Diagnosis

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The first step in diagnosing drug-resistant TB is to recognize that the patient is at risk and to expedite the laboratory diagnosis of TB.

The diagnosis of tuberculosis (TB) frequently requires a high index of suspicion, especially in low-prevalence areas. Once TB is considered, sputum or other specimens for acid-fast bacilli (AFB) smear, growth detection, and susceptibility testing are collected. The possibility of drug-resistant TB should be considered simultaneously with specimen collection and selection of the initial treatment regimen. Failure to consider the possibility of drug-resistant TB until drug susceptibility tests return weeks to months later can result in unnecessarily inadequate drug regimens.

Risk Assessment for Drug Resistance

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

- Treat the patient with the most appropriate empiric regimen
- Minimize transmission
- Minimize potential drug side effects
- Provide the best chance of cure
- Prevent further drug resistance
- Offer appropriate care to contacts

Predicting who is at risk prior to the return of susceptibility test results is the first step in early detection of drug resistance.

The most important predictors of drug-resistant TB are:

- A previous episode of TB treatment
- Progressive clinical and/or radiographic findings while on TB therapy
- Origin from, history of residence in, or frequent travel to a region/country with high rates of drug resistance
- Exposure to an individual with infectious drug-resistant TB, including in facilities where drug resistance has occurred; e.g., correctional institutions, homeless shelters, or other congregate settings
Risk Factors in Persons with a History of TB

Suspicion for drug-resistant TB should be HIGH if the patient has 1 or more of the following characteristics on current or prior treatment:

- **Large bacillary load** with extensive (bilateral or cavitary) disease
- **Lack of conversion** of cultures to negative during therapy
- **Lack of improvement or only partial improvement** in TB symptoms
- **Worsening of TB symptoms** or radiograph findings
- **Nonadherence** or intermittent or erratic ingestion of prescribed anti-TB regimen
- **Lack of directly observed therapy (DOT)** or poorly supervised therapy
- **History of an inappropriate treatment regimen**, including:
  - Administration of single-drug therapy
  - Too few effective drugs
  - Inadequate drug doses

Risk Factors in Persons without Prior TB History

Clinical suspicion of drug resistance should occur when a patient with TB symptoms and signs has a history of 1 or more of the following:

- **Exposure** to a person with documented drug-resistant TB
- **Residence in or travel to a region** with high rates of drug-resistant TB
- Residence or work in an **institution** or setting in which drug-resistant TB is documented
- **Treatment of pulmonary problems with a prolonged course of multiple medicines or an injectable agent** for more than a few weeks in a foreign country; i.e., the patient may not realize that he/she was treated for TB
- **Treatment of a pulmonary problem with a fluoroquinolone**
- **Previous treatment for latent TB infection (LTBI)** when signs of TB disease were not recognized
Questions to Ask Your Patient

Soliciting history of previous TB treatment requires a great deal of patience and attention to detail. In a culturally sensitive and confidential setting, allow plenty of time, utilize an accurate and unbiased medical interpreter (if necessary), and be willing to repeat or rephrase a question to obtain the information. Give the patient encouragement to reveal accurate information by asking and responding in a nonjudgmental manner. Ask the patient if he/she has ANY written information regarding his/her treatment, any old radiographs, etc.

- Have you been told you had TB before?
- Have you been treated for TB?
- Have you received injections for a lung problem?
- Have you purchased and used medicated cough syrups in a foreign country?

If your patient answers “yes” to any question(s) that indicate he or she may have been previously treated for TB:

- Where were you treated?
- What drugs did you receive?
- How many different drugs? How many pills each day? What size and colors were the pills/capsules?
- Did you receive injections?
- How long were you on treatment?
- When did you start?
- When did you stop? Why did you stop (completed treatment, adverse reaction)?
- It’s hard to remember to take medicine everyday. Did you take medications daily? Every pill?
- TB medicine is expensive. Were you ever without medication?
- Did you miss medication sometimes? How often?
- Did healthcare workers observe you taking your medications?
- Did your urine turn orange?
- Did you feel better?
- Did you ever have a sputum examined? What was the result?
- If positive, did your subsequent sputa test negative?
- 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 drug resistance?
- Did your TB symptoms return after finishing treatment?

