

Laboratory Services

CONTENTS

Introduction.....	11.2
Purpose.....	11.2
Policy	11.2
Available Laboratory Tests.....	11.4
Specimen Collection	11.6
How to perform spontaneous sputum collection at a healthcare facility	11.7
How to direct a patient to perform spontaneous sputum collection at home.....	11.8
Induced sputum collection at a healthcare facility	11.9
How to collect gastric aspirates	11.9
Bronchoscopy or collection of extrapulmonary specimens	11.9
Specimen Shipment	11.10
Resources and References	11.12

Introduction

Purpose

Use this section to do the following:

- Obtain contact information for laboratories.
- Determine which tests are available and the tests' turnaround times.
- Identify which laboratory can perform a specific test.

The diagnosis of tuberculosis (TB), management of patients with the disease, and public health TB control services rely on accurate laboratory tests. Laboratory services are an essential component of effective TB control, providing key information to clinicians (for patient care) and public health agencies (for control services).¹

Policy

Public health laboratories should ensure that clinicians and public health agencies within their jurisdictions have ready access to reliable laboratory tests for diagnosis and treatment of TB.²

Effective TB control requires timely, complete, and accurate communication among the laboratory system, TB control program, and healthcare provider.

Program Standards

RECOMMENDED NATIONAL STANDARDS FOR AFB LABORATORY TESTING

- *Rapid delivery of specimens to the laboratory (Delivery in lab within 24 hours from specimen collection)*
- *Use fluorescent acid-fast staining and microscopic examination (Report AFB slide result within 24 hours from receipt of specimen in lab)*
- *Use rapid broth system for primary culture detection of AFB (Report culture detection of AFB within 14 days)*
- *Use rapid ID, i.e. HPLC/genetic probes (Report TB ID within 14-21 days from receipt of specimen in lab)*
- *Use rapid broth susceptibility testing (Report susceptibility to primary anti-TB drugs within 21-28 days from receipt of specimen in lab)*
- *Report susceptibility results to attending physician as soon as available, i.e. by phone or FAX*

INFORMATION PERTAINING TO AFB LAB TESTING

SPECIMEN (collect in sterile leak proof container)

1. Requires three specimens on three consecutive days.
2. Best results come from 5-10 ml material expectorated by patient soon after awakening in the morning.
3. Specimen should be delivered to the laboratory within 24 hours.

AFB SLIDE EXAMINATION (ONE DAY)

1. Least sensitive of all AFB Tests.
2. Requires 100,000 AFB/ml for a slide to be positive.
3. If positive the patient can infect others.
4. Positive Slide – does not determine whether TB or MOTT.
5. Report within 24 hours of receiving the specimen in the laboratory.

DIRECT SPECIMEN PCR TB PROBE TEST (1-2 DAYS)

1. Very sensitive and very specific test.
2. Requires only one AFB to produce a positive result.
3. Performed only on non-bloody, pulmonary specimens.
4. Performed on the new patient's first slide positive specimen (not performed during treatment)
5. Report within 24 hours of the positive slide report.
6. Positive result (very reliable) – *Mycobacterium tuberculosis* complex (not MOTT).
7. Test not available in most laboratories.

AFB CULTURE (7-10 DAYS)

1. More sensitive than the AFB slide test.
2. Only requires 10 AFB/ml of specimen to produce a positive result.
3. Culture may be AFB positive even though the slide test was reported negative for AFB.
4. Rapid broth testing – normally positive within 1-2 weeks.

5. Positive culture result may be either *Mycobacterium tuberculosis* complex or MOTT
6. Negative culture results require 6 weeks to report.

AFB IDENTIFICATION (10-12 DAYS)

1. Performed as soon as culture becomes positive with growth of AFB.
2. Rapid preliminary identification may be reported within 1-3 days of a positive culture report.
3. Preliminary identification is based upon HPLC or genetic probe.
4. Genetic probe (growth based/non-PCR) reports *M.tuberculosis* complex or NOT *M.tuberculosis* complex.
5. HPLC – Identifies many *Mycobacterium* spp.

