

## Acid-Fast Bacilli (AFB), Smear and Culture w/ Reflex to Isolate Identification

### Use

To identify the presence of acid-fast bacilli (AFB) in clinical specimens, aid in the diagnosis of infections caused by *Mycobacterium* species, determine the presence or absence of *Mycobacterium tuberculosis* complex (MTBC) versus nontuberculous mycobacteria, and evaluate MTBC for drug resistance when indicated.

Per the [Oklahoma Administrative Code, Title 310 Chapter 515-1-4](#), AFB-positive smear results should be “...transmitted to the OSDH within one (1) working day (Monday through Friday, state holidays excepted) of diagnosis or positive test” if (1) “...no additional testing is performed ...” or (2) “... subsequent testing is indicative of *Mycobacterium tuberculosis* Complex”.

### Clinical Significance

Diagnosing, monitoring, and characterizing tuberculosis (TB) specimens in a public health laboratory are essential for effective disease control and prevention. Accurate and timely laboratory diagnosis supports appropriate clinical management and initiation of treatment, reducing morbidity and limiting transmission within the community. Ongoing monitoring through culture, drug susceptibility testing, and molecular characterization allows public health programs to detect drug resistance, assess treatment effectiveness, and identify potential transmission networks or outbreaks. Together, these laboratory functions provide critical data for surveillance, guide public health interventions, and strengthen efforts to control and ultimately eliminate TB. Unfortunately, reported cases of TB have increased in recent years in the United States (up by 7.9% in 2024 over 2023) due to a combination of factors, including disruptions in diagnosis, treatment, and screening, increased migration and travel, growth in higher risk groups (e.g., immunocompromised, homeless, incarcerated, drug-users), and incomplete treatment of latent TB.

Additional information about TB can be found at the OSDH Infectious Disease [website](#).

### Methodology

Specimens from non-sterile sites undergo chemical decontamination to reduce non-acid-fast competing flora. Decontaminated specimens, along with specimens from sterile sites, are concentrated, prepared on slides, and stained using the fluorochrome stain Auramine rhodamine. Slides are examined by fluorescence microscopy and number of bacilli counted per field of view appropriate for the degree of positivity (e.g., the higher the smear positivity, the fewer the fields evaluated). Results are reported using a semi-quantitative 0 to 4+ scale based on the average number of AFB per high-dry (x400 magnification) field.

AFB smear-positive respiratory specimens from newly diagnosed patients are automatically reflexed to MTBC/rifampin (RIF) real-time PCR testing. This assay detects the presence of MTBC DNA and, when detected, screens for genetic mutations associated with rifampin resistance. Specimens that are MTBC/RIF-positive are automatically reflexed to comprehensive molecular detection of drug resistance (MDDR) testing at the Centers for Disease Control and Prevention (CDC), which identifies mutations associated with resistance to first-line, additional second-line, and newer or repurposed anti-tuberculosis drugs.

Each specimen is also cultured for six (6) weeks to eight (8) weeks. Cultures demonstrating growth are evaluated by fluorochrome staining to confirm the presence of AFB and exclude contaminating flora. Cultures in which contaminating organisms are identified are reported as indeterminate. AFB-positive cultures are automatically reflexed for mycobacterial isolate identification, with MTBC isolates reflexed to drug resistance testing. Refer to [Acid-Fast Bacilli \(AFB\), Isolate Identification with Reflex to Mycobacterium tuberculosis Complex Drug Resistance Testing](#) for additional details.

## Specimen Type

- Wound and abscess fluids/tissues, sterile body fluids, respiratory, stool, tissues

## Minimum Volume/Size

- Abscess fluid: 5 mL minimum; > 5 mL preferred (aspirate preferred over drainage)
- Sterile body fluids: 5 mL minimum; 5-10 mL preferred, especially for CSF
- Bronchoalveolar lavage (BAL): 5 mL minimum; 10-20 mL preferred
- Bronchial Wash: 5 mL minimum; 10-20 mL preferred
- Stool: 5 mL minimum; 5-10 mL preferred
- Tissue biopsy: 0.5 cm<sup>3</sup> minimum; ≥ 1.0 cm<sup>3</sup> preferred (fresh tissue only)
- Fine needle aspirate (FNA): ≥ 1.0 mL minimum; ≥ 5 mL preferred (submit aspirate, not needle)

**Note:** When specimen volume is limited, testing will be prioritized for mycobacterial culture, followed by molecular testing and AFB smear as volume allows.