If the patient was previously treated for TB in the United States or Mexico, records detailing his/her treatment should be obtained from the local jurisdiction or CureTB (see Appendix 2, “Contact Information for Selected Organizations Working to Control and Prevent TB in the International Arena”). If the patient was treated in Western Europe or by a private provider in a developed country, records may be available and should be sought. Appendix 3, “International Resources for TB Treatment and Policies,” lists Websites that may be helpful in identifying TB policies in selected countries.
If your patient answers “no” to the questions that indicate he or she may have been previously treated for TB:

- Have you been exposed to or had contact with anyone with TB?
- If yes, when was that?
- What is that patient’s name and birthdate? Where was he/she treated? How long was he/she treated? Was he/she cured?
- Did you have a skin test? 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?

Obtain records when possible regarding treatment of a presumed source case.
Multidrug-resistant TB (MDR-TB) refers to an isolate which is resistant to at least isoniazid and rifampin. Extensively drug-resistant TB (XDR-TB) refers to an MDR-TB isolate which is also resistant to a fluoroquinolone and at least one of the following injectable drugs: amikacin, kanamycin or capreomycin.

Testing for TB Infection

Suspicion or evaluation of TB sometimes begins with the use of the tuberculin skin test (TST) or an interferon gamma release assay (IGRA) such as QuantiFERON®-TB Gold (QFT-G) or T-SPOT.TB. Neither the IGRA nor the TST have 100% sensitivity nor specificity for diagnosis of TB infection, and their ability to detect TB disease may be even less. Studies suggest that among newly diagnosed, culture-positive TB cases, the TST and QFT-G may be positive in as few as 80% and 70% of cases respectively. A negative TST or IGRA test does not rule out TB disease.

Laboratory Diagnosis

The role of the laboratory is critical in the diagnosis of TB, and even more so for drug-resistant TB. Definitive diagnosis of drug-resistant TB requires that *M. tuberculosis* be isolated and drug susceptibility results be completed and conveyed to the clinician. Prompt turnaround time for laboratory results is of paramount importance in rapid diagnosis and appropriate treatment of drug-resistant TB, including multidrug-resistant TB and extensively drug-resistant TB.

The optimal laboratory diagnosis of TB begins with a close relationship and open dialogue between the healthcare provider, TB control, and the TB laboratory. The laboratory’s results are essential for TB patient management, infection control, and public health. This dual charge, serving patient care and public health, must be viewed within the context of the social, political, economic, scientific, and technical changes that are occurring in industrialized countries. All the stakeholders should form a synergistic network, making the whole of the virtual organization more effective than the sum of its parts. The laboratory diagnosis of TB begins with the requisition form. The healthcare provider, TB control, and TB laboratory need to design forms that serve all parties involved, benefitting patient care and control of tuberculosis. The following information is needed in order to optimize scarce resources in the current healthcare environment and to optimize the laboratory’s contribution:

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

The laboratory is charged with informing its partners of the conditions for optimum testing, such as sample volume requirements, transit conditions, and test limitations. While public health laboratories can fine-tune their operations with careful communication with local TB control programs, this may pose a challenge for commercial laboratories serving the entire country.
Laboratory Turnaround Times

Growth detection and identification of *M. tuberculosis* complex may take a few weeks. Drug susceptibility testing of a TB isolate requires an additional **1 to 3 weeks**. Slow growth of some mycobacterial strains (a common characteristic noted in many MDR-TB strains) further lengthens the time to identification and susceptibility testing. Delays in the return of reports of culture confirmation and susceptibility results will delay the diagnosis of drug-resistant TB and initiation of appropriate treatment.

Fortunately, newer laboratory strategies and technologies are impacting TB care. One of the *Healthy People 2010* goals for TB care is to reduce the average time for a laboratory to confirm and report TB cases from 21 days in 1996 to 2 days for 75% of cases. To overcome the poor sensitivity and specificity of smear microscopy, the use of nucleic acid amplification in facilities such as the Orange County Public Health Laboratory in California has demonstrated the ability to nearly reach this goal (75% of cases detected within 4 days). Novel technologies, such as line probe assays and molecular beacon assays, have been studied or implemented, enabling rapid screening for MDR-TB by direct testing of sputum sediment without waiting for growth from cultures.

