UCSF University of California San Francisco

CURRY INTERNATIONAL TUBERCULOSIS CENTER

CDPH California Department PublicHealth

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Diagnosis

Contributors to 2022 updates: **ADITHYA CATTAMANCHI,** MD, MAS & **LISA CHEN,** MD

SUMMARY OF KEY UPDATES (2022)

- Updated recommendations more strongly support use of rapid molecular tests for both identification of *Mycobacterium (M.) tuberculosis* and drug susceptibility testing (DST)
- Recommendation for expanded DST for second-line agents includes newer/repurposed drugs and addition of targeted next-generation sequencing technology

The first step in diagnosing drug-resistant TB is to use rapid molecular tests.

The diagnosis of tuberculosis (TB) frequently requires a high index of suspicion, especially in low-prevalence areas. The index of suspicion should be based on consideration of clinical and epidemiological risk factors, symptoms, physical examination (e.g., enlarged lymph nodes or other findings suggestive of possible extrapulmonary involvement), and radiographic findings. Once TB is considered, sputum or other specimens are collected for TB diagnostic testing. **The possibility of drug-resistant TB should be considered simultaneously with specimen collection and selection of the initial treatment regimen.** Optimal clinical practice would include the use of nucleic acid amplification tests (NAAT) with rapid molecular DST for rifampin (RIF) with or without isoniazid (INH), as a universal practice to both identify *M. tuberculosis* and rapidly identify key first-line drug-resistance. If initial rapid tests identify RIF and/or INH resistance, more extensive rapid molecular DST would be indicated. Failure to consider the possibility of drug-resistant TB until growth-based (conventional, phenotypic) DST results return weeks to months later can result in inadequate drug regimens, amplification of drug resistance, and additional disease transmission.

Rapid identification of drug resistance in a person with TB is critical to:

- Treat with the most appropriate regimen
- Minimize transmission
- Prevent acquisition of further drug resistance
- Offer appropriate care to contacts
- Provide the best chance of cure

Risk assessment for drug resistance

While ideal, NAATs are not universally available in all settings for rapid diagnosis of TB and testing resistance to key first-line drugs. It is therefore important to understand who is at higher risk for drug resistance and to prioritize rapid molecular testing for drug resistance when risks are identified. Understanding risk factors for drug resistance is also important for interpretation of DST results, e.g., raising suspicion for false-positive or inaccurate results when risk factors for drug resistance are not present.

The most important predictors of drug-resistant TB are:

- **Previous episode(s)** of TB treatment
- **Worsening clinical and/or radiographic findings** while on TB therapy
- History of **residence** in, or frequent travel to, a **region or country with a high prevalence of drug-resistant TB** (see **Chapter 1, *Epidemiology***)
- **Exposure to a person with known (or highly suspected) infectious drug-resistant TB,** or exposure to individuals in congregate settings where drug resistance has been documented

Suspicion for missed or acquired drug-resistant TB should be high and rapid molecular DST should be obtained for people with TB who have one or more of the following characteristics:

- Lack of anticipated improvement, or worsening of TB symptoms or radiographic findings despite TB treatment
- Cultures that remain persistently positive after 2-3 months while on treatment, despite initial phenotypic DST showing susceptible results, or reversion to positive after initial culture conversion
- Non-adherence or intermittent or erratic ingestion of prescribed anti-TB medications
- Lack of consistent verification of treatment adherence or ingestion (e.g., directly observed therapy [DOT], electronic or video DOT [eDOT or vDOT], or other medication monitoring strategies)
- Relapse after treatment completion when TB is considered cured. Note: programmatic data suggests the risk for relapse with acquired resistance is significantly lower if DOT (5 days/week x 6 months for drug-sensitive TB), with an appropriate regimen can be verified, but a rapid molecular test should still be obtained
- History of an inappropriate treatment regimen, including:
  - Administration of single-drug therapy
  - Too few effective drugs
  - Inadequate drug dosing

Questions to ask

Soliciting a history of previous TB treatment requires a great deal of patience and attention to detail. In a confidential setting, allow plenty of time, use an accurate and unbiased medical interpreter (helpful even if the person seeking care speaks some English, but as a secondary language), and be willing to repeat or rephrase a question to obtain the information. Give encouragement to reveal accurate information by asking and responding in a nonjudgmental manner. Initial questions include:

- Have you been told you had TB before?
- Have you been treated for TB? Were you sick from the TB (TB disease) or were you treated for latent TB infection (often described as "sleeping" TB)?
- Have you received injections or antibiotics for many months for a lung problem? Were you hospitalized for a lung condition? Tell me about that.

