

Mycobacteria Identification and Susceptibility Testing

Disease Overview

- More than 100 mycobacteria species have been identified; some may cause disease in the immunocompromised host, while others are saprophytes.
- *Mycobacterium tuberculosis* is the causative agent of tuberculosis (TB), a global disease. 9.4 million new TB cases occurred worldwide in 2009.
- Disseminated *M. avium intracellulare* is one of the opportunistic infections seen in acquired immune deficiency syndrome (AIDS). In individuals with AIDS, the organism has been cultured from lung, brain, cerebrospinal fluid, liver, spleen, intestinal mucosa, and bone marrow.

Specimen Collection

- Collection of early morning sputum specimens on three consecutive days is optimal. Specimens should be collected in a sterile, leak-proof container, labeled appropriately, and transported refrigerated to the laboratory as soon after collection as possible. Blood specimens should be collected in SPS tubes and transported at room temperature. Swabs are not acceptable for AFB culture. Each specimen should be placed in its own sealed bag. This decreases the risk of cross-contamination of patient specimens and employee exposure in case of leakage. Specimens may be rejected or may require a disclaimer if multiple specimens are transported in a single bag and leakage occurs. Source information is critical for appropriate processing protocol used in the laboratory and should always be provided.
- Isolates submitted for mycobacterial identification and/or susceptibility testing must be treated as infectious
- substances for shipping, as described in the ARUP Laboratory Test Directory. Isolates must be submitted in tubed media with a screw cap and placed inside an individually sealed plastic bag. Agar plates and mixed cultures are unacceptable and will be rejected. Please indicate source of isolate.
- Volumes for selected specimen types necessary to optimize patient results are shown in the table below. If less than the minimum acceptable volume is received, the specimen will be rejected due to insufficient quantity. Swabs are not appropriate and will be rejected.

Specimen Type	Quantity		Comments
	Optimal	Minimum	
Respiratory specimens (sputum, BAL, BW, tracheal aspirate, etc)	5-10 mL	1 mL	Three specimens on three separate days; early morning collection is preferred. When the volume received is below optimal, a short volume disclaimer will be added to the smear report.

Specimen Type	Quantity		Comments
Gastric lavage	5-10 mL	1 mL	Must be neutralized with 100 mg sodium carbonate prior to shipping. The pH of the submission should be between 6 and 8. pH values below this range may cause a false negative result of culture.
Blood/bone marrow	5 mL	1 mL	Collect 5 mL in a BACTEC™ Myco/F Lytic tube. AFB smears are not performed on blood. For bone marrow, collect as much volume as possible in an SPS tube. AFB stains are not performed on bloody specimens.
CSF	5-15 mL	1 mL	Stain is not performed if volume received is less than 5 mL.
Abscess (wound) aspirates	5-15 mL	1 mL	Source is required.
Body fluids/aspirates	5-15 mL	1 mL	Source is required.
Urine	30-50 mL	10 mL	Stain is not performed if volume received is less than 40 mL.
Stool		1 gm	Stain is not performed on stool specimens
Tissue	Visible	N/A	Source is required.

Clinical Significance and Methodology

1. Direct detection of *M. tuberculosis* complex by nucleic acid amplification (NAA)
 - Direct detection of *M. tuberculosis* complex (MTB) using NAA techniques allows for rapid diagnosis of TB. ARUP uses the GEN-PROBE® AMPLIFIED™ Mycobacterium Tuberculosis Direct Test (MTD), which is the only commercial NAA test cleared by the FDA for testing both smear-positive and smear-negative respiratory specimens. Although ARUP accepts both processed and unprocessed respiratory specimens, **unprocessed specimens are preferred** for two reasons:
 - Processed specimens must be tested by MTD within 72 hours, which can be problematic if transport delays occur.
 - The phosphate buffer used for resuspension of processed sediments can affect amplification. A phosphate buffer above 67 mM (e.g., Alpha-Tec Systems, Inc. NAC-PAC™ XPR-plus™ A. F. B. Buffer) and the concentration of NaOH may interfere with amplification of the MTD test, causing false-negative results.

