

Clinical Testing Mycobacteriology

Specimen Requirements

Refer to “Instructions for Packaging and Shipping Primary Specimens.”

Information Required

Complete the Communicable Diseases Laboratory Test Requisition (IL482-1039). Information must be completed (full name of patient, submitter, provider code, collection date, source, test request, etc.) for testing.

Collection Materials for Mycobacteriology (TB) Testing

Mycobacteriology specimen collection containers (see Table 4). See Appendix C for supplies.

Interfering Substances

Propylene glycol, waxed containers, tap water (may contain saprophytic *Mycobacteria*), antimicrobial therapy, food particles, mouthwash

Criteria for Rejection

Preserved specimens; specimen submitted more than 10 days after collection; specimens leaking in transit; quantity not sufficient (no visible specimen in the container); specimens not properly labeled or without corresponding requisition form, patient name/ID number on specimen tube does not match requisition form, blood collected in ethylene diamine tetra-acetic acid (EDTA), specimens collected in formalin or other preservative, 24-hour pooled respiratory (sputum) specimens

Test Methodologies

1. Acid fast bacilli are detected using Auramine-Rhodamine Fluorescence Microscopy
Detects and quantifies the presence of acid fast bacilli
Does not distinguish between live and dead organisms
Does not distinguish *Mycobacterium tuberculosis* complex from non-tuberculous (NTM) bacilli
2. Innogenetics Line Probe (LiPa) assay detecting the following *Mycobacterium* species:
 - M. avium*
 - M. celatum*
 - M. chelonae/abscessus* complex
 - M. fortuitum*
 - M. genavense*
 - M. gordonae*
 - M. haemophilum*
 - M. kansasii*
 - M. malmoense*
 - M. marinum/ulcerans*
 - M. scrofulaceium*
 - M. simiae*
 - M. smegmatis*
 - M. tuberculosis* complex
 - M. xenopi*
3. Sequence analysis of the heat shock protein 65 (HSP65) to identify species not detected by the Line Probe assay.
4. Real-time polymerase chain reaction assay to distinguish *M. tuberculosis* from *M. bovis* BCG and *M. bovis*
5. First Line Drug susceptibility analysis for *M. tuberculosis* isolates:
 - Ethambutol tested at 5.0 µg/mL
 - Isoniazid tested at 0.1 µg/mL and 0.4 µg/mL
 - Pyrazinamide tested at 100.0 µg/mL
 - Rifampin tested at 1.0 µg/mL
6. PCR based Spoligotyping and MIRU@ tandem repeat analysis; IS6110-restriction fragment length polymorphism (RFLP) analysis for genotypic typing of *M. tuberculosis* (performed at the Michigan Department of Community Health)
Note: All *M. tuberculosis* isolates state wide must be sent to the Chicago Illinois Department of Public Health mycobacteriology laboratory in Chicago for genotyping.

Availability

Mycobacteriology testing performed at Chicago laboratory only. For Real-time Polymerase Chain Reaction see “Molecular Diagnostics, Instructions for Detection of *M. tuberculosis* Complex by Real-time Polymerase Chain Reaction - Specimen Collection and Submission.”

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Instructions for Mycobacteria Specimen/Referred Culture Collection/Submission