If the answer is **"yes"** to any question(s) that indicate previous treatment for TB, ask the following types of questions to obtain more information:

- Did your provider give you any written reports about your TB care or copies of radiographs (X-rays)?
- Where and when (what years) were you treated?
- What medicines did you receive? Do you have a written list of names?
- How many different medicines? How many pills each day? What sizes and colors were the pills/capsules?
- Did you receive injections?
- How many times were you treated for TB?
- How long were you on treatment? When did you start/stop? Why did you stop (completed treatment, bad reaction to the medicine)?
- It's hard to remember to take medicine every day. Did you miss medication sometimes? How often?
- Did you take medications daily? Every pill? Did healthcare workers watch you take your medications?
- TB medicine can be expensive. Were you ever without medication?
- Did your urine turn orange?
- Did you feel better? Did your TB symptoms return after finishing treatment?
- Did you ever have sputum collected for testing? What was the result?
- Did your doctor ever tell you: That you had to be treated for TB longer? That you had a return of TB? That you had TB that was drug resistant?

If patients were previously treated for TB in the United States (U.S.), records detailing their treatment should be obtained directly from the appropriate state and/or local jurisdictions. Information about **prior treatment outside the U.S. may be available** through CureTB (see ***Resources*** at the end of this chapter). If they were treated in Western/Northern Europe, Canada, or other high-resource country, records should be available and can be sought directly from the providers or through the appropriate national/regional programs. Immigration health records (e.g., class B1 records) may also be informative and should be obtained.

Many resource-limited countries, particularly those documented as among the highest TB burden countries, use standardized World Health Organization (WHO) TB regimens and diagnostic algorithms that may differ from recommended U.S.-based practices. See ***Resources*** at the end of this chapter for WHO reference documents and other websites that may clarify TB policies in selected countries.

If a person answers **"no"** to the questions that indicate previous treatment for TB, the following types of questions should be asked to evaluate if there was exposure to drug-resistant TB:

- Have you been exposed to or had contact with anyone with TB (or who may be sick with a chronic cough)? Has anyone in your family had TB?
- If yes, when was that? Where were you exposed? How long were you exposed?
- What is that person's name and age? Where were they treated? How long were they treated for? Were they cured?
- Did you have a skin or blood test for TB? Do you know the results?
- Did you have a chest X-ray? Do you know the results?
- Did you receive medications to prevent TB? If so, what drugs and for how long? Did you come to a clinic for the medications where a healthcare worker observed you take the pills, or did a healthcare worker meet you and provide medications?
- Did you have cough, fever, weight loss, or other symptoms?
- If yes, when did those symptoms start?
- Have you ever given sputum specimens to check for TB?

Whenever possible, obtain records regarding treatment of a presumed source case.