SUSCEPTIBILITY (WITHIN 21 DAYS)

1. Performed as soon as growth from culture has been identified as *M.tuberculosis* complex.
2. Primary antibiotic susceptibility report may be expected one week from the *M.tuberculosis* identification report.
3. Pyrazinamide susceptibility report may be expected one week after the primary drug report.
4. Secondary antibiotics may be requested to be tested and are reported approximately three weeks after initiated.

Available Laboratory Tests

The laboratory tests listed below in Table 1 are available where noted.

Table 1: AVAILABLE LABORATORY TESTS

Test	Laboratory	Turnaround Time
Diagnosis		
QuantiFERON®-TB Gold (QFT-G)	<i>Sparrow Hospital in Lansing Borgess Hospital in Kalamazoo University Hospital in Ann Arbor</i>	<i>Check with individual hospital.</i>
Acid-fast (AFB) bacilli smear	<i>Michigan Department of Community Health</i>	Within 24 hours from receipt in laboratory ³
Culture	<i>Michigan Department of Community Health</i>	Mycobacterial growth detection by culture within 14 days from date of specimen collection Identification of cultured mycobacteria within 21 days from date of specimen collection ^{4,5}
Drug susceptibility	<i>Michigan Department of Community Health</i>	Within 28 days from date of specimen collection ^{6,7}
Nucleic acid amplification (NAA) test	<i>Michigan Department of Community Health</i>	Within 1 day from date of positive AFB slide report
Epidemiologic Monitoring		
Genotyping	<i>Michigan Department of Community Health</i>	Spoligo/MIRU-10 days from TB identification

Laboratories should report positive smears or positive cultures, and primary healthcare providers should report suspected or confirmed cases of TB to the health department, as specified in the “Reporting Tuberculosis” topic in the Surveillance section. Prompt reporting allows the health department to organize treatment and case management services and to initiate a contact investigation as quickly as possible.⁸

Laboratories which perform AFB slide and culture are required to submit, as soon as possible, the first isolate of *Mycobacterium tuberculosis* cultured from a patient to the MDCH TB laboratory unit for susceptibility testing and genotyping (Michigan

Communicable Disease Rules, R 325.179;

http://www.state.mi.us/orr/emi/admincode.asp?AdminCode=Single&Admin_Num=32500171&Dpt=&RngHigh=32).



For information on reporting, see the “Reporting Tuberculosis” topic in the Surveillance section.



For laboratory services available at MDCH and private laboratories located in Michigan contact the MDCH TB laboratory at (517) 335-9636.

Specimen Collection

Sputum is phlegm from deep in the lungs. The important characteristics needed in sputum specimens are freshness and actual sputum, rather than saliva. Three specimens collected early in the morning on three successive days will provide the most reliable results.

To isolate mycobacteria from clinical materials successfully, handle specimens carefully after collection. For optimal results, collect specimens in clean, sterile, leak proof containers and keep them in refrigerated conditions to inhibit the growth of contaminating organisms, since most specimens will contain bacteria other than mycobacteria.⁹

Refer to Table 2 to review the methods used to collect various specimens and the type of specimens obtained for pulmonary tuberculosis (TB).



NOTE: During specimen collection procedures in which aerosols may be produced, use appropriate respiratory protection and environmental controls. For more information, refer to the CDC's "Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-care Settings, 2005" (*MMWR* 2005;54[No. RR-17]) at this hyperlink: <http://www.cdc.gov/mmwr/pdf/rr/rr5417.pdf> .

