Chapter 11: Laboratory Services

Contents

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 health care provider.[3]

For roles and responsibilities, refer to the "Roles, Responsibilities, and Contact Information" topic in the Introduction.

Available laboratory tests

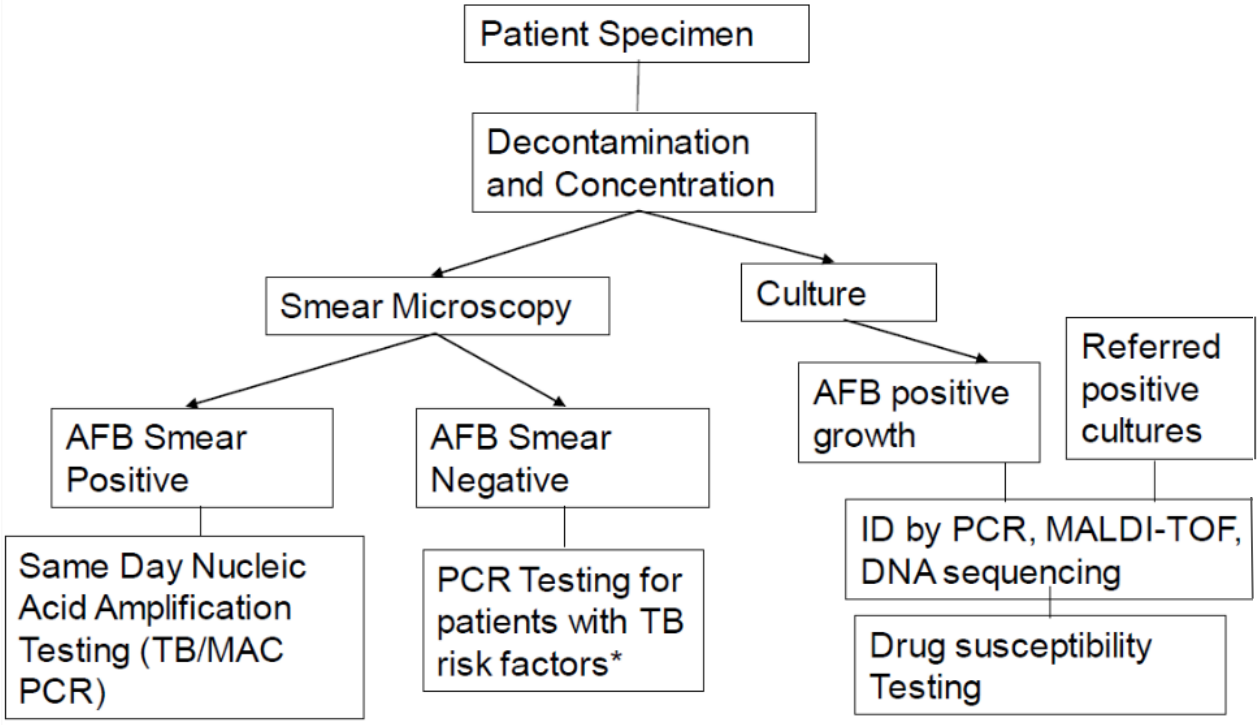
The laboratory tests listed below in Table 11.1 are available where noted.

Table 11.1: Available laboratory tests

Test	Laboratory	Turnaround time
Diagnosis		
IGRA (QFT or T.spot)	IGRA tests are not available through Wisconsin State Laboratory of Hygiene and should be ordered by private providers. Departments should explore contacts/ lab services	

Test	Laboratory	Turnaround time
	based on local availability and demand.	
Acid-fast (AFB) bacilli smear	Wisconsin State Laboratory of Hygiene or private laboratory	Within 24 hours from receipt in laboratory[4]
Culture	Wisconsin State Laboratory of Hygiene or private laboratory	Mycobacterial growth detection by culture 7–21 days after primary specimen identification/ isolation. Specimens are held for 6–8 weeks for culture growth by WSLH.
Drug susceptibility	Wisconsin State Laboratory of Hygiene and a few private laboratories. If additional susceptibility testing is necessary, WSLH will send to CDC or another reference laboratory at request of the state TB program staff.	2 to 3 weeks after positive culture isolated.
Nucleic acid amplification (NAA) test	Wisconsin State Laboratory of Hygiene	Within 2 days of receipt of AFB smear positive specimen.
Epidemiologic Monitoring		
Whole Genome Sequencing (WGS)	Wisconsin State Laboratory of Hygiene	On average, the turnaround time can be anywhere from 3 weeks to 8 weeks. This does not need to be ordered or requested and will automatically be sent out.