- **The processing status of all samples must be provided.** There is no clinical relevance in continuing testing with AMTD after a TB diagnosis is made, as NAA does not provide information indicating successful therapy. In most patients, NAA will remain positive for an extended period after the initiation of therapy.
- Paraffin block specimens are unacceptable for MTD.
- The MTD is also used to test CSF and pleural fluid specimens. These sources have been validated at ARUP Laboratories. Bloody specimens should not be tested with MTD, since blood may produce non-specific fluorescence that leads to equivocal or false-positive results. Repeat testing is not performed on bloody specimens with equivocal results.
- The Centers for Disease Control and Prevention suggests the following strategies when using amplification tests for direct detection of MTB in respiratory specimens:¹
 - At least one specimen, preferably the first diagnostic specimen, should be tested by NAA. NAA test results should be interpreted in correlation with AFB smear results.
 - If NAA result is positive and AFB smear result is positive, presume patient has TB and begin anti-TB treatment while awaiting culture results. The positive predictive value of FDA-approved NAA tests for TB is >95 percent in AFB smear-positive cases.
 - If NAA is positive and AFB smear result is negative, use clinical judgment to determine whether to begin anti-TB treatment while awaiting culture results. Consider testing additional samples using NAA. Pending culture results, a patient can be presumed to have TB if two or more specimens are NAA- positive.
 - If NAA result is negative and AFB smear result is positive, the presence of inhibitors will be ruled out. If inhibitors are detected, the NAA test is of no diagnostic help for this specimen.
 - A patient can be presumed to have an infection with non-tuberculous mycobacteria (NTM) if a second specimen is smear-positive and NAA-negative, as well as negative for inhibitors.
 - If NAA result is negative and AFB smear result is negative, use clinical judgment to determine whether to begin anti-TB treatment while awaiting culture results. Currently available NAA tests are not sufficiently sensitive (detecting 50–80 percent of AFB smear-negative, culture-positive pulmonary TB cases) to exclude the diagnosis of TB in AFB smear-negative patients suspected to have TB.

II. Mycobacterial acid-fast stain

- All specimens requested for culture receive an AFB stain, except as noted in the table above. Stains are performed on processed, concentrated material. An acid-fast stain, as a stand-alone test without a culture, is appropriate only when following respiratory specimens on a previously diagnosed patient. Client-submitted slides of unprocessed specimens are unacceptable for AFB stain.

III. Mycobacterial culture

- All respiratory or potentially contaminated source specimens are digested/decontaminated and concentrated by standard NALC-NaOH processes. As is recommended by the CDC, mycobacterial culture at ARUP includes both liquid (e.g., MGIT, Becton Dickinson, Sparks, MD) and solid media. Specimens from wounds, abscesses, and other cutaneous lesions are set up at both 35°C and 30°C.
- If *M. haemophilum* is the suspected pathogen, please notify the ARUP Mycobacteriology Laboratory, as this organism requires hematin for growth. The laboratory should also be notified when the presence of *M. genavense* is suspected, as this organism will not grow on media routinely used for the isolation of *Mycobacteria* spp. *M. leprae* is unculturable in the laboratory. PCR testing is available in select reference laboratories for the diagnosis of *M. leprae* (contact the CDC for additional information). When aerobic actinomycetes (e.g., *Nocardia* and *Streptomyces* species) are isolated from AFB culture, they will be identified and reported.

IV. Mycobacterial culture with reflex to amplified detection

- This testing option is available when an AMTD test is wanted for all acid-fast stain-positive respiratory specimens or if required by state regulations (e.g., New York). For patients with multiple smear-positive specimens, the MTD will be repeated until MTD is positive or an organism has been identified from culture. Repeat testing with MTD has no clinical relevance once a TB diagnosis has been made. Therefore, repeat cultures with reflex to NAA should not be ordered after the patient is positive for MTD testing.

V. Mycobacterial identification

- Organisms submitted for identification must be submitted in pure culture and shipped according to infectious substance shipping regulations. When the submitted organism is determined to be a “mixed culture,” ARUP will contact the client for guidance. At that point, the client has the following options:
 - Submit a new isolate in pure culture.
 - Request to cancel the test (a processing fee will be charged).
 - Request that ARUP attempt to isolate the submitted organisms at an additional charge (AFBISO, ARUP test code 0060725).
- At ARUP, the MTB AccuProbe (Gen-Probe, Inc.) is used for the identification of *M. tuberculosis* and *M. avium* complex. When the probes are negative, 16S rRNA gene sequencing is performed. If the referring laboratory has already tested any AccuProbe, please include the results on the requisition to avoid duplicate testing.
- Some mycobacteria species cannot be differentiated from one another using 16S rRNA gene sequencing. These organisms will preliminarily be reported as a “slash call” and will be further evaluated based on growth rate, pigment production, and/or additional PCR assays.
- *M. chelonae/abscessus* complex is further identified to the species level using a PCR assay. Identification to the species level is required for *M. chelonae/abscessus* when susceptibility testing is requested. Speciation will be performed on the first identified isolate and referred on all other isolates within 90 days of collection.
- *M. tuberculosis* complex isolates can be identified to the species level. This is not performed routinely when *M. tuberculosis* complex is submitted for susceptibility testing.