**To ensure rapid diagnosis** of TB and drug-resistant TB, the following turnaround times—set by national standards—should be achieved by laboratories:

- Clinical specimens should reach the laboratory **within 24 hours of collection**
- AFB smear reports should reach physicians **within 24 hours of specimen receipt** in the laboratory
- Positive culture results should be reported **within 14 days of specimen collection**
- Isolate should be definitively identified as *M. tuberculosis* complex **within 17 to 21 days of specimen collection**
- Antibiotic susceptibility results should be reported to the physician **within 28 days of specimen collection**

Because drug susceptibility results are essential for prescribing appropriate regimens for treating drug-resistant TB, **second-line susceptibility tests should be requested as soon as drug resistance is suspected or identified**. Consult with the laboratory about which second-line susceptibility tests (if any) are performed and which reference laboratory it is using. See Appendix 4, “Laboratory Resources,” for tests performed by some of the public health and reference laboratories. 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, if necessary. In some jurisdictions, molecular methods are available to rapidly detect some drug resistance.

If drug resistance is strongly suspected based on the patient’s prior treatment history or exposure to drug-resistant disease, concerns should be discussed immediately with the laboratory director.

Molecular susceptibility testing or conventional direct susceptibilities can sometimes be performed upon request, which may hasten the results. (See Appendix 4, “Laboratory Resources,” and Appendix 5, “Direct Method.”) Second-line susceptibility tests should be ordered even before the first-line results have been returned in these circumstances. The laboratory should notify the clinician of preliminary results as soon as it is confident of the validity and not wait for final confirmation.
• When drug resistance to rifampin (RIF) or more than one first-line drug (isoniazid [INH], RIF, pyrazinamide [PZA], ethambutol [EMB] or streptomycin [SM]) is found, susceptibility tests should be requested for the full spectrum of second-line agents. Amikacin or kanamycin, capreomycin, a fluoroquinolone (ofloxacin, levofloxacin or moxifloxacin), and ethionamide are the minimum second-line drugs. Fewer laboratories perform testing against cycloserine, para-aminosalicylate (PAS), rifabutin, and other agents, but these too may be required.

• Timely and frequent communication with the laboratory is essential. If the laboratory that cultured the isolate has limited capacity for susceptibility testing, the provider should arrange to send the isolate to a reference laboratory immediately.

• The clinician should know the name, telephone number, and contact person for each laboratory that will process and perform drug susceptibility testing on isolates for patients with suspected drug resistance.

False-Positive Results

False-positive results for isolation of M. tuberculosis complex or detection of drug resistance may occur. When there is a question regarding laboratory results, it is important to discuss the situation with the laboratory.

• **When would a clinician suspect a false-positive result?**
  - When the patient’s clinical manifestations do not seem to be compatible with the laboratory finding
  - When only 1 culture is positive among several specimens collected or there are discrepant results among different cultures from the same patient

• **When would the laboratory suspect a false-positive result?**
  - When a culture is late to turn positive (at 5 or 6 weeks), especially if there is close proximity to another strongly positive culture (suggesting possible cross-contamination)
  - When unusual drug resistance patterns are found in unrelated patients suggesting possible misinoculation or mislabeling of specimens

Possible causes of discrepant results, pseudo-outbreaks and misdiagnosis of drug-resistant TB include:

• **Errors may occur at the specimen collection site:**
  - Mislabelling of specimens at the clinic, ward, or bronchoscopy suite
  - Contamination of medical devices used for collecting specimens, such as inadequate sterilization of bronchoscopy tubing

• **Errors may occur in the laboratory:**
  - Mislabelling 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 drug susceptibility testing
  - Malfunctioning biosafety cabinet
  - Malfunction of laboratory test systems
  - Cross-contamination due to poor technique or using a common vessel to add reagents among specimens.
• Failure to check for contamination with bacteria
• Failure to check for mixed infection with nontuberculous mycobacteria (NTM)
• Result-entry errors

• When investigating discrepant results, check all possible sources of errors.
  • If possible, test another isolate from the same patient.
  • Repeat testing on original sample (if still available).
  • Repeat susceptibility testing by using another method or another laboratory.
  • Consult with experts. It may take a team effort, with candid communication between the healthcare provider and laboratory personnel, to find a solution.