Testing algorithm for specimens received at Wisconsin State Laboratory of Hygiene (WSLH)



Laboratories should report all positive smears, NAAT/PCRs, and positive cultures, and primary health care 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.[5]

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

For laboratory services available in Wisconsin, contact the Wisconsin Tuberculosis Program at 608-261-6319.

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. An early morning specimen is best; therefore, when collecting a set of three sputum specimens, at least one of them should be an early morning specimen.

To isolate mycobacteria from clinical materials successfully, handle specimens carefully after collection. For optimal results, collect specimens in clean, sterile containers and keep them in refrigerated conditions to inhibit the growth of contaminating organisms, since most specimens will contain bacteria other than mycobacteria.[6]

Refer to Table 11.2 to review the methods used to collect various specimens and the type of specimens obtained for pulmonary TB.

The Wisconsin Tuberculosis Program offers job aids for nebulized induction and spontaneously produced sputum collection.

Public Health Madison & Dane County has a step-by-step video guide to home sputum collection available in multiple languages.

During procedures in which aerosols may be produced, including sputum collection spontaneously or via induction, 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]).

WSLH specific logistics

To order WSLH Kit #8, sputum collection, call 800-862-1013. Kits are free for Wisconsin public health entities. It is recommended to have kits available and on hand at the health department as it can take a few days to order and receive the kits when needed.

Kit includes the following: Sterile tube with label, absorbent pad, specimen transport bag, cold pack, instruction sheet, and insulated mailer with labels.

Requisition form A should be included, one order form per specimen is needed. Select "MM00252 Mycobacteria (AFB) Smear and Culture" when ordering.

Courier service to WSLH is available via **Gold Cross Courier Service, please call 763-233-0099** to set up an account and schedule pickups.

Table 11.2: 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.	5–10 ml of sputum from deep in the lung
Induced sputum collection should be considered if a patient needs assistance in	5–10 ml of sputum from deep in the lung

bringing up sputum.*	
Gastric aspirates can be submitted for the diagnosis of pulmonary TB in young children who cannot produce sputum.	50 ml of gastric contents
Bronchoscopy can be used in the following situations: • If a patient cannot produce sputum by the above three methods[7] or • If a patient has a substantial risk of drug-resistant TB and has initial routine studies that are negative[8] or • In a patient in whom there is suspicion of endobroncheal TB[9] or • If a variety of clinical specimens for the diagnosis of pulmonary TB or other possible diseases need to be obtained	• Bronchial washings • Bronchoalveolar lavage • Transbronchial biopsy
* It is important to specify if the sputum is induced or not, because induced sputum is “more watery” and appears to be just saliva. Some laboratories may throw out induced sputum and report it as an inadequate specimen.	

Refer to Table 11.3 for collection methods and specimen types for extrapulmonary TB.

Table 11.3: Specimen collection methods and types for extrapulmonary tuberculosis

Extrapulmonary tuberculosis		
Collection method	**Specimen type**	
Extrapulmonary specimen collection from tissue and other body fluids can be submitted for the diagnosis of extrapulmonary tuberculosis.	**Examples of tissues (biopsy)*** • Lymph node • Pleural • Bone/joint • Kidney • Peritoneal • Pericardial	**Examples of fluids** • Pleural • Cerebrospinal • Blood • Urine • Synovial • Peritoneal • Pericardial
* **Do not place specimens in formalin. Formalin fixed sample may be sent to CDC for NAAT/PCR testing, although this takes several weeks. Specimens placed in saline can generally be tested faster.**		

How to perform spontaneous sputum collection at a health care facility

1. Collect the specimen in an airborne isolation room or a negative pressure sputum collection booth with the door shut. If these are not available, the client should be outdoors for specimen collection. **N95 masks must be worn by health care personnel for AFB cough-producing 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.

        i. Instruct the client to gently brush their teeth, gingival margins, tongue and buccal surfaces using sterile, filtered water or normal saline to rinse.

        ii. **Do not use tap water, unfiltered water, toothpaste, commercial mouth wash preparations, nose drops, or any medications containing alcohol or oil. Instruct the client to avoid taking oral antibiotics or any other medications immediately before the sputum collection procedure.**

        iii. Instruct the client to gargle several times with sterile, filtered water or normal saline after brushing. **Do not** use tap water or water from any unfiltered source.

        iv. Coach the client and observe the first sputum collection, at a minimum, in order to obtain a good quality sputum specimen. The client should understand that sputum is material that is brought up from the lungs and that nasal secretions and saliva or spit are not acceptable specimens.

        v. Instruct the client to inhale deeply, as far as possible, and then exhale slowly three times. After the third breath, direct the client to inhale completely and try to cough hard to produce sputum from deep within the lungs. The client may feel a rattle or tickle as the sputum moves up the lungs and into the throat.

        vi. Instruct the client to expectorate the sputum into the sterile specimen container. Repeat this process until at least 5ml of sputum has been collected.