Testing for TB disease

- All persons in whom a **clinical suspicion for active pulmonary TB disease** exists should, at a minimum, have **their initial sputum specimen examined by NAAT**. In comparison to acid-fast bacilli (AFB) smear microscopy, NAATs are superior in sensitivity and specificity for the diagnosis of TB. If feasible, some programs and experts suggest 2 specimens for NAAT. When NAATs are not available, or not available in a timely fashion, smear microscopy remains the mainstay for initial TB diagnostic testing.
- AFB smear microscopy continues as a standard test used in TB evaluation, and grading of smears has been used to suggest degree of infectious risk and to track response to treatment. The 2017 American Thoracic Society/Infectious Disease Society of America/Centers for Disease Control and Prevention (ATS/IDSA/CDC) *Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children*, recommends 3 specimens each be collected at least 8 hours apart. (Note: WHO guidelines suggest 2 sputum specimens collected 1 hour apart may be sufficient.)
- Culture remains the current gold standard for the diagnosis of TB— it is the most sensitive test and enables comprehensive DST. All sputum specimens should be submitted for culture regardless of whether NAAT is ordered.
- All persons in whom a clinical **suspicion for active drug-resistant pulmonary TB disease** exists should have a **sputum specimen submitted for rapid molecular testing for drug resistance.** The most readily accessible tests check for either RIF-resistance (e.g., Xpert MTB/RIF) or RIF- and INH-resistance (line probe assays). These tests can be accessed through local, state or reference laboratories. If resistance is identified, more extensive molecular testing should be obtained (available through CDC and regional reference laboratories). For more information, see section: ***Testing for drug resistance,*** and **Chapter 3, *Laboratory***.
- Persons in whom **extrapulmonary disease** is suspected should also have specimens from the site of disease examined by AFB smear, culture, and histopathology (when applicable). Rapid molecular tests may be performed on some non-respiratory specimens in laboratories that have validated these assays. Specialized reference laboratories have the capacity to attempt molecular diagnosis through DNA extraction when formalin-fixed tissue is the only available specimen. For more information regarding molecular tests for extrapulmonary specimens, refer to **Chapter 3, *Laboratory***.
- If rapid molecular testing for drug resistance is available and suspicion for drug resistance is high, providers may choose to wait for rapid results before starting a regimen in stable situations with appropriate isolation precautions. For severely ill persons in whom suspicion of TB disease is high and timely rapid testing is not available, consider an empiric treatment regimen because culture with DST can often take more than 4 weeks before a result is obtained. For more information on choosing an appropriate empiric treatment regimen, refer to **Chapter 4, *Treatment***.
- Although tuberculin skin tests (TST) or interferon gamma release assays (IGRA) are sometimes performed as part of an evaluation for active TB disease, these tests are of limited value because **a negative TST/IGRA result does not rule out TB disease, and a positive result does not distinguish between TB infection and TB disease.**

Molecular assays for identification of *M. tuberculosis complex*

NAATs are molecular assays that have been available since 1995, but initially had limited uptake due to cost and availability. Fortunately, with improved access, molecular assays are having a significant impact on TB care. The ATS/IDSA/CDC 2017 diagnostic guidelines **promote the use of NAATs in all persons for whom a diagnosis of TB is being considered** to prevent delays in diagnosis and treatment initiation. The type of NAAT used will vary based on local availability, but most commonly it will be a polymerase chain reaction (PCR)-based method.

- NAATs have high specificity (i.e., few false positives) and high positive predictive value for identifying TB.
- NAATs are more sensitive (i.e., more likely to be positive in a person with TB) than sputum smear microscopy but a negative NAAT does not rule out active TB disease. If clinical suspicion for TB is high, NAAT testing should be repeated on a second specimen to increase sensitivity for diagnosing TB. When NAAT results are negative, decisions regarding empiric anti-TB treatment should be based on clinical suspicion of TB, risk of adverse outcomes, and public health considerations.
- A positive smear with a negative NAAT suggests presence of a nontuberculous mycobacterium (NTM).
- The Xpert MTB/RIF assay is a NAAT that was approved by the U.S. Food and Drug Administration (FDA) in 2013 and can rapidly identify the presence of *M. tuberculosis* complex. The assay also rapidly identifies mutations in the *rpoB* gene that confer RIF resistance. See section: ***Molecular assays for identification of drug resistance.***
    - The Xpert MTB/RIF assay can be performed on raw sputum specimens, requires minimal sample preparation, provides results within 2 hours and is highly accurate for detecting *M. tuberculosis* complex.
    - Note: **False-negative results** can occur, particularly in paucibacillary specimens (i.e., specimens with low bacterial load). **False-positive** results for identifying *M. tuberculosis* complex are less common but do occur particularly when someone has had a previous episode of TB disease (or very rarely with NTM). For more information, see **Chapter 3, *Laboratory***.
- WHO recommends the use of Xpert MTB/RIF (or other commercial rapid molecular tests currently not available in the U.S., see **Chapter 3, *Laboratory***) as the initial diagnostic test for **all persons** evaluated for pulmonary TB and for some forms of extrapulmonary TB.

Testing for drug resistance

Definitive diagnosis of drug-resistant TB requires that *M. tuberculosis* complex be identified and drug-susceptibility results completed and conveyed to the clinician. State and/or local TB control officials should be notified promptly when drug resistance is either strongly suspected or confirmed.

- If laboratory results suggest drug resistance, consult with experts at the local or state TB control programs or regional CDC TB Centers of Excellence for guidance.