Susceptibility Testing
Susceptibility Interpretations

The interpretation of susceptibility testing 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 in the patient, 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 be either within or outside of human cells; 2) mycobacteria have a long generation time and may exist in a dormant or active state; and 3) mycobacteria can live in a variety of tissue types for which drugs may have different penetration levels.

In interpretation of M. tuberculosis complex susceptibility results, clinical trials have ascertained that when more than 1% of organisms within a population are mutants resistant to a given drug, clinical success with that drug is less likely. The concentration 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 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.

The agar proportion method using Middlebrook 7H10 agar was used for the early clinical efficacy trials. In the United States, this method is used as the standard by which to compare all newer susceptibility methods. Each method sets the critical concentration for each drug based on M. tuberculosis growth compared to growth on 7H10 agar. (See Appendix 6, “Critical Concentrations.”)

If more than 1% of the strain’s population grows at the critical concentration of the drug for that particular medium, consider the isolate to be resistant to that drug and plan on using other drugs. (Be aware that INH could be tested at both low and high level and it may be possible to still use INH in the event of low-level INH resistance.)
Susceptibility Methods

Susceptibility testing of mycobacteria utilizes the same solid media, broths, and inoculation methods as culture techniques. The systems are supplemented with anti-tuberculosis drugs. Growth of the organisms in the presence of anti-tuberculosis drugs is compared to controls in order to interpret susceptibility or resistance. (For examples and details about susceptibility testing and each of the following methods, see Appendices 5 to 11.)

**Agar proportion method:** The clinical specimen (direct method) or a subculture of mycobacterial growth (indirect method) is used to inoculate agar plates containing either an anti-TB drug or no drug (control). The growth of colonies on the drug-containing quadrant is compared to the control quadrant as a proportion (percent resistance). This process typically takes at least 3 to 4 weeks. (See Appendix 8, “Proportion Method.”)

**Direct method:** The clinical specimen (usually AFB smear-positive sputum) is processed and then inoculated directly onto agar plates containing various anti-TB drugs. (See Appendix 5, “Direct Method.”)

**Indirect method:** After the *M. tuberculosis* grows from a clinical specimen, a suspension is prepared and inoculated onto drug-containing agar plates or into broth bottles or tubes. (See Appendix 9, “Indirect Method.”)

**Broth methods:** A cell suspension of *M. tuberculosis* is inoculated into vials or tubes of broth containing either the critical concentration of an anti-TB drug or no drug (control). The growth of the organism in the drug-containing medium is compared to the growth in the control. Broth methods are preferred for first-line testing as they are much faster (typically 5 to 10 days) than the proportion method using agar media.

**BACTEC 460 TB method:** *M. tuberculosis* is grown in bottles of broth containing $^{14}$CO$_2$-labeled substrate. The BACTEC 460 TB system is well standardized and very reliable; however, it is a radioactive test system and requires the use of needles/syringes and is not fully automated. (See Appendix 10, “BACTEC 460 TB Method.”)

**Newer broth methods:** Other broth systems have been developed to detect mycobacterial growth in a fully automated system. In addition, these systems can be used for drug susceptibility testing. These include the following:

- **BACTEC MGIT 960** is a nonradiometric antimicrobial susceptibility system for testing *M. tuberculosis* complex from broth culture. It has been validated to provide results for SM, INH, RIF, EMB (SIRE) and PZA in a time frame close to the BACTEC 460 TB system. MGIT 960 has been Food and Drug Administration (FDA) approved.

- **VersaTREK** is an automated method that was first developed for blood cultures and later adapted for the recovery and drug susceptibility of mycobacteria. It has been validated for performing qualitative susceptibility testing with INH, RIF, EMB and PZA with *M. tuberculosis* complex isolates.

- **MB/BacT ALERT 3D** is a nonradiometric antimicrobial susceptibility system for testing *M. tuberculosis* complex isolates. It was developed to provide susceptibility results for SM, INH, RIF, and EMB, but recently, critical concentrations of the drugs listed were modified and a new acidified vial for standardized PZA testing was introduced. However, no antimycobacterial drugs have been cleared for susceptibility testing with this system by the FDA.
**Molecular methods:** DNA is extracted from the bacteria and amplified. Certain mutations associated with drug resistance can be detected. (See Appendix 7, “Molecular Methods.”)