VI. Susceptibility testing for *M. tuberculosis* complex (MTBC)

- Susceptibility testing for the primary anti-tuberculosis agents is performed for the first MTBC isolate identified at ARUP per patient, regardless of request.
- For MTBC, the CLSI Subcommittee on Antimycobacterial Susceptibility Testing recommends initial testing of the primary anti-tuberculous agents: isoniazid (INH), rifampin, ethambutol, and pyrazinamide (PZA). Streptomycin is now considered a secondary agent. Susceptibility testing of the secondary anti-tuberculosis agents (including streptomycin) should be performed if an isolate of *M. tuberculosis* is resistant to rifampin or any two primary agents. Clients will be notified of any resistant results.
- At ARUP, the Mycobacteria Growth Indicator Tube (MGIT) method is used for susceptibility testing. Susceptibility results are expressed as concentrations rather than minimum inhibitory concentrations (MICs): isoniazid: 0.1 µg/mL; rifampin: 1.0 µg/mL; ethambutol: 5.0 µg/mL; and pyrazinamide: 100 µg/mL. Streptomycin is tested as a primary agent for New York clients only.
- All resistant results are confirmed by repeat testing. When drug resistance is first detected, an initial report is issued to indicate that resistance findings are preliminary and confirmatory testing is underway.
- Susceptibility will be performed on the first identified isolate and referred on all other isolates, including those from other sources, within 90 days of collection.
- Susceptibility for the secondary anti-tuberculous agents is automatically referred to the National Jewish Medical and Research Center in Denver, Colorado whenever an isolate is confirmed to be resistant to rifampin or any two of the other

primary drugs. Isolates with monoresistance to isoniazid should also be tested for susceptibility to secondary agents if the physician is planning to use a fluoroquinolone in the treatment regimen. This testing must be specifically requested.

- The following second-line agents are tested at the stated concentrations:
 - Amikacin: 6 µg/mL
 - Capreomycin: 10 µg/mL
 - Cycloserine: 60 µg/mL
 - Ethionamide: 10 µg/mL
 - Kanamycin: 6 µg/mL
 - PAS: 8 µg/mL
 - Streptomycin: 1 and 4 µg/mL.
- MIC is performed for levofloxacin and moxifloxacin (2, 4, and 8 µg/mL). Additional agents can be tested by specific request.

VII. Susceptibility testing for non-tuberculosis *Mycobacterium* species

- Susceptibility testing, when specifically requested, will be performed on:
 - Isolates from significant sources (e.g., blood, CSF, tissues, and other normally sterile body fluids).
 - The following species are tested: *M. avium-intracellulare* complex, *M. kansasii*, *M. marinum*, *M. chelonae-abscessus* complex, *M. fortuitum* complex, and *M. immunogenum*.
 - Complete identification of the organism is required to perform susceptibility testing. When identification is not provided, ARUP will proceed with species identification at an additional charge.
- Susceptibility testing for other species and/or other sources will not be automatically performed. Direct communication between the requesting physician and the laboratory is required for the laboratory to perform susceptibility testing for isolates and sources not listed above.
 - The Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) susceptibility testing of mycobacteria (M24-A2) was approved in March 2011. Testing and results interpretation are based on the information published in that document.
 - For *M. avium* and *M. intracellulare*, which make up the *M. avium* complex (MAC), the only antimicrobial agents for which a correlation between in vitro susceptibility results and clinical response has been demonstrated in controlled clinical trials are the macrolides (clarithromycin and azithromycin). CLSI guidelines state that clarithromycin should be the only drug tested and reported routinely. Clarithromycin results predict azithromycin. In vitro MIC results for the rifamycins and ethambutol have shown poor correlation with clinical response; therefore, results for these drugs are not reported. For macrolide-resistant MAC isolates or for patients intolerant to macrolide therapy, secondary agents such as the fluoroquinolones, linezolid, and amikacin may be considered. ARUP routinely reports clarithromycin, linezolid, ciprofloxacin, moxifloxacin, and amikacin. Interpretive breakpoints exist for clarithromycin only. Tentative breakpoints have been proposed for linezolid and moxifloxacin. All other reporting requests must be directly communicated to the laboratory.
 - Testing of *M. kansasii* is similar to that of MAC. CLSI guidelines state that isolates of *M. kansasii* should initially be tested against rifampin only. Rifampin-susceptible isolates