Growth-based (conventional, phenotypic) DST

Different techniques of **growth-based DST** may be used and are outlined in more detail in **Chapter 3,** *Laboratory*. With all techniques, growth detection and identification of *M. tuberculosis* complex may take a few weeks, and growth-based DST requires an additional 1 to 3 weeks. Slow growth of some mycobacterial strains (a common characteristic noted in many multidrug-resistant [MDR]-TB strains) further lengthens the time to identification and DST. Delays in reporting of culture confirmation and/or drug-susceptibility results to the treating provider can further delay the diagnosis of drug-resistant TB and initiation of appropriate treatment, resulting in ongoing transmission risk.

- The interpretation of growth-based DST results for mycobacteria is somewhat different than that for most other pathogens. In the latter case, the clinician compares the **minimum inhibitory concentration (MIC)** of the pathogen with the achievable serum level. If a safe dose of the antibiotic will kill the bacteria, the drug can be successfully used. The interpretation of susceptibility testing for mycobacteria is not as straightforward; several variables complicate the process: 1) mycobacteria may reside within or outside of human cells; 2) mycobacteria have a long replication time and may exist in a continuum between dormant and active states; and 3) mycobacteria can live in a variety of tissue types for which drugs may have different penetration levels.
- The concentration of drug that constitutes the breakpoint between a resistant and susceptible strain is called the "critical concentration." **The critical concentration is the level of drug that inhibits a wild-type (a strain that has not been exposed to TB drugs) *M. tuberculosis* complex strain but does not appreciably suppress the growth of a resistant strain.** The critical concentration may be different depending on the medium used for the assay.
- **If, on solid media, more than 1% of the strain's population grows at the critical concentration of the drug for that particular medium, the isolate is considered to be resistant** to that drug and other drugs must be used in the regimen. Be aware that INH, streptomycin (SM), and fluoroquinolones may be tested at both low and high concentrations, and it may be possible to still consider use of drugs that display low-level resistance only.

More detailed discussions regarding critical concentrations, use of high- and low-level testing of selected drugs, and interpretation of MICs can be found in **Chapter 3, *Laboratory***.

Molecular assays for identification of drug resistance

Molecular assays for identification of drug resistance can hasten the time from weeks to hours to identify the presence of drug resistance once the sample is received in the laboratory. All molecular assays detect mutations in mycobacterial DNA that are known to cause resistance to a specific anti-TB drug. Because resistance mechanisms at the molecular level are not fully understood, current molecular assays cannot detect all drug resistance. Growth-based DST should be performed when isolates are available.

Commonly available molecular assays for identification of drug resistance include:

- **Xpert MTB/RIF assay:** In addition to detecting *M. tuberculosis* complex, Xpert MTB/RIF also detects mutations in the *rpoB* gene that confer RIF resistance and is currently the only non-sequencing molecular test for drug resistance approved by the FDA. The detection of RIF resistance is predictive of MDR-TB because RIF mono-resistance is relatively uncommon. Although accuracy is high, false-positive results for RIF resistance can occur. **Positive results that are unexpected for the clinical circumstance (e.g., positive result for RIF resistance in a person without risk factors for drug resistance) need closer review; consider consultation with experts.** Further consultation with the laboratory can help determine whether the result is likely to be false-positive; e.g., if cycle threshold values are high and end-point fluorescence values are low, or when RIF resistance is detected by multiple probes (rare occurrence due to NTM). In this situation, confirmation using a sequence-based test (see ***Sequencing-based assays***) should be done. For more details regarding test performance of the Xpert MTB/RIF assay, refer to **Chapter 3, *Laboratory***.
- **Line-probe assays (LPAs):** LPAs are currently performed primarily outside of the U.S. but are available at some U.S. reference laboratories for rapid identification of INH and RIF (Hain GenoType MTBDR*plus*) or fluoroquinolones, ethambutol (EMB), and injectable agents (Hain GenoType MTBDRsl).
- **Sequencing-based assays:** Molecular assays that use a sequencing technique (e.g., Sanger sequencing, pyrosequencing, targeted next-generation sequencing) have the advantage of reporting on the actual mutations, which can be useful in interpretation. These tests can have a lower sensitivity compared to Xpert MTB/RIF, particularly when used on smear-negative specimens. These are available through reference laboratories or through the CDC Molecular Detection of Drug Resistance (MDDR) service.
    - Targeted next generation sequencing is replacing pyrosequencing and Sanger sequencing in some reference laboratories, including CDC. This method has the ability to detect a wider range of mutations and heteroresistance. It has similar or slightly lower analytical sensitivity (i.e., more *M. tuberculosis* DNA is required for a valid result) than pyrosequencing and Sanger methods. For more details see **Chapter 3, *Laboratory***.
- Additional rapid molecular assays are available for use primarily outside of the U.S. (and some select U.S. reference laboratories) and are endorsed for use by WHO. See **Chapter 3, *Laboratory***.