Instructions for Collection of Primary Specimens

1. See Table 4 for primary specimens, optimal collection requirements and special instructions.
2. Successful isolation of the pathogen requires that the best specimen be properly and promptly collected.
3. If possible, collect specimens before chemotherapy is started.
4. Collect specimens in sterile, leak-proof containers; **DO NOT** use waxed containers. See Appendix C for available collection kits and supplies.
5. Collect specimens aseptically, minimizing contamination with indigenous micro biota.
6. Avoid interfering substances including propylene glycol, waxed containers, tap water (may contain saprophytic *Mycobacterium*), antimicrobial therapy, food particles, and mouthwash.
7. Collect sufficient materials for the test requested. Refer to “Table 4, Specimen Requirements for Mycobacterial Isolation,” for additional information.
8. Sputum specimens
 - a. At least one specimen on any given day in the early morning
 - b. Collect on three consecutive mornings. Do not pool specimens.
 - c. Collect sputum from the lung after a deep, productive cough for optimal pulmonary specimens (not saliva or nasopharyngeal discharge).
 - d. Label induced sputum specimens as “Induced” since they resemble saliva.
9. Other acceptable pulmonary specimens are bronchial washing, bronchial biopsies, bronchial brushing and transtracheal aspirate.
10. Collect body fluids aseptically, such as spinal, pleural, pericardial, synovial, as ascitic fluids, blood, pus and bone marrow. Consider collecting gastric aspirate if other methods fail to produce a valid specimen (see special instructions in Table 4).
11. Collect laryngeal swabs only from patients who are unable to produce sputum or from children. Swabs are not recommended for the isolation of *Mycobacteria*. They are acceptable **only** if a specimen cannot be collected by other means. This must be stated on the test requisition. The laboratory smear report will state that the specimen was submitted on a swab and another specimen should be transmitted as soon as possible.
12. Urine specimens
 - a. Submit a single first morning specimen.
 - b. Wash the external genitalia before the specimens are collected.
 - c. Process the urine immediately or refrigerate.
 - d. Urine specimens are not recommended for the isolation of *Mycobacteria*.
13. Swabs are not recommended for the isolation of *Mycobacteria*. They are acceptable **only** if a specimen cannot be collected by other means. This must be stated on the test requisition. The laboratory smear report will state that the specimen was submitted on a swab and another specimen should be submitted as soon as possible.

Criteria for Rejection

1. Primary specimens received more than 10 days after collection
2. Specimens leaking in transit
3. Blood collected in ethylene diamine tetra-acetic acid (EDTA).
4. Specimens collected in formalin or other preservative
5. 24-hour pooled respiratory (sputum) specimens
6. Specimens not properly labeled or without corresponding requisition form
7. Specimen name/ID number on specimen tube does not match requisition form

Instructions for Submission of Referred Cultures

1. Submit referred specimens on **solid medium**, in screw-cap tubes or *Mycobacterium* pellets in screw-cap tubes. Secure the cap with tape. Never ship cultures on petri-dishes or in liquid medium.
2. Referred specimen must have sufficient growth for testing.
3. Only *M. complex* isolates may be referred to the Illinois Department of Public Health laboratory for drug susceptibility testing.
4. *M. tuberculosis* isolates **MUST** be forwarded to the Department’s mycobacteriology laboratory for genotyping analysis as part of the ongoing national epidemiological surveillance to track the spread of tuberculosis.

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Instructions for Packaging and Shipping Primary Specimens

Primary specimens must arrive at the Illinois Department of Public Health within 24 hours of collection. Failure to do so will compromise the isolation of *Mycobacterium* cultures for identification and drug susceptibility tests. If the specimen(s) cannot be shipped immediately, store at 4 C to 8 C.

1. By ground transport
 - a. Wrap specimen(s) individually in absorbent material.
 - b. Place wrapped specimen(s) into a biohazard labeled bag and seal securely.
 - c. Place the test requisition(s) in the biohazard bag outside pouch so that it does not come in contact with the specimen sealed inside the bag.
 - d. Place the sealed biohazard bag and test requisition(s) inside the shipping container.
 - e. The shipping container must be rigid such as a cooler and labeled with the UN 3373 Biological Substance Category B marking.
 - f. Close securely.
2. Commercial carrier by ground/air transport
 - a. Wrap specimen(s) individually in absorbent material.
 - b. Place the wrapped specimen(s) inside a biohazard labeled 95 kPa bag and seal following the instructions on the bag.
 - c. Place the test requisition(s) in the 95 kPa bag outside pouch so that it does not come in contact with the specimen sealed in the bag.
 - d. Place the sealed 95 kPa bag and completed test requisitions(s) inside the outer shipping container and close securely.
 - e. Label the outer shipping container with the appropriate Illinois Department of Public Health laboratory address.
 - f. Complete the return address section to include the name of the person shipping the package, business name and address and a business phone number.
 - g. The shipping container must include the UN3373 Biological Substance Category B marking.