Table 2: SPECIMEN COLLECTION METHODS AND TYPES FOR PULMONARY TUBERCULOSIS

Pulmonary Tuberculosis	
Collection Method	Specimen Type
Spontaneous sputum collection occurs when the patient can cough up sputum without extra assistance.	<ul style="list-style-type: none"> ▪ 5–10 ml of sputum from deep in the lung
Induced sputum collection should be considered if a patient needs assistance in bringing up sputum.*	<ul style="list-style-type: none"> ▪ 5–10 ml of sputum from deep in the lung
Gastric aspirates can be submitted for the diagnosis of pulmonary tuberculosis (TB) in young children who cannot produce sputum. (Must be neutralized to pH7 and delivered to the laboratory within 72 hours of specimen collection)	<ul style="list-style-type: none"> ▪ 50 ml of gastric contents
Bronchoscopy can be used in the following situations: <ul style="list-style-type: none"> ▪ If a patient cannot produce sputum by the above three methods¹⁰ or ▪ If a patient has a substantial risk of drug-resistant TB 	<ul style="list-style-type: none"> ▪ Bronchial washings ▪ Bronchoalveolar lavage ▪ Transbronchial biopsy

<p>and has initial routine studies that are negative¹¹ or</p> <ul style="list-style-type: none"> ▪ In a patient in whom there is suspicion of endobroncheal TB¹² or ▪ If a variety of clinical specimens for the diagnosis of pulmonary TB or other possible diseases need to be obtained 	
<p>* It is important to specify if the sputum is induced or not, because induced sputum is “more watery” and appears to be just saliva. The laboratories may alter the testing process based upon the watery nature of the specimen.</p>	

Refer to Table 3 for collection methods and specimen types for extrapulmonary TB.

Table 3: SPECIMEN COLLECTION METHODS AND TYPES FOR EXTRAPULMONARY TUBERCULOSIS

Extrapulmonary Tuberculosis		
Collection Method	Specimen Type	
<p>Extrapulmonary specimen collection from tissue and other body fluids can be submitted for the diagnosis of extrapulmonary tuberculosis.</p>	<p>Examples of tissues (biopsy)*</p> <ul style="list-style-type: none"> ▪ Lymph node ▪ Pleural ▪ Bone/joint ▪ Kidney ▪ Peritoneal ▪ Pericardial 	<p>Examples of fluids</p> <ul style="list-style-type: none"> ▪ Pleural ▪ Cerebrospinal ▪ Blood ▪ Urine ▪ Synovial ▪ Peritoneal ▪ Pericardial
<p>* Do not place specimens in formalin.</p>		

How to Perform Spontaneous Sputum Collection at a Healthcare Facility

1. Collect the specimen in a specialized room or booth designed for cough-inducing procedures.
2. Instruct the patient on how to collect the sputum sample.
 - a. Put a mark at the 5 ml level on the sputum tube (if not already marked) to show the patient the minimum amount of sputum needed. (Most laboratories consider 5 to 10 ml an adequate amount.)
 - b. Review with the patient how to collect sputum.
3. Make sure that both the specimen container and laboratory requisition are filled out completely before shipping. If either the test requisition or the specimen are not labeled correctly, the specimen cannot be tested.

- a. On the specimen container, record the patient name and the date and time of collection.
4. Make sure the specimen and laboratory requisition are packaged into appropriate shipping containers, per laboratory instructions.
5. If possible, send the specimen on the day it is collected. If this is not possible, refrigerate the specimen until it is sent on the next day.
6. Do not delay sending specimens in order to send all three on the same day.
7. Use the most rapid transport to the laboratory: yourself, courier, overnight carrier, or US mail.



NOTE: Make every effort to submit specimens to the laboratory within 24 hours of collection. Normal flora can overgrow any mycobacteria in the specimen and make it unusable. If specimens cannot be submitted within 24 hours, keep in mind that most laboratories will not run a specimen over five days old. Know how long it takes the specimen to get to the laboratory from the time it leaves your hands, and submit specimens accordingly.

How to Direct a Patient to Perform Spontaneous Sputum Collection at Home

If a patient will be collecting sputum specimens at home, provide the following guidance.

1. Put a mark at the 5 ml level on the sputum tubes (if not already marked) to show the patient the minimum amount of sputum needed. (Most laboratories consider 5 to 10 ml an adequate amount.)
2. Review with the patient how to collect sputum.
3. Make arrangements for a healthcare worker to pick up the specimen or for the patient, a family member, or a friend to drop off the specimen.