When drug resistance has been identified by non-sequencing molecular assays (e.g., Xpert MTB/RIF or LPA) in low-incidence settings, results must be confirmed by a sequencing-based method because non-sequencing assays can report silent mutations as drug resistant. Sequencing-based assays also offer additional drug-resistance information on an expanded panel of drugs.

For more details regarding different types of molecular assays for drug resistance identification and the genes and mutations associated with drug resistance, see **Chapter 3, *Laboratory***.

When to use rapid molecular tests for drug resistance

Ideally, rapid molecular tests that identify both TB and resistance to RIF (e.g., Xpert MTB/RIF) should be used as the initial diagnostic test in all persons suspected of having TB. Anyone diagnosed with TB should also have rapid molecular tests performed to identify INH resistance (e.g., LPA or sequencing). In settings where rapid molecular tests are not routinely available, such tests should be requested for all persons with TB who are identified as at-risk for drug resistance (Table 1). Rapid identification of drug resistance is also indicated when earlier identification of resistance confers a significant medical or public health advantage or in specific situations where molecular methods may have an advantage over conventional laboratory testing. Request a sequencing-based assay when a NAAT (such as Xpert MTB/RIF) identifies resistance to first-line anti-TB drugs.

TABLE 1. **Priority situations for rapid molecular resistance testing**

Increased risk for drug resistance	• Persons born in or who have spent significant time (more than 1 year) in countries with at least a moderate TB incidence (≥20 per 100,000) or a high primary MDR-TB prevalence (≥2%) • Known contact to a drug-resistant TB case (or case with features highly suspicious for drug resistance) • Persons not responding to the current regimen • Persons who were treated previously
Increased consequences of drug resistance	• Persons with TB in a congregate or other setting with large numbers of contacts (e.g., correctional facilities, healthcare facilities, schools) • Persons in whom unidentified drug resistance may have significant consequences because of young age (less than 5 years old), immunocompromised status, or need for alternate regimen for drug-susceptible TB • Persons with TB with contacts that have risks for rapid progression of TB disease and in whom effective preventive "window" treatment is needed (such as young children less than 5 years old or immunocompromised persons)
Laboratory issues	• Cultures are mixed with other bacteria (growth-based DST would be delayed or not possible to perform at all) • AFB smear-positive but culture-negative (molecular tests can provide drug-susceptibility results despite lack of growth) • Pathology specimens not initially sent to mycobacteriology laboratory
Program priorities	• Universal MDR-TB screening via rapid molecular testing for all NAAT- or smear-positive TB may be available in some jurisdictions to identify drug-resistant TB cases earlier

- Although there are significant advantages offered by the addition of rapid molecular assays, growth-based susceptibility testing remains an integral diagnostic test to confirm molecular results and to investigate susceptibility to drugs for which molecular detection of resistance is incomplete or not yet possible.

Communication with the TB laboratory

If drug resistance is suspected, immediately discuss concerns with your TB laboratory.

- Timely and frequent communication with the laboratory is essential.
- Some laboratories do not perform DST unless a specific and/or separate order for DST has been submitted. Clarify the process if the laboratory's protocols are not well known to you.
- If the laboratory that provides mycobacterial culture services has limited capacity for DST, the provider should arrange for the isolate to be sent to a reference laboratory immediately. Consult with the laboratory about which second-line DSTs are performed and which reference laboratory is being used.
- If necessary, clinicians should contact their state or local TB programs or an expert in MDR-TB for assistance in identifying a qualified public health/reference laboratory.
- The CDC MDDR service offers molecular sequencing services for rapid detection of drug resistance, as do other reference laboratories. (See **Chapter 3, *Laboratory*, Table 3** for a list of reference laboratories and available testing). In some jurisdictions, molecular methods are also available locally.
- The clinician should know the name, telephone number, and contact person for each laboratory that will process and perform DST on isolates for persons with suspected drug resistance.
- The clinician should expect growth-based DST results within 1-3 weeks of TB identification on culture and should contact the laboratory if reports are not received in this time period. For a list of expected turnaround times for mycobacteriology laboratory services, see **Chapter 3, *Laboratory*, Table 1.**