## Collection Instructions

- **General**
  - Use aseptic technique for all specimen collections.
  - Swabs are unacceptable for AFB testing.
  - Collect specimens prior to initiation of antimycobacterial therapy whenever possible.
  - Submit specimens in sterile, dry, leak-proof containers (e.g., 50 mL conical tube) with no fixatives or preservatives.
  - Clearly label containers with two unique patient identifiers, collection date, and the specimen type (e.g., Abscess fluid; Sterile fluid; BAL) and indicate the anatomic source.
- **Closed Wounds and Abscesses**
  - Skin preparation: Thoroughly disinfect overlying skin using sterile saline or 70% ethanol and standard antiseptic technique.
  - Collection:
    - Fluid: Using a sterile needle and syringe, aspirate purulent material directly from the abscess cavity. Collect as much specimen as feasible (minimum 5-10 mL fluid; larger volumes improve sensitivity). Transfer aspirated fluid into a sterile, dry, leak-proof container. Do not add saline or transport media, as dilution may reduce organism recovery. Clearly label the specimen source as "Abscess fluid" and indicate the anatomical site.
    - Tissue biopsy (if performed): Place tissue directly into a sterile, dry, leak-proof container with 1-5 mL saline. Clearly label the specimen source as "Abscess biopsy" and indicate the anatomical site.
- **Open Wounds and Abscesses**
  - Site preparation: Gently remove surface debris or exudate.
  - Collection:
    - Fluid: Using a sterile needle and syringe, aspirate purulent material from the deepest portion of the abscess. Collect as much specimen as feasible (minimum 5-10 mL fluid; larger volumes improve sensitivity) in a sterile, dry, leak-proof container. Clearly label the specimen source as "Abscess fluid" and indicate the anatomical site.

**Note:** Do not collect superficial drainage due to poor diagnostic yield and avoid swab collection.
    - Tissue: Alternatively, collect tissue from the abscess wall or base during debridement. Submit fluid or tissue in a sterile, dry, leak-proof container with 1-5 mL saline. Clearly label the specimen source as "Abscess tissue" and indicate the anatomical site.
- **Body Fluids** (sterile) e.g., cerebral spinal fluid (CSF), pericardial fluid, peritoneal fluid, pleural fluid, synovial fluid, etc.
  - Aseptically collect 5-10 mL of fluid in a sterile, dry, leak-proof container, such as a 50 mL conical tube

container. Clearly label the specimen source as “Sterile fluid” and indicate the anatomical site. Do not add saline or transport media, as dilution may reduce organism recovery. Do not submit a swab.

- **Respiratory**, e.g., bronchoalveolar lavage (BAL), bronchial brush, bronchial wash, sputum (expectorated or induced)
  - BAL or Wash: Collect 5–10 mL (larger volumes preferred) into a sterile, dry, leak-proof container, such as a 50 mL conical tube.  
**Note:** *Ensure specimens represent lower respiratory tract secretions, not upper airway contamination.*
  - Brushings: Place brush and brushings in a sterile, dry, leak-proof container, such as a 50 mL conical tube, with 5-10 mL of sterile saline.
  - Sputum: Collect 5–10 mL of thick, mucoid or purulent sputum (saliva is unacceptable) into a sterile sputum trap (expectorated) or dry, leak-proof container, such as a 50 mL conical tube.  
**Note:** *It is recommended that sputum specimens collected for the determination of pulmonary TB be submitted in a series of 3 specimens. Each specimen should be collected on one of three consecutive days with at least one of them collected immediately after the patient wakes up. The first AFB smear will be positive in 85.8% of patients with active TB, the second smear will be positive for an additional 11.9% of active TB patients, and the final smear will be positive for the remaining 2.3% of active TB patients.*
- **Stool**
  - Collect 5–10 mL or a quarter-sized amount of stool in a sterile, dry, leak-proof container, such as a 50 mL conical tube.
- **Tissue**
  - Aseptically collect tissue in a sterile, dry, leak-proof container, such as a 50 mL conical tube. Add 1–5 mL sterile saline to tissue for transport.

#### Common Causes for Rejection

- Quantity not sufficient for testing
- Swab or superficial drainage
- Specimen in formalin or fixatives
- Frozen specimen
- Specimen leaked in transit
- Specimen received outside of stability window (> 6 days)

#### Shipping and Storage

Store and ship refrigerated (2-8°C) for delivery as soon as possible following collection. Ensure cap of container is tightly secured, then wrap cap with Parafilm or other barrier film to reduce risk of leaking during transport. Place each specimen in an individually sealed biohazard polybag with sufficient absorbent material to contain any leaks. Place documentation for the sample in the outside pocket of the biohazard bag to avoid contamination.