Induced Sputum Collection at a Healthcare Facility

If the patient cannot produce sputum spontaneously, then make arrangements for an induced sputum to be collected at a facility. Facilities where sputum can be collected include the respiratory therapy department of a local hospital, TB clinic, or laboratory. Facilities should have appropriate respiratory protection, environmental controls, and policies and procedures.

How to Collect Gastric Aspirates

The following are basic guidelines for collecting gastric aspirates:

- Collect the specimen after the patient has fasted for eight to ten hours and, preferably, while the patient is still in bed.
- Collect a specimen daily for three consecutive days.



For additional information on how to collect a gastric aspirate and prepare the specimen for transport, see the guide and Francis J. Curry National Tuberculosis Center's online video *Pediatric TB: A Guide to the Gastric Aspirate (GA) Procedure* at this hyperlink:

http://www.nationaltbcenter.ucsf.edu/products/product_details.cfm?productID=ONL-06 .

Bronchoscopy or Collection of Extrapulmonary Specimens

If TB staff are consulting with physicians before the specimens are collected, the physician should be reminded to send part of the specimen (not in formalin) to the microbiology laboratory for acid-fast bacilli (AFB) smear and culture, in addition to any other tests or pathology examinations the physician plans to obtain. In addition, a post-bronchoscopy sputum specimen should be sent for AFB smear and culture.

- **Bronchoscopy:** Refer the patient to a local specialist.
- **Extrapulmonary specimens:** These specimens will be collected by the physician performing the diagnostic work-up.

Specimen Shipment

In order to transport TB specimens or cultures, there are two primary categories of infectious substances, and each category has different packaging requirements to provide increased levels of protection against leaks and contamination.

Category A mycobacterial cultures (culture isolates suspected of being *Mycobacterium tuberculosis* complex) are Category A Infectious Substances and are only to be transported by a medical courier or shipped by private carrier as dangerous goods. Category A Infectious Substances cannot be mailed through the United States Postal Service (USPS).

Category B Infectious Substances (raw diagnostic specimens, such as sputum, blood, or tissue) can be mailed through the USPS, shipped by private carrier (e.g., Federal Express, Airborne Express, etc.), or transported by a medical courier.

Shipment of dangerous goods by the USPS is regulated by the United States Department of Transportation. Specific shipping instructions from the Centers for Disease Control and Prevention (CDC) can be found in the publication by the United States Department of Health and Human Services (DHHS) *Public Health Mycobacteriology: A Guide for the Level III Laboratory*. Packaging and shipment of specimens by USPS should meet the following regulations:

- Office of Health and Safety. "Interstate Shipment of Etiologic Agents" [Web page] (Centers for Disease Control and Prevention Website):
<http://www.cdc.gov/od/ohs/biosfty/shipregs.htm>
- United States Postal Service. Domestic Mail Manual:
http://pe.usps.com/text/dmm300/dmm300_landing.htm
- United States Postal Service. 135 Mailable Dangerous Goods (International Mail Manual): http://pe.usps.gov/text/lmm/immc1_013.htm
- National Archives and Records Administration. Code of Federal Regulations Title 39—United States Postal Service (U.S. Government Printing Office Website):
http://www.access.gpo.gov/nara/cfr/waisidx_03/39cfrv1_03.html
- National Archives and Records Administration. Code of Federal Regulations Title 49—Transportation (U.S. Government Printing Office Website):
http://www.access.gpo.gov/nara/cfr/waisidx_04/49cfrv2_04.html
- U.S. Department of Labor, Occupational Safety & Health Administration (OSHA): Occupational Health and Safety Standards 29 CFR 1910.1030:
http://www.osha.gov/pls/oshaweb/owastand.display_standard_group?p_toc_level=1&p_part_number=1910¹³

For shipments by private carriers, follow International Air Transportation Association (IATA) instructions. *Mycobacterium tuberculosis* cultures are defined as infectious substances/etiologic agents when shipped by private carrier and must be shipped in packaging approved by the United Nations (UN), according to IATA Packing Instruction