When to order second-line drug testing

- **When resistance to RIF or INH is found, DST should be requested for the second-line agents.** CDC can provide DST for fluoroquinolones (levofloxacin and moxifloxacin), linezolid, clofazimine, bedaquiline, para-aminosalicyclic acid, rifabutin, ethionamide and injectable agents. Some state public health labs offer many of these same tests. Fluoroquinolone resistance testing should be obtained in INH mono-resistant cases. See **Chapter 3, *Laboratory*, Table 3** for a complete listing of reference laboratories for second-line molecular and phenotypic testing.
- **When co-morbidities or drug intolerance warrant a non-standardized regimen, drug testing should be done to confirm the isolate is sensitive to any second-line agents under consideration for use.**

False-positive results

When there is a question regarding laboratory results, it is important to discuss the situation with the laboratory.

False-positive results may be suspected when:

- The clinical manifestations do not seem to be compatible with the laboratory findings, particularly when only one culture is positive among several specimens collected
- Unusual drug resistance patterns are found in unrelated persons suggesting possible errors in inoculation or mislabeling of specimens

Cross-contamination can cause false-positive results for isolation of *M. tuberculosis* complex or detection of drug resistance. For example, with growth-based methods, cross-contamination could occur when more than one sample tube is open or a common vessel of reagents is used for all the specimens (both practices are no longer acceptable laboratory practice). Clinicians can ask the laboratory whether *M. tuberculosis* was cultured from a sample processed together with another person's sample that is smear-positive to assess this risk.

Other potential causes for false-positive results

Errors at the specimen collection site:

- Mislabeling of specimens or mistakes in entry of demographic data at the clinical site of collection
- Contamination of medical devices used for collecting specimens, such as inadequate cleaning of bronchoscopy tubing

Errors in the laboratory:

- Mislabeling of specimens or media:
  - when transferring a specimen from the original container to a centrifuge tube
  - when inoculating media with specimen
  - when working up a positive culture for identification or DST
- Malfunctioning biosafety cabinet
- Malfunction of laboratory test systems (e.g., releasing results when controls failed)
- Cross-contamination due to poor technique or using a common vessel to add reagents to all specimens
- Failure to check for contamination with non-AFB microorganisms
- Failure to check for mixed infection with NTM; if present, the NTM may be the source of the drug resistance pattern
- Data entry errors or errors in electronic reporting
- When MIC is close to the critical concentration

False-positive results for culture or growth-based DST are documented to occur in 1-3% of cases in the U.S.

Rapid molecular tests for drug resistance may report RIF-resistant results that are false-positive due to the presence of **silent mutations**. These are mutations identified within a gene associated with drug resistance that do not confer *in vitro* resistance. For example, rapid molecular testing with Xpert MTB/RIF may report RIF resistance for a specimen later found to be RIF sensitive by growth-based (phenotypic) DST. The next-generation Xpert MTB/RIF Ultra cartridge was re-designed in a manner that silent mutations are less likely to be detected, but is currently not available in the U.S. Nonetheless, further investigation using DNA sequencing can help to identify the responsible mutation as a silent mutation, and thus confirm that the molecular test result should be considered a false-positive. False-positive molecular results may also occur in persons with prior TB after completing treatment (e.g., residual DNA from "dead" bacilli). For more discussion on silent mutations and causes for discordant results, see **Chapter 3, *Laboratory***.

Investigating results of questionable validity

When investigating results of questionable validity, check all possible sources of errors.

- If possible, collect another specimen or test another isolate from the same person under evaluation.
- Repeat testing from the original sample (if still available).
- Repeat DST by using another method or another laboratory.
- Request genotyping to help identify false-positive culture results due to cross-contamination, such as when the strain under investigation matches the isolate from another case diagnosed in the same laboratory and there is no epidemiologic link between the two cases.
- Consult with laboratory experts. It may take a team effort, with candid communication between the healthcare provider and laboratory personnel, to find a solution.