Instructions for Packaging and Shipping Referred Cultures

1. Ship *M. tuberculosis* complex isolates by a private carrier (I.e. FedEx)
2. Package and ship as a Category A infectious substance using United Nations (U.N.) certified 6.2 packaging according to DOT 49 Parts 171 to 178 and the U.S. Postal Service regulations. See Appendix G.
3. Follow packaging manufacturer's instructions for packaging and labeling.
4. Compliance with the requirements of the regulations is the responsibility of the shipper.

Susceptibility Testing

Testing will be performed on isolates identified as *M. tuberculosis*. Multiple drug resistant isolates will be referred to CDC for the detection of susceptibility to second-line antimycobacterial drugs.

Send all specimens and referred cultures to:
Illinois Department of Public Health Laboratory
Mycobacteriology Laboratory
2121 W. Taylor St.
Chicago, IL 60612-4224
Phone: 312-793-1063
Fax: 312-793-7764

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Table 4. Primary Specimen Requirements for Mycobacterial Isolation

Specimen Type	Optimal Specimen Requirement's	Special Instructions
Abscess content, aspirated fluid	>1mL in sterile screw capped tube	Cleanse skin with alcohol before aspirating sample. Disinfect site as routine.
Blood	10 mL (yellow top) blood collection tube	Blood culture. Mix tube contents immediately after collection.
Body fluids	As much as possible in a sterile container	Volumes of <10 ML may be directly inoculated into MGIT tubes.
Bone	Bone in sterile container without fixative	
Bronchoalveolar lavage or bronchial washing	>5 mL in sterile container	Collect aseptically.
Bronchial brushing	Bronchial brush in sterile container	
Cerebral Spinal Fluid (CSF)	>2 mL in sterile container	Use maximum volume attainable.
Gastric aspirate	>5-10 mL in sterile container	Collect early morning specimen on three consecutive days. Adjust pH. Add 100 MG of sodium carbonate following collection.
Lymph node	Node or portion without fixative	Collect aseptically.
Skin lesion	Submit biopsy in sterile container	Collect biopsy from periphery of lesion or aspirate material from under margin of lesion.
Sputum	5 mL in sterile, wax-free disposable container. Collect an early morning specimen from deep productive cough on at least three different days. Do not pool specimens. For follow up on patient therapy, collect at weekly intervals beginning three weeks after initiation.	Instruct patient how to produce sputum. Have patient rinse mouth with water before collecting.
Stool	>1 g of stool in sterile container	Collect specimen directly into container.
Tissue biopsy sample	> 1 g of tissue, in sterile container	Collect aseptically.
Urine	Minimum 40 mL of first morning specimen	Collect first morning specimen on three different days. Accept only one specimen/day.
Trans-tracheal aspirate	As much as possible in sterile container	

Table 5. Mycobacteriology Tests

Test	Specimen	Performed At	TAT (Days)
Detection of Acid Fast Bacilli (Direct Smear)	Primary specimen	Ch	1
Identification of <i>Mycobacterium</i> species	Primary specimen	Ch	14-21
Identification of <i>Mycobacterium</i> species	Referred cultures	Ch	3-10
<i>M. tuberculosis</i> drug susceptibility-First Line	Primary specimen	Ch	21-28
<i>M. tuberculosis</i> drug susceptibility-First Line	Referred cultures	Ch	10-21
Direct detection of <i>M. tuberculosis</i> by real-time polymerase chain reaction	Primary specimens (respiratory only)	Ch	3
<i>M. tuberculosis</i> genotyping	Primary specimen and referred culture	² See footnote	³ See footnote

²The Illinois Department of Public Health laboratory triages *M. tuberculosis* isolates to the Michigan Department of Community Health for genotyping analysis.

³Epidemiology study