602: http://oregonstate.edu/vetmed/pdf/iata_602.pdf . Diagnostic specimens are defined as human or animal specimens, including excreta, secretions, blood and its components, tissue, tissue fluids, and cultures of nontuberculous mycobacteria being transported for diagnostic or investigational purposes. Diagnostic specimens must be packaged according to IATA Packing Instruction 650:

<http://www.iata.org/NR/ContentConnector/CS2000/SiteInterface/sites/whatwedo/cargo/file/PI650.pdf> .¹⁴



To obtain specimen collection and transport supplies, contact MDCH by phone @ (517) 335-9867 or by e-mail @ <http://www.michigan.gov/mdchlab>

Resources and References

Detailed descriptions of recommended laboratory tests; recommendations for their correct use; and methods for collecting, handling, and transporting specimens have been published. For more information on laboratory testing for tuberculosis (TB), see the following:

- ATS, CDC, IDSA. "Controlling Tuberculosis in the United States: Recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America" (*MMWR* 2005;54[No. RR-12]). Available at: <http://www.cdc.gov/mmwr/PDF/rr/rr5412.pdf> .
- ATS, CDC, IDSA. "Diagnostic Standards and Classification of Tuberculosis in Adults and Children" (*Am J Respir Crit Care Med* 2000;161[4 Pt 1]). Available at: <http://www.cdc.gov/tb/pubs/PDF/1376.pdf> .
- National Committee for Clinical Laboratory Standards. *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard* [Document no. M24-A] (Wayne, PA; 2003).

References

- ¹ ATS, CDC, IDSA. Controlling tuberculosis in the United States: recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. *MMWR* 2005;54(No. RR-12):18.
- ² ATS, CDC, IDSA. Controlling tuberculosis in the United States: recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. *MMWR* 2005;54(No. RR-12):19.
- ³ ATS, CDC, IDSA. Controlling tuberculosis in the United States: recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. *MMWR* 2005;54(No RR-12):19; and Tenover, R., et al. The resurgence of tuberculosis: is your laboratory ready? *Journal of Clinical Microbiology* 1993:767–770.
- ⁴ ATS, CDC, IDSA. Controlling tuberculosis in the United States: recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. *MMWR* 2005;54(No RR-12):19; and Tenover, R., et al. The resurgence of tuberculosis: is your laboratory ready? *Journal of Clinical Microbiology* 1993:767–770.
- ⁵ CDC. National plan for reliable tuberculosis laboratory services using a systems approach - recommendations from CDC and the Association of Public Health Laboratories Task Force on Tuberculosis Laboratory Services. *MMWR* 2005;54(No. RR-6):2.
- ⁶ ATS, CDC, IDSA. Controlling tuberculosis in the United States: recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. *MMWR* 2005;54(No RR-12):19; and Tenover, R., et al. The resurgence of tuberculosis: is your laboratory ready? *Journal of Clinical Microbiology* 1993:767–770.
- ⁷ CDC. National plan for reliable tuberculosis laboratory services using a systems approach - recommendations from CDC and the Association of Public Health Laboratories Task Force on Tuberculosis Laboratory Services. *MMWR* 2005;54(No. RR-6):2.
- ⁸ CDC. Diagnostic microbiology. In: Chapter 5: diagnosis of TB. *Core Curriculum on Tuberculosis (2000)* [Division of Tuberculosis Elimination Web site]. Updated November 2001. Available at: <http://www.cdc.gov/tb/pubs/corecurr/index.htm> . Accessed November 1, 2006.
- ⁹ ATS, CDC, IDSA. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med*. 2000;161:1376–1395.
- ¹⁰ Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
- ¹¹ Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
- ¹² Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
- ¹³ National Jewish Medical and Research Center. *How to Mail Specimens and Cultures to the National Jewish Mycobacteriology Laboratory*. Denver, CO; March 2005:2.
- ¹⁴ National Jewish Medical and Research Center. *How to Mail Specimens and Cultures to the National Jewish Mycobacteriology Laboratory*. Denver, CO; March 2005:5–7.