While gathering additional information from the laboratory or waiting for repeat DST results, **decisions regarding the drug regimen should be based on individual and public health factors.** Consider empiric expansion of the drug regimen for individuals who have not responded well to standard therapy, or who have extensive disease or risk factors for poor outcomes. When the risk of transmission is high (e.g., residence in a congregate setting), empiric expansion of the drug regimen may be considered to reduce the risk of extended isolation if drug resistance is confirmed. On the other hand, when individual or public health risk is low, standard or current therapy can be continued.

Discordant results

Discordant test results can arise in a variety of situations: discordance between different laboratories, different molecular assays, or between molecular and growth-based assays. Although new methods are validated against the standard method, perfect agreement cannot always be achieved. Discrepancies in results due to differences in methodology, medium, and critical concentrations are inevitable.

Discordant growth-based DST results between different laboratories may occur if:

- Tests are not performed using the same specimen.
- Tests involve strains of *M. tuberculosis* complex that have MICs close to the critical concentration. Experience over time has shown that the reproducibility for testing of these strains can be suboptimal.
- Errors occur during growth-based DST. Examples include:
  - Failure to use a standardized, well homogenized inoculum
  - Failure to add a drug to the broth medium
  - Adding the wrong drug or concentration
  - Inoculation errors
- Failure to recognize a **mixed infection** (*M. tuberculosis* complex and an NTM) which is more difficult to detect in broth systems
- Failure to recognize **contamination with a non-AFB microorganism,** which is more difficult to recognize in broth systems
- Changes in the performance of growth-based DST or support of mycobacterial metabolism can occur when a new lot of culture media is made or received, and when a new lot of drug solutions is prepared, or a new drug kit is received. Laboratories should perform proper quality control to ensure that DST performs as expected.
- A subculture must be made for DST, and the microbiologist must take growth from various parts of a slant or a plate to assure that the organisms tested are diverse enough to be representative of the initial population.
- In the case of possible **emerging resistance,** testing different populations may result in different resistance patterns. If emerging resistance is suspected due to known risks for acquired resistance or inadequate regimen, a change in regimen may be indicated.

Discordant results may also arise when different methods for testing drug resistance are used. Early results from molecular tests for drug resistance may on occasion be discordant with results reported later from the growth-based DST, which is currently considered the gold standard.

- In a 2014 evaluation comparing results of isolates tested through the CDC MDDR service with matching results using growth-based DST from public health laboratories, overall concordance for resistance was 93.9% for RIF and 90% for INH.
- Current molecular methods for detecting INH resistance test primarily for *inhA* and *katG* mutations, which identify approximately 85% of resistant strains. Additional testing for less common mutations (e.g., *fabG1*, *ahpC*) is routinely

performed by the CDC MDDR service and some reference labs, but the mutations associated with resistance remain unknown in 10-15% of cases. Whole genome sequencing (WGS) is likely to improve INH molecular resistance detection, but clinical DST analysis using WGS is not yet widely available.

- For RIF resistance, the discordance between molecular and growth-based DST can be complex. Emerging evidence suggests that sequence-based testing may prove to be a better reference standard for determining resistance. The identification and implications of silent and borderline resistance (disputed) mutations that may be the source of test discordance are areas of ongoing investigation. For more details, see **Chapter 3, *Laboratory***.
- Some discordance may be attributed to **heteroresistance**, i.e., the presence of more than one population of *M. tuberculosis* (drug-resistant and drug-susceptible, or more than one drug-resistant strain) present within the same person. Laboratory methods differ in their ability to detect mixed populations.
  - Growth-based DST can detect small populations of drug-resistant organisms.
  - Targeted next-generation deep sequencing assays may be able to detect drug-resistant subpopulations that are below the threshold of growth on culture. This can occur most commonly when <1% of the bacillary population is drug resistant.
  - PCR-based assays, in general, have the least ability to detect small mixed populations.

Because the ramifications of RIF resistance or MDR are so significant, always have the resistance pattern confirmed by the public health or reference laboratory.

