

Acid-Fast Bacilli (AFB), Isolate Identification with Reflex to *Mycobacterium tuberculosis* Complex Drug Resistance Testing

Use

Confirmation and identification of *Mycobacterium* spp. isolates.

Per the [Oklahoma Administrative Code, Title 310 Chapter 515-1-8](#), “*pure isolates of Mycobacterium tuberculosis shall be sent to the OSDH Public Health Laboratory for additional characterization, typing or confirmation within two (2) working days (Monday through Friday, state holidays excepted) of final identification or diagnosis*”.

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

Mycobacterium isolates are identified to genus/species level using matrix-assisted laser desorption/ionization, time of flight, mass spectroscopy (MALDI-ToF MS). Isolates identified as MTBC are then subject to phenotypic drug susceptibility testing (DST), genotypic DST, and whole genome sequencing in compliance with [CDC TB Genotyping Information Management System \(GIMS\)](#) requirements. MTBC cultures that test resistant to first-line (rifampin, isoniazid, PZA, and ethambutol) phenotypic DST may be subject to second-line phenotypic DST.

Specimen Type

- Pure viable isolate on species-appropriate solid media (e.g., Lowenstein-Jensen (LJ), 7H10, or 7H11) with visible growth
- Pure viable isolate in species-appropriate liquid media (e.g., Middlebrook 7H9 (MGIT) broth) with visible growth

Specimen source and suspected organism information is required.

Minimum Volume/Size

- 1 solid media plate or slant
- 1 mL liquid media

Collection Instructions

Note: DST and sequencing are only performed on patient samples with a **new MTBC diagnosis** or on previously diagnosed patient samples **collected > 12 months since last DST/sequencing testing** (i.e., once per patient per 12 months). Samples submitted before 12 months have lapsed, will only be subject to culture and identification.

- Per OAC 310:515-1-8 ([What to Report \(oklahoma.gov\)](http://www.oklahoma.gov)), specimens must be submitted within two (2) working days (Monday through Friday, State holidays excepted) of final identification or diagnosis.

Common Causes for Rejection

- No growth
- Non-viable
- Frozen
- Leaked in transit

Shipping and Storage

Store and ship at ambient temperatures (18-30°C) for delivery within 2 days of subculture. Seal liquid media containers using Parafilm or other barrier film to reduce the risk of leaking during transport. Place each specimen in an individually sealable biohazard polybag.

Turn-around Time

Test results are reported within 15 working days from receipt. Second-line phenotypic DST may delay availability of final results.

Reference Range

- Mycobacterial Identification
 - Genus/Species
- MTBC Phenotypic DST
 - Susceptible
- MTBC Genotypic DST
 - No mutation associated with resistance detected

Reportable Results

Sequencing results are used for surveillance purposes only and are not reported to the submitter.

- Mycobacterial Identification:
 - Genus/species identified
 - Nontuberculosis mycobacteria (if unable to speciate)
- MTBC Phenotypic DST
 - Susceptible
 - Resistant
- MTBC Genotypic DST
 - Mutations associated with resistance detected
 - The detected mutation(s) have uncertain significance; resistance cannot be ruled out
 - No mutation associated with resistance detected

Interpretation

MALDI-ToF-MS identifies the genus/species of mycobacteria associated with the infection. Phenotypic DST determines whether MTBC isolates are susceptible or resistant to anti-tuberculosis drugs based on growth in the presence of antimicrobial agents. Detection of resistance-associated genetic mutations predicts resistance of MTBC isolates to the corresponding drug; however, absence of detected mutations does not exclude resistance due to uncommon or unknown mechanisms. Genotypic DST results should always be correlated with phenotypic DST results and vice versa. Discordance between phenotypic and genotypic DST can occur. All laboratory results should be interpreted in conjunction with clinical findings, particularly when managing suspected or confirmed drug-resistant tuberculosis.

Limitations/Interferences

- General
 - Submission of scant growth or culture on media other than LJ or MGIT broth may delay testing and the availability of results.
 - Results may be delayed or unattainable if a mixed culture is submitted for testing.
- Mycobacterial Culture/Identification:
 - MALDI-ToF-MS is only able to identify those mycobacteria in its microbial reference library.
 - Organisms will not be identified if there is no reference pattern in the reference library for that test organism.
- MTBC Phenotypic DST:
 - Phenotypic DST is only performed on MTBC organisms.
 - Phenotypic DST requires viable organism growth and may take weeks for slow-growing organisms such as *M. tuberculosis*, delaying treatment decisions.
 - Results are based on fixed drug concentrations that may not fully reflect in vivo drug exposure, pharmacokinetics, or achievable tissue levels.
 - Minor resistant subpopulations may not be detected if they are present below the assay's limit of detection, potentially leading to false-susceptible results.
 - For some agents, particularly second-line or newer anti-tuberculosis drugs, phenotypic results may not correlate well with clinical response or known resistance mechanisms.
 - Previous or ongoing antimicrobial therapy can suppress growth or select for resistant subpopulations, complicating interpretation.
 - Results may be delayed or unattainable if a mixed culture is submitted for testing.
- MTBC Genotypic DST:
 - Genotypic DST is only performed on MTBC organisms.
 - A negative result (i.e., no mutation detected) does not rule out the presence of resistance-conferring mutations elsewhere in the genome. Heteroresistance below the level of detection of this assay may also contribute to false-negative results.
 - Inhibitory substances in the specimen may prevent successful DNA amplification.
 - Inability to generate successful sequences in targeted drug-resistance associated loci or negative MTBC identification result do not preclude the presence of MTBC.
 - Genotypic DST results should not be used as the sole criterion for the diagnosis of drug susceptible or resistant TB but can be used in conjunction with other clinical data.

CPT Code

CPT codes vary depending on organism identified and methods used.

Notes

MTBC susceptibility testing for first-line and second-line drugs are laboratory-developed tests. The MALDI-ToF-MS system is approved for *in vitro* diagnostic use by the U.S. Food and Drug Administration.