### Collection / Transport

#### Collection Requirements:

- Indicate specimen source: BAL, CSF, Sputum, Sputum (induced), Stool, Urine, Tissue (specify), Processed Specimen (specify) or Other Other Body Fluid (specify).
- Indicate if patient is a Cystic Fibrosis patient (will require different decontamination), or if sample is environmental (contact laboratory before collection), or veterinary (animal type).

#### Specimen Preparation:

- **Sputum:** >5 mL of unprocessed (raw) sputum. Collect the first early morning expectoration in a sterile 50 mL polypropylene centrifuge tube capable of withstanding 3,000 x g (i.e., Falcon, Greiner, etc).
- **Body Fluids or Cerebrospinal Fluid (CSF):** As much body fluid as possible is aseptically collected by aspiration or during a surgical procedure. CSF should be maximum volume obtained, ideally at least 2 mL.
- **Gastric Aspirates:** Must be neutralized (pH 7) with sodium carbonate if transport time exceeds four hours from collection. Transfer 5-10 mL to a sterile container.
- **Tissue:** =10 g or as much as possible with a biopsy. Transfer to a sterile container (without formalin or preservatives).
- **Urine:** = 40 mL to a sterile container.
- **Stool:** = 1 g in sterile, wax-free disposable container.
- **Blood:** =5 mL adult; =1 mL child. If blood has to be transported before inoculation of the medium, sodium polyanethol sulfate (SPS), heparin, or citrate may be used as anticoagulants.

Ship the specimen on the day of collection via airmail or by overnight courier.

#### Patient Preparation:

- Patients who are suspected of having pulmonary TB based on clinical evaluation and who have received no antituberculosis therapy, <7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated with NAAT.
- **Sputum:** Three sputum specimens at 8-24 hour intervals and at least one first-morning specimen. Have patient rinse mouth with water before collecting sputum to minimize contaminating specimen with food particles, mouthwash, or oral drugs, which may inhibit the growth of mycobacteria.
- **Induced sputum:** Use sterile hypertonic saline. Avoid sputum contamination with nebulizer reservoir water which may contain saprophytic mycobacteria from tap water. Specify Sputum (Induced) on requisition form, since it may resemble saliva which is not an acceptable specimen.
- **Bronchoalveolar Lavage (BAL):** Avoid contaminating bronchoscope with tap water.
- **Cerebrospinal Fluid (CSF):** Use maximum volume attainable, ideally at least 2 mL.
- **Gastric lavage:** Aspiration of swallowed sputum from the stomach by gastric lavage may be necessary for infants, young children, and the obtunded. Fasting, early-morning specimens are recommended in order to obtain sputum swallowed during sleep. Samples of 5 to 10 mL, adjusted to neutral pH, should be collected on 3 consecutive days.
- **Stool:** Collect specimen directly into container, or transfer from bedpan or plastic wrap stretched over toilet bowl.
- **Urine:** Collect first morning specimen on 3 consecutive days to provide the best yield. Accept only one specimen/day.

#### Unacceptable Conditions:

Swabs are not recommended for the isolation of mycobacteria, since they provide limited material. They are acceptable only if
a specimen cannot be collected by other means. Negative results obtained from swab specimens are unreliable.

Samples contained in waxed containers are not acceptable. Waxed containers may produce false-positive smear results.

Tissue specimens submitted in formalin are not acceptable.

Gastric aspirates that have not been neutralized are not acceptable.

Frozen sputum or stool specimen is not acceptable.

24-hour pooled urine or sputum specimen is not acceptable.

Urine from catheter bag is not acceptable.

<40 mL of urine, unless larger volume cannot be obtained, is not acceptable.

Blood collected in EDTA, ACD, or in conventional blood culture bottles and coagulated blood are not acceptable.

Specimen which leaked in transit is not acceptable.

**Storage Transport Temp:**

If specimen cannot be mailed on the day of collection, keep the specimen refrigerated until the day of shipment. Specimen can be shipped at room temperature or cold, using a cool-pack.