### Variation in Results

Discrepancies in test results can occur between different laboratories. Reasons include:

- Although new methods are validated against the standard method, perfect agreement cannot be achieved. Discrepancies in results due to differences in methodology, medium, and critical concentrations are inevitable.
- Some strains of *M. tuberculosis* complex have MICs that are close to the critical concentration. Long experience has shown that the reproducibility for testing of these strains can be poor.
- The different laboratories may not have actually used the same specimen.
- Errors can occur during drug-susceptibility testing:
  - **Failure to use a standardized inoculum** (well-dispersed suspension)
  - **Failure to add a drug to the 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 another organism**, which is more difficult to recognize in broth systems
- Changes in drug activity or support of mycobacterial metabolism can occur between different lots of culture media. Ideally, laboratories should check new batches of medium ingredients to verify that the medium they produce has the same drug activity as previous, validated lots of medium.
- If a subculture is tested, it may not represent the entire initial population.

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

- Scrutinize results and assess whether they fit the clinical and epidemiological picture.
- Talk to the laboratorian and discuss reasons for conflicting results.
  - **Ask how the laboratory ruled out mixed infection with NTM**
  - **Ask how the laboratory ruled out any contamination with non-AFB organisms**
  - **If in doubt, your public health laboratory should repeat the test using the most recent isolate available**
Use of Strain Typing

Molecular genotyping of *M. tuberculosis* complex can be useful in:

- Detecting unrecognized outbreaks or confirming outbreaks under investigation
- Investigating or identifying possible false-positive culture results
- Distinguishing between relapse or reinfection (if a previous isolate is still available for genotyping)
- Documenting the amplification of initial monoresistance to MDR-TB versus reinfection with a more resistant strain

Isolates with matching strain types can have different drug susceptibility patterns. This is because TB due to a specific strain may initially be susceptible to a panel of drugs, but with inappropriate or inadequate treatment, the population of resistant organisms will flourish. **The genotype does not change because drug resistance has developed.**

Overview of the CDC Tuberculosis Genotyping Program

Two public health genotyping laboratories, one in Michigan and one in California, are under contract with CDC to provide genotyping services to TB programs in the United States. TB programs, through their state public health laboratories, may submit one isolate from each culture-positive TB patient to a genotyping laboratory.

The genotyping laboratories will use three genotyping methods:

- **Spoligotyping**
- Mycobacterial interspersed repetitive units (MIRU) analysis
- **IS6110**-based restriction fragment length polymorphism (RFLP) analysis

Spoligotyping and MIRU analysis are polymerase chain reaction (PCR)-based genotyping methods. The genotyping laboratories will analyze all the submitted isolates by both PCR-based genotyping tests. Under certain circumstances and upon the request of the TB program, isolates that have matching genotypes by both spoligotyping and MIRU analysis can be further typed by RFLP. The genotyping services are free to TB programs, but neither CDC nor the genotyping laboratories will pay the packaging and shipping costs.

The objectives of universal TB genotyping are:

1. To determine the extent and dynamics of ongoing transmission in order to focus program interventions in specific areas and populations
2. To assess TB transmission in outbreaks and to refine contact investigations
3. To identify nosocomial transmission not identified by conventional methods
4. To investigate possible false-positive culture results so that clinicians can be notified of diagnostic errors quickly, allowing for termination of unnecessary TB treatment

Therefore, it is particularly important that all drug-resistant TB isolates are genotyped.
Summary

- Patients at highest risk of drug-resistant TB are those who:
  - Previously have been treated for TB
  - Came from or traveled to regions/countries with high rates of drug resistance
  - Have been exposed to individuals with drug-resistant TB
  - Are failing TB treatment

- Each TB patient should be assessed for risk of drug resistance.

- The laboratory is crucial in the diagnosis and management of drug-resistant TB.

- Communication to the laboratory that drug resistance is suspected is essential for rapid susceptibility testing and optimal patient care.

- Drug-resistant TB should be confirmed by a public health laboratory or experienced reference laboratory.

- Proper control of TB transmission requires timely performance of all laboratory testing and close communication between the clinician and the laboratory.

- All drug-resistant TB isolates should be genotyped.
References