#### Turn-around Time

- AFB Smear: within 3 working days from specimen receipt
- MTBC PCR: within 4 working days from specimen receipt
- AFB Culture: within 45 working days from specimen receipt
- MDDR Testing: as made available by reference laboratory; may delay availability of final results

#### Reference Range

- AFB Smear:
  - Negative for AFB
- MTBC/RIF PCR:
  - *Mycobacterium tuberculosis* complex Not Detected

- Rifampin resistance Not Detected
- AFB Culture
  - Culture Negative

### Reportable Results

AFB Smear, AFB Culture, MTBC/RIF PCR, and MDDR are reported to the submitter.

- AFB Smear:
  - No AFB present in smear
  - 1+ AFB found: 4-40 per 100 high power fields
  - 2+ AFB found: 4-40 per 10 high power fields
  - 3+ AFB found: 4-40 per high power field
  - 4+ AFB found: > 40 per high power field
- MTBC/RIF PCR:
  - *Mycobacterium tuberculosis* complex Detected or Not Detected
  - Rifampin resistance Detected or Not Detected
  - Indeterminate
- AFB Culture:
  - Culture Positive
  - Culture Negative
  - Culture Indeterminate
- MDDR Testing:
 

See CDC [Molecular Detection of Drug Resistance \(MDDR\) in Mycobacterium tuberculosis Complex by DNA Sequencing User Guide | Tuberculosis \(TB\) | CDC](#)

### Interpretation

Results of this test should be interpreted in conjunction with other laboratory and clinical findings and epidemiological information.

- **AFB Smear:** AFB smear microscopy detects acid-fast organisms in clinical specimens but does not differentiate MTBC from nontuberculous mycobacteria (NTM).
  - **AFB Not Detected (Negative):** No AFB observed after examination of the required number of microscopic fields and scan of slide. A negative smear does not exclude mycobacterial infection, particularly in patients with low organism burden.
  - **AFB Detected (Positive):** AFB observed and reported using a semi-quantitative grading scale based on the average number of organisms per field (e.g., 1+, 2+, 3+, 4+). Higher grade smear positivity correlates with higher organism load and increased likelihood of transmission in respiratory specimens.
- **MTBC/RIF PCR:** Detection of MTBC supports a diagnosis of tuberculosis but does not distinguish viable from nonviable organisms. Culture remains necessary for definitive diagnosis, full drug susceptibility testing, and epidemiologic typing.
  - **MTBC Detected; RIF Resistance Not Detected:** MTBC DNA is present. No mutations associated with rifampin resistance were identified. This result suggests infection with rifampin-susceptible MTBC; however, phenotypic susceptibility testing is required for confirmation and to assess resistance to other drugs.
  - **MTBC Detected; RIF Resistance Detected:** MTBC DNA is present, and mutations associated with rifampin resistance were identified. Rifampin resistance is a strong predictor of multidrug-resistant tuberculosis (MDR-TB). This result warrants prompt clinical and public health action and reflex testing for comprehensive drug resistance.

- **MTBC Detected; RIF Resistance Indeterminate:** MTBC DNA is detected, but rifampin resistance could not be reliably determined (e.g., low bacterial load or assay inhibition). Additional testing by culture-based or alternative molecular methods is recommended.
- **MTBC Not Detected:** MTBC DNA was not detected. This result does not exclude tuberculosis, particularly in specimens with low organism burden or in patients receiving antimycobacterial therapy.
- **Invalid / Inconclusive:** The assay did not yield a valid result due to inhibition, inadequate specimen quality, or technical failure. Repeat testing with a new specimen is recommended.
- **AFB Culture:** AFB culture is the gold standard for the detection of mycobacteria, allowing recovery of viable organisms for identification and drug susceptibility testing.
  - **No Growth (Negative):** No AFB isolated after completion of the incubation period. A negative culture does not exclude mycobacterial infection, particularly in specimens with low organism burden, extrapulmonary disease, or patients receiving antimycobacterial therapy.
  - **Positive for Acid-Fast Bacilli:** Growth consistent with acid-fast organisms detected. Culture confirmation establishes viable organism presence and enables species identification and molecular and phenotypic drug susceptibility testing. Further testing is required to identify the organism to species level (e.g., MTBC vs. NTM) and to determine drug susceptibility as indicated.
    - **Positive for *Mycobacterium tuberculosis* Complex (MTBC):** MTBC isolated and confirmed. This result is diagnostic of tuberculosis and triggers public health reporting and drug resistance testing per policy.
    - **Positive for Nontuberculous Mycobacteria (NTM):** NTM isolated and identified. Clinical significance varies by species and specimen source and should be interpreted in conjunction with clinical and radiographic findings.
  - **Contaminated / Indeterminate:** Culture overgrown with non-acid-fast organisms or otherwise uninterpretable, preventing reliable detection of mycobacteria. Repeat specimen collection is recommended.