Public Health Madison Dane County has developed a step-by-step home sputum collection video guide.

3. Make sure the specimen container and laboratory requisition form (see details above for obtaining form and kit) are filled out completely before shipping.

    a. On the specimen container, record the patient name and the date and time of collection.

It is especially important to **specify if the sputum is induced or not,** because an induced sputum generally is “more watery” and appears to be just saliva. Some private laboratories may throw out the specimen and report it as an “inadequate specimen.”

4. Make sure the specimen and laboratory requisition are packaged into appropriate shipping containers, per laboratory instructions.

Refer to the “Specimen Collection and Shipment Supplies” topic in the Supplies, Materials, and Services section, and see the “Specimen Shipment topic,” which follows.

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 U.S. mail.

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. See section above and consider showing them the suggested video which is available in multiple languages for education on proper collection technique.

3. Make arrangements for a health care worker to pick up the specimen or for the patient, a family member, or a friend to drop off the specimen to designated location.

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 10 hours and, preferably, while the patient is still in bed.
- Collect a specimen daily for three 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.

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

For transportation, 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.

Pure mycobacterial cultures (or culture isolates suspected of being mycobacteria) are Category A Infectious Substances and can be transported only 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 (for example, Federal Express, Airborne Express), or transported by a medical courier. Courier service to WSLH is available via Gold Cross **Courier Service, please call 763-233-0099** to set up an account and schedule pickups.

Please contact the Wisconsin State Laboratory of Hygiene's Customer Service line at 800-862-1013 for more information about currently available courier delivery services, and for sputum collection kits, and requisition forms for Wisconsin State Laboratory of Hygiene.

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): Interstate Shipments of Etiologic Agents.
- United States Postal Service Domestic Mail Manual
- United States Postal Service. 135 Mailable Dangerous Goods (International Mail Manual)
- National Archives and Records Administration. Code of Federal Regulations Title 39—United States Postal Service (U.S. Government Printing Office Website)
- National Archives and Records Administration. Code of Federal Regulations Title 49—Transportation (U.S. Government Printing Office Website).
- U.S. Department of Labor, Occupational Safety & Health Administration (OSHA): Occupational Health and Safety Standards 29 CFR 1910.1030[10]

For shipments by private carriers, follow International Air Transportation Association (IATA) instructions. *Mycobacterium tuberculosis* pure 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. Diagnostic specimens are defined as human or animal specimens, including excreta, secreta, 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.

For more information on shipping specimens in Wisconsin, contact the Wisconsin State Lab of Hygiene's Customer Service line at 800-862-1013.

To obtain specimen collection and transport supplies, see the topic on "Specimen Collection and Shipment Supplies" in the Supplies, Materials, and Services section.

Resources and references

Resources

Public Health Madison Dane County has a step-by-step home sputum collection video guide available.

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 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]).
- ATS, CDC, IDSA. "Diagnostic Standards and Classification of Tuberculosis in Adults and Children" (*Am J Respir Crit Care Med* 2000;161[4 Pt 1]).
- 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] Association of Public Health Laboratories. *The Future of TB Laboratory Services: A framework for integration/collaboration/leadership* [Association of Public Health Laboratories Web site]. 2004. Available at The Future of TB laboratory services : a framework for integration, collaboration, leadership : a report from an APHL task force (cdc.gov):.

[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. Diagnostic microbiology. In: Chapter 5: diagnosis of TB. *Core Curriculum on Tuberculosis (2000)* [Division of Tuberculosis Elimination Web site]. Updated November 2001. Available at: Core Curriculum on Tuberculosis: What the Clinician Should Know | Tuberculosis (TB) | CDC

[6] ATS, CDC, IDSA. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med*. 2000;161:1376–1395.

[7] Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.

[8] Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.

[9] Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.

[10] National Jewish Medical and Research Center. *How to Mail Specimens and Cultures to the National Jewish Mycobacteriology Laboratory.* Denver, CO; March 2005:2.