should respond to standard therapy: rifampin, ethambutol, and isoniazid. In HIV-infected patients being treated with protease inhibitors, rifabutin is used in place of rifampin. *M. kansasii* isolates susceptible to rifampin are also susceptible to rifabutin. Rifampin-resistant *M. kansasii* are tested against the following secondary agents: amikacin, ciprofloxacin, clarithromycin, ethambutol, linezolid, moxifloxacin, rifabutin, streptomycin, and trimethoprim/sulfamethoxazole. Currently, there are no CLSI interpretive criteria for moxifloxacin and only the MIC is reported. INH will be reported by request only.

- *M. marinum* has a predictable susceptibility pattern. Routine susceptibility testing is not necessary. Clarithromycin, doxycycline or minocycline, rifampin, and trimethoprim-sulfamethoxazole have been used successfully. Susceptibility testing of *M. marinum* should be considered for patients who fail several months of therapy and remain culture-positive. The following agents are reported for *M. marinum*: amikacin, clarithromycin, doxycycline, ethambutol, rifampin, and trimethoprim-sulfamethoxazole. Interpretation is based on proposed CLSI guidelines.
- Slowly growing non-tuberculous mycobacteria (other than those listed above) are tested against rifampin and the secondary drugs recommended for testing *M. kansasii* as indicated by CLSI.
- Rapidly growing mycobacteria are tested against amikacin (not for *M. chelonae*), ciprofloxacin, clarithromycin, ceftazidime, doxycycline, imipenem (tentative break points reported), linezolid, minocycline (no interpretation), moxifloxacin, trimethoprim-sulfamethoxazole, tobramycin (reported for *M. chelonae* only), and tigecycline (reported by request only, no interpretation). The final susceptibility result for clarithromycin is read at 14 days to ensure detection of inducible resistance unless resistance (MIC ≥ 8 µg/mL) is recognized earlier, at which time the result will be finalized.

References

1. Center for Disease Control. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *MMWR* 2009;58(1):7–10.
2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twentieth information supplement. <http://www.clsi.org/source/orders/free/m100-s20.pdf> (accessed on July 14, 2010).
3. Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes; approved standard. <http://www.clsi.org/source/orders/free/m24-aa.pdf> (accessed on July 15, 2010).
4. Cloud JL, et al. Interpretative criteria for use of AccuProbe for identification of *Mycobacterium avium* complex directly from 7H9 broth cultures. *J Clin Microbiol* 2005;43:3474–8.
5. Murray PR, et al. 2007. *Manual of clinical microbiology*, 9th ed. Washington DC: ASM Press.
6. Clinical and Laboratory Standards Institute. Criteria for microorganism identification by DNA target sequencing; approved guideline. <http://www.clsi.org/source/orders/free/MM18-a.pdf> (accessed on July 15, 2010).

Test Information

- 0060095** **Mycobacterium tuberculosis Amplified Direct Detection (MC MTD)**
- 0060063** **Mycobacterium tuberculosis Amplified Direct Detection, CSF (MC MTDCSF)**
- 0060152** **AFB Culture (includes AFB stain 0060151)**
- 0060738** **AFB Culture (includes AFB stain 0060151) with Reflex to Mycobacterium tuberculosis Amplified Direct Detection (0060095)**
- 0060060** **Blood Culture, AFB**
- 0060024** **Blood Culture, AFB & Fungal**
- 0060999** **AFB Identification**
- 0060997** **AFB Identification with Reflex to Susceptibility**
- 0060725** **AFB Identification from Mixed Culture (by specific request only)**
- 0060217** **Antimicrobial Susceptibility—AFB/Mycobacteria**
- 0060347** **Antimicrobial Susceptibility—AFB/Mycobacterium tuberculosis Primary Panel**
- 0060348** **Antimicrobial Susceptibility—AFB/Mycobacterium tuberculosis Secondary Panel**
- 0060328** **Mycobacterium chelonae-abscessus Id by PCR**
- 0060771** **Mycobacterium tuberculosis Complex Speciation**

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.