### Limitations/Interferences

- **AFB Smear:**
  - Although slow-growing mycobacteria like *M. tuberculosis* are consistently acid-fast, rapidly growing mycobacteria may be variably acid-fast or even negative for acid-fastness especially with the fluorochrome stain. Negative results do not preclude the presence of *Mycobacteria* spp. or other clinically relevant organisms. AFB smear microscopy requires ~5,000 to 10,000 bacilli/mL for detection in sputum; whereas culture can detect ~10 to 100 viable mycobacteria per mL. Specimens with low bacillary burden may yield false-negative results, especially in early disease, extrapulmonary disease, or patients receiving antimycobacterial therapy. Poor specimen collection (e.g., salivary sputum, superficial swabs) may result in false-negative findings.
  - Positive results demonstrate the specimen is positive for AFB only and do not indicate TB infection. The AFB smear cannot distinguish MTBC from NTM or other acid-fast organisms (e.g., *Nocardia*, *Rhodococcus*, *Legionella micdadei*, cysts of *Cryptosporidium* species, and *Isospora* spp.), which may cause false positive results. Definitive diagnosis and species identification require culture and/or molecular testing. Smear microscopy detects both viable and nonviable AFB and does not indicate organism viability or infectiousness.
- **MTBC/RIF PCR:**
  - Positive MTBC results only indicate the presence of MTBC DNA and not viable organisms; PCR detects DNA from both viable and nonviable organisms and results may remain positive after initiation of therapy.
  - Negative MTBC results do not exclude the possibility of MTBC infection or infection by other

mycobacterial species. False-negative results may occur in specimens with low bacillary burden, extrapulmonary disease, or suboptimal specimen collection.

- Positive rifampin resistance results infer resistance to rifampin; however, variable resistance may occur depending on the mutation and breakthrough may occur at higher doses for some mutations. Although rifampin resistance is highly associated with MDR-TB, resistance to other first- or second-line drugs must be confirmed by additional molecular or phenotypic testing.
- Negative rifampin resistance results do not preclude the presence of rifampin resistant organisms. The assay detects only known mutations within targeted regions associated with rifampin resistance; rare or novel resistance mechanisms may not be detected. Blood, mucus, residual decontamination reagents, or other substances may inhibit amplification and lead to invalid or false-negative results. Low-level resistant subpopulations may not be detected, potentially resulting in false-susceptible RIF results.
- AFB Culture:
  - Time to detection varies by species and bacterial load, specimen collection methods, patient factors including the presence of symptoms and prior treatment, and the method of specimen processing.
  - False negative or indeterminate results may occur due to poor specimen collection or processing, antimycobacterial treatment of the patient, and antimicrobials used in the growth medium to control overgrowth by non-mycobacterial species.
  - Some mycobacteria have fastidious growth requirements and may not grow under standard culture conditions.
  - Non-mycobacterial species may overgrow mycobacteria present.
  - Isolation of NTM from skin or wounds may represent colonization or environmental contamination rather than true infection and must be interpreted in clinical context.
  - The consistency of microscopic morphology of AFB in culture medium has not been established.
  - The acid-fastness of mycobacteria may vary depending on strain, age of culture, and other variables.
  - Presence of multiple mycobacterial species may complicate isolation and identification. Faster growing mycobacteria may develop positive fluorescence prior to slower mycobacteria.
  - Inoculation of culture tubes with more than 0.5 mL of sample can increase contamination or otherwise adversely affect culture results.
  - Recovery of mycobacteria from MGIT tubes has only been established for: respiratory specimens, gastric aspirates, tissue, stool, and sterile body fluids except blood.
- MDDR:
  - Results are reported for the sample as received.
  - Samples with low numbers of MTBC may not amplify.
  - Heteroresistance may not be detected.
  - The results of MDDR should not be used to rule out the presence of MTBC in a sample.

### **CPT Code**

CPT codes will vary depending on organism identified and methods used.

### **Notes**

The AFB smear and AFB culture are laboratory-developed tests. The Cepheid Xpert® MTBC/RIF assay is approved for *in vitro* diagnostic use by the U.S. Food and Drug Administration.