

San Joaquin County Public Health Laboratory Services

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Introduction

Purpose

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).

Bacteriological examination of sputum is the only diagnostic tool that provides definitive proof if a client suffers from active pulmonary tuberculosis and is considered the "gold standard" for the diagnosis of TB disease. Without bacteriological confirmation, drug sensitivities cannot be obtained making it difficult to select an appropriate drug regime.

Policy

San Joaquin County Public Health Laboratory purpose is to ensure that clinicians and other health agency's within our county jurisdiction have ready access to reliable laboratory tests for diagnosis and treatment of TB.

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

State Laws and Regulations

Title 17, California Code of Regulations (CCR), Section 2505 REPORTABLE CONDITIONS: NOTIFICATION BY LABORATORIES requires laboratories and clinician offices to report cases of tuberculosis to the local Public Health Department with in one day of diagnosis or evidence of laboratory identification of *Mycobacterium tuberculosis*. Additionally, any laboratory that isolates *Mycobacterium tuberculosis* from a patient specimen must submit a culture to the local public health laboratory for the local health jurisdiction in which the health care provider's office is located as soon as available from the primary isolate on which a diagnosis of tuberculosis was established.

Laboratory Contact Information

San Joaquin County Public Health Laboratory (SJCPHL)	1601 East Hazelton Ave Stockton CA 95205 209-468-3460 209-468-0639 Fax
Michigan Dept. of Community Health TB Laboratory – Genotyping Lab	927 Terminal Rd Lansing, MI 48906

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)	SJCPHL	Test performed weekly, contact laboratory for collection protocol
Acid-fast (AFB) bacilli smear	SJCPHL	Within 24 hours from receipt in laboratory
Culture	SJCPHL	Cultures are incubated for 8 weeks before reporting Negative. Time to detection is dependent on growth rate and quality of specimen. Identification of mycobacteria is usually within 7 – 21 days from date of positive culture.
Drug susceptibility Primary Drugs*	SJCPHL	Within 30 days from date of positive culture.
Nucleic acid amplification (NAA) test**	SJCPHL	Within 24 hours from receipt in laboratory
Treatment Monitoring		
Hepatic enzymes or up to 8 clinical, multichannel chem panel (that includes aspartate aminotransferase [AST], alanine aminotransferase [ALT], lactate dehydrogenase [LDH], total and direct bilirubin, alkaline phosphatase, uric acid, and calcium)	Available at Most Clinical Laboratories	Usually available same day
Uric acid		
Complete blood count (CBC) and platelets		
Kidney function		

Test	Laboratory	Turnaround Time
Epidemiologic Monitoring		
Genotyping***	Michigan Depart. Of Community Health TB Laboratory 927 Terminal Road Lansing, MI 48906	Within 10-14 working days after culture received by laboratory

*If Multiple drug resistance is suspected or detected with primary drug panel (Rifampin, INH, PZA, and Ethambutol – RIPE) specimens will be forwarded to California Dept. Public Health Microbial Disease Laboratory (CDPH-MDL) and/or Center for Disease Control (CDC) for testing of secondary drug susceptibility panel.

**Nucleic Acid Amplification testing (NAAT). All initial smear positive sputum specimens will be reflexed to a NAAT test using a real time MTB/Rifampin PCR assay. Smear negative respiratory specimens will be tested upon request of submitter. Pyrosequencing of smear positive specimens from patients suspected to have drug resistant tuberculosis may be forwarded for testing to CDPH-MDL.

***All primary isolates of *M. tuberculosis* identified or received by SJCPHL are forwarded to Michigan Dept. Of Community Health for genotyping.

Laboratories should report positive smears or positive cultures, and primary healthcare providers should report suspected or confirmed cases of TB to San Joaquin County Public Health Services (PHS) Tuberculosis Control Program, 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.¹



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

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. Specimens must be collected no sooner than 8 hours apart with at least one specimen 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.²

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



During procedures in which aerosols may be produced, use appropriate respiratory protection and environmental controls. Ref 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.	<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 methods³ or ▪ If a patient has a substantial risk of drug-resistant TB and has initial routine studies that are negative⁴ or ▪ In a patient in whom there is suspicion of endobroncheal TB⁵ or ▪ If a variety of clinical specimens for the diagnosis of pulmonary TB or other possible diseases need to be obtained 	<ul style="list-style-type: none"> ▪ Bronchial washings ▪ Bronchoalveolar lavage ▪ Transbronchial biopsy

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 outside or in a specialized room designed for cough-inducing procedures. Label specimen container before giving to patient.
2. Instruct the patient on how to collect the sputum sample.
 - a. Put a mark at the 5 ml level on the sputum container 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. Fill out laboratory requisition completely before shipping specimen to laboratory.
 - a. On the specimen container, record the patient name and the date and time of collection.
 - b. Use SJCPHL Requisition form. Required information: patient name, address, date of birth, date and time of specimen collection, type of specimen, submitter and physician name. If testing is being billed please provide medical provider information and billing information.



