typing (wgMLST)**
 - Routinely performed for all submitted isolates
 - Replaces GENType and PCRTyping previously derived from conventional genotyping.
 - Analyzes 2,690 genes in the *M. tuberculosis* genome; isolates that match at ≥99.7% of genes will form a cluster and assigned a wgMLSType.
- **Whole-genome single nucleotide polymorphism (wgSNP) comparison**
 - Performed upon request and when transmission is suspected (not universally done)
 - Used to distinguish isolates within a genotype-matched cluster or wgMLSType. wgSNP analysis, combined with epidemiologic investigation, can assist in investigating potential chains of transmission.
 - Analyzes approximately 90% of the 4.4 million nucleotides in the *M. tuberculosis* genome.

Molecular surveillance can also help investigate possible false positive isolation of *M. tuberculosis* complex which is reported to occur in 1-2% of isolates. Most laboratories are vigilant in detecting false positives, but they may still be undetected. If false-positive culture results are suspected by the clinician, the clinician should communicate with the laboratory and request the isolate be sent for WGS as soon as possible.

Molecular surveillance has become increasingly important, and programs are encouraged to submit all isolates for sequencing.

SUMMARY

- Two-way communication between clinician and laboratory is crucial to ensure appropriate testing and optimal turnaround time.
- Appropriate and adequate specimen collection and handling ensures the most clinically useful laboratory results.
- Both conventional growth-based and molecular tests have important roles in diagnosis of TB and drug resistance.
- A critical concentration is not a minimum inhibitory concentration (MIC). Requesting an MIC determination can be helpful in some situations.
- Some mutations do not confer resistance. To maximize the information obtained from molecular testing, results from probe-based molecular tests for drug resistance showing resistance should be confirmed by sequence-based tests.
- Discordance in susceptibility test results can occur across test types and laboratories. Discordance can have multiple possible causes and can be clinically confusing. Expert consultation is recommended.
- Molecular assays may be performed on extrapulmonary specimens and on fixed specimens at certain laboratories.
- Therapeutic drug monitoring (TDM) can play an important role in managing patients with drug resistance, but requires care in specimen collection, handling, documentation, and interpretation.

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