**Notes:**

AFB smear and culture isolation from sputum or other clinical specimens. AFB smear performed using fluorescence microscopy. No smear is performed on blood, bone marrow, stool and urine specimens.

Culture performed using 1 liquid (MGIT) and 3 solid (LJ and 7H11 bi-plate) media.

Nucleic Acid Amplification Test (NAAT; AFB2) is performed on the first specimen or by request for subsequent specimens. The Centers for Disease Control and Prevention (CDC) recommends that NAAT should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.

If AFB smear and NAAT are positive, MTB1 (direct 10-drug agar proportion antimicrobial susceptibility testing (AST) and MTB4 (molecular MDR-TB screen) will be performed. In Fall 2012, the Association of State and Territorial Health Officials (ASTHO) recognized the use of a universal laboratory screening test for multidrug-resistant tuberculosis (MDR-TB). Reflex testing is beneficial, as it allows the most rapid detection of a MDR-TB patient.

AFB identification (AFB4) is performed on culture-positive specimens.

If colony morphology on solid media resembles Mycobacterium tuberculosis (MTB) complex or if no previous clinical history indicating nontuberculosis infection available on a liquid culture, a line probe assay to detect M. tuberculosis will be performed. If positive, first-line drug antimicrobial susceptibility testing (AST; MTB2) will be performed on all initial isolates. The laboratory will provide at no charge a subculture to the respective state laboratory for genotyping upon request. All other mycobacteria are identified using line probe assay or DNA sequencing (i.e., rpoB gene and 16S rRNA gene sequencing).

Notify laboratory when the presence of M. genavense, M. haemophilum and other fastidious mycobacteria are suspected, as these organisms will not grow on media routinely used for mycobacterium growth detection.

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**Overview**

**Performed:**

Mon-Sat; Sunday upon request.

**Methodology:**

AFB Smear, growth detection (culture), and molecular detection

**Reported:**
AFB smear reported within 24 hours upon receipt.
NAAT reported within 24 hours upon receipt or called-in request order.
Culture isolation reported in two to eight weeks, depending on growth.
Culture identification dependent on testing methods.

Lab Department:
Mycobacteriology Lab (TB)

Synonyms:
1a & 2a

Related Tests:
AFB2, AFB3, AFB4, AFB5, MTB1, MTB2, MTB3, MTB4, MTB5, MTB6, NTM1, NTM2, NTM3, NTM4, NTM5

Result Interpretation

Interpretive Data:
Potential results for AFB Fluorochrome smear (40X lens):

- **NOAFB-** No AFB seen
- **TO4-** 1 to 4 AFB/Field
- **TO10-** 5 to 10 AFB/Field
- **TO20-** 11 to 20 AFB/Field
- **20MORE-** Greater than 20 AFB/Field

Nucleic Acid Amplification Test (NAAT) is performed on first specimen or by request for subsequent specimens; result: (MTB complex rRNA detected or No MTB complex rRNA detected).

**AFB Culture:** 4 media are inoculated for growth detection (1 broth, 1 Lowenstein-Jensen slant and a 7H11 biplate). Negative cultures are reported after 6 weeks of incubation; LJ slants are incubated for 8 weeks. In the event of growth detection beyond 6 weeks, an amended report will be issued.

**Results:** Culture negative for AFB or Identification performed on AFB culture positive specimens.
Antimicrobial susceptibility testing (AST) is performed on nontuberculous mycobacterium (NTM) isolates by request only.

Per Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases (2007), M. gordonae is frequently encountered in the environment and in clinical laboratories but is almost always considered nonpathogenic; therefore, AST for M. gordonae is performed by specific request only.

CPT Codes

**CPT Code:**
87015, 87116, 87206, 87556
CPT codes for identification vary based on methods (see AFB4). CPT codes for AST vary based on drug panel performed (see MTB1–NTM5).
Test Name: Acid-fast Bacilli (AFB) Smear & Culture (clinical specimen only) NAAT on first specimen or by request
Test Code: AFB1