What to do if discordant test results are found:

- Assess whether results fit the clinical and epidemiological picture.
- Talk to the laboratory director and discuss reasons for conflicting results.
- Ask how the laboratory ruled out mixed infection with NTM or contamination with non-AFB microorganisms.
- If in doubt, your public health laboratory or a reference laboratory should repeat the test using the most recent isolate available.
- Discordance between rapid molecular tests and the growth-based DST results should be investigated further with a sequence-based method.
- When the clinical level of suspicion for resistance is strongly at odds with the initial rapid molecular test (Xpert MTB/RIF or LPA) results, confirmation using a sequence-based method is recommended.
- For more detailed discussions, see sections: ***Molecular tests for drug resistance*** and **Difficulties interpreting results from molecular tests** in **Chapter 3, *Laboratory***.

Use of strain typing / molecular surveillance

Molecular surveillance of *M. tuberculosis* complex through genotyping or WGS can be useful in:

- Detecting unrecognized outbreaks or confirming outbreaks under investigation
- Investigating or identifying false-positive results (e.g., laboratory cross-contamination)
- Distinguishing between relapse or reinfection (if a previous isolate was genotyped or is still available for genotyping)
- Documenting the progression of acquired drug-resistant TB versus reinfection with a drug-resistant strain
    - TB due to a specific strain may initially be susceptible to a panel of drugs, but with inappropriate or inadequate treatment, a subpopulation of drug-resistant TB organisms will flourish. In such instances, the resistant and susceptible populations are part of the same strain and therefore, have the same genotype. However, the drug-resistant bacteria will have acquired mutations that confer drug resistance. Reinfection with a resistant strain is likely to demonstrate a different genotype.

For more details see **Chapter 3, *Laboratory***.

SUMMARY

- People at highest risk of drug-resistant TB are those who:
  - Previously have been treated for TB
  - Resided in or traveled to regions/countries with high rates of drug resistance
  - Have been exposed to individuals with known or high risk for drug-resistant TB
  - Are not improving with or failing TB treatment
- All persons with TB should be assessed for risk of drug resistance.
- Ideally, rapid molecular tests that identify both TB and resistance to RIF (e.g., Xpert MTB/RIF) should be used as the initial diagnostic test in all persons suspected of having TB. Anyone diagnosed with TB should also have rapid molecular tests performed to identify INH resistance. In settings where rapid molecular tests are not routinely available, such tests should be requested for all persons with TB who are identified as at-risk for drug resistance
- If initial rapid tests identify RIF and/or INH resistance, verify results with a sequenced-based method and obtain a more extensive panel of rapid molecular DST.
- For rapid DST and optimal patient care, it is essential to communicate with the laboratory that drug resistance is suspected.
- Proper control of TB transmission requires timely performance of all required laboratory tests.
- Drug-resistant TB should be confirmed by a public health laboratory or reference laboratory.
- If a patient is suspected or confirmed to have MDR-TB, consultation with an expert in TB for further management and treatment is recommended.

Resources

BCG Atlas
Detailed information on current and past bacille Calmette-Guérin (BCG) vaccination policies and practices for over 180 countries.
www.bcgatlas.org

Centers for Disease Control and Prevention
False-Positive Investigation Toolkit: A Resource for Mycobacteriology Laboratories.
https://www.cdc.gov/tb/publications/guidestoolkits/false_positive/False-Positive.htm

CureTB: Binational TB Referral Program
Connects people with TB to healthcare services as they move between the U.S. and other countries, a collaboration between the County of San Diego's Tuberculosis Control Program and the CDC's Division of Global Migration and Quarantine (DGMQ).
https://www.sandiegocounty.gov/hhsa/programs/phs/cure_tb/

Geneva Foundation for Medical Education and Research (GFMER) Guidelines Clearinghouse, TB
https://www.gfmer.ch/Guidelines/Tuberculosis/Tuberculosis_mt.htm

Global TB Community Advisory Board: TB Guidelines from Countries and International Organizations
https://www.tbonline.info/guidelines/

The International Standards for Tuberculosis Care (ISTC); the Patients' Charter for Tuberculosis Care; ISTC Handbook; and **ISTC Training Materials** (includes translated materials)
https://www.currytbcenter.ucsf.edu/international-research#guidelines

Pill Identifier
https://www.drugs.com/pill_identification.html

World Health Organization Global Tuberculosis Programme, Country, Regional, and Global Profiles.
https://www.who.int/teams/global-tuberculosis-programme/data

All resources accessed November 30, 2022.

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