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. If specimen being couriered to SJCPHL than place specimen inside ziplock bag and requisition form in outer pouch of zip lock specimen bag.



Refer to the “Specimen Collection and Shipment Supplies” topic in this section.

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. Have patient or health care person deliver specimen to laboratory as soon as possible.



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 container 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 deliver specimen to the laboratory.
4. Provide patient with zip lock specimen bag, with completed laboratory requisition form and paper bag for transport. Have patient fill in date and time of collection on requisition form.

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 Hospital facility that has appropriate respiratory protection, environmental controls, policies and procedures in place.

How to Collect Gastric Aspirates

The following are basic guidelines for collecting gastric aspirates:

- Gastric aspirates are usually collected from children who are unable to produce sputum specimens. Hospitalization is usually required for collection.
- Early morning specimens just after patient gets out of bed and prior to eating are the optimal time to collect respirator specimens that have been swallowed by patient during the night.
- Collection of more than one sample is best
- Specimen must be submitted to laboratory within 4 hours of collection as gastric samples must be neutralized in order to preserve mycobacteria present in sample. Specimens not neutralized within 4 hours are unsatisfactory for testing.



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 Collection and Shipping Supplies

San Joaquin County provides specimen containers and shipping supplies by request.

Specimen Collection and Shipping supplies provided by the San Joaquin County Public Health Services Laboratory				
Item	Who May Order/Use	Cost to Patient	How to Obtain	Contact
Specimen Collection Supplies				
Sputum Collection Containers	Health care providers	No cost	Use copy of requisition found in Recourses and Reference section.	SJCPHS Laboratory @ (209)-468-3460 or fax no. (209) 468-
Blood Collection Tube	Health care providers	No cost	Use copy of requisition found in Recourses and Reference section.	SJCPHS Laboratory @ (209)-468-3460 or fax no. (209) 468-0639.
IGRA testing	Health care providers	Medical can be billed or fee payed by requestor or by Public Health Order	Contact Public Health Laboratory. Special collection protocols are required	SJCPHS Laboratory @ (209)-468-3460 or fax no. (209) 468-0639.
Shipping Supplies				
Specimen transport bags or shipping	Health care providers	No cost	Use copy of requisition can be found in Recourses and Reference section.**	SJCPHS Laboratory @ (209)-468-3460 or fax no. (209) 468-

** Diagnostic shipping box may be provided or assistance will be given in selecting proper containers.

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 (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:

- Public Health Service/CDC: 42 CFR, Part 72—Interstate Shipment of Etiologic Agents at <http://www.cdc.gov/od/ohs/biosfty/shipregs.htm>
- USPS: 39 CFR and USPS Domestic Mail Manual C023.1.1, International Mail Manual 135, and USPS Publication 52.
- US Department of Transportation: 49 CFR, Parts 171–180 (August 14, 2002) at http://www.access.gpo.gov/nara/cfr/waisidx_04/49cfrv2_04.html
- The Department of Labor, Occupational Safety and Health Administration (OSHA):29 CFR 1910.1030⁶

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 or assistance please contact Public Health Laboratory
@ 209-468-3460

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 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.
 - ¹ ATS, CDC, IDSA. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med*. 2000;161:1376–1395.
 - ¹ Iseman, MD. *A Clinician’s Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
 - ¹ Iseman, MD. *A Clinician’s Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
 - ¹ Iseman, MD. *A Clinician’s Guide to Tuberculosis, 2000*. 1st ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
 - ¹ National Jewish Medical and Research Center. *How to Mail Specimens and Cultures to the National Jewish Mycobacteriology Laboratory*. Denver, CO; March 2005:2.
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FOR LAB USE ONLY:

DATE REQUEST RECEIVED/INITIALS _____

DATE SUPPLIES FILLED/INITIALS _____

DATE SUPPLIES SENT/INITIALS _____

LABORATORY SUPPLY REQUEST

FAX OR MAIL COMPLETED FORM TO:

**PUBLIC HEALTH SERVICES SAN JOAQUIN COUNTY
PUBLIC HEALTH LABORATORY (SPECIMEN PROCESSING)
P.O. BOX 2009
STOCKTON, CA 95201
TELEPHONE: (209) 468-3460 FAX: (209) 468-0639**

SPECIMEN CONTAINER:	NO. REQUESTED:	NO. SENT
Acid Fast Bacilli culture bottle		
ENTERIC		
OVA & PARASITES		
HIV: Serum kits		
Oral Fluid Kits		
Chlamydia: Culture M4 (Viral Transport Media)		
HERPES/VIRUS ISOLATION (M-4)		
Chlamydia/GC NAAT Kits:		
Urine collection kit:		
Unisex Swab collection kit		
Water		
Blood Collection Tubes:		
Gold top with serum separator		
Yellow Top – TB Blood		
LAB FORMS ONLY (SPECIFY)		
SPECIMEN TRANSPORT BAGS		
OTHER (SPECIFY)		

REQUESTED BY:

COUNTY

STREET

CITY

ZIP