

# Specimen Collection and Laboratory Services

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# Introduction

## Purpose

Use this section to

- get 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).<sup>1</sup>

Effective TB control requires timely, complete, and accurate communication among the laboratory system, TB control program, and healthcare provider.<sup>2</sup>



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

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# Laboratory Contact Information

To locate and contact a laboratory, refer to Table 1: **Laboratory Contact Information**. For the list of the tests performed at each laboratory, refer to Table 2: **Available Laboratory Tests**.

TABLE 1: LABORATORY CONTACT INFORMATION

| Role and Responsibilities  | Contact Information   |
|--|---|
| <p>State Laboratory</p> <p>Accepts primary specimens for AFB smear and culture, and referral isolates for identification and susceptibility testing of <i>Mycobacterium tuberculosis</i>. Serves as a resource for questions involving mycobacterial laboratory testing.</p> | <p>Idaho Bureau of Laboratories (IBL)</p> <p>Colleen Greenwalt</p> <p>2220 Old Penitentiary Road</p> <p>Boise, ID 83712</p> <p>Phone: (208)334-2235</p> |
| <p>Private Laboratories</p> <p>Provide primary specimen smear and culture for mycobacterium. Refer isolates for identification and susceptibility testing.</p>   | <p>This varies greatly. Check your local hospitals for services currently available.</p>  |

# Available Laboratory Tests

The laboratory tests listed below in Table 2 are available. See also <http://www.healthandwelfare.idaho.gov/site/3528/default.aspx> for Laboratory Submission Guidelines, which are also located in the Investigative Guidelines.

When performing tests for the diagnosis of mycobacterial infection, both acid-fast bacilli smear and culture should be ordered. Culture identification is automatically performed on any isolate, and first-line susceptibility testing is automatically performed on all initial *Mycobacterium tuberculosis* complex isolates. *Nucleic acid amplification testing (NAAT) may increase the speed of diagnosis on new suspected TB patients and can be ordered on a case-by-case basis after discussion with laboratory staff.*

TABLE 2: AVAILABLE LABORATORY TESTS

| Test                          | Laboratory   | Turnaround Time  |
|-------------------------------|--|--|
| Diagnosis                     |  |  |
| QuantiFERON®-TB Gold (QFT-G)  | Not currently available in Idaho but some adjacent states (Washington, Utah) do have it available—contact the Idaho Bureau of Laboratories or State TB Program to discuss. |  |
| Acid-fast (AFB) bacilli smear | Idaho Bureau of Laboratories and many private clinical laboratories  | Generally within 24 hours from receipt in laboratory   |
| Culture                       | Idaho Bureau of Laboratories; some private clinical laboratories accept specimens for culture but will generally forward them to the IBL or another reference laboratory   | Cultures are incubated for up to 6 weeks before reported as negative. Time to detection of mycobacterial growth is dependent on growth rate. Ideally, growth of <i>Mycobacterium tuberculosis</i> should be detected within 14 days of specimen inoculation, although this is dependent on many factors. |
| Culture identification        | Idaho Bureau of Laboratories   | Ideally, <i>M. tuberculosis</i> complex should be identified within 21 days of specimen inoculation.   |
| Drug susceptibility           | Idaho Bureau of Laboratories   | Ideally, results of first-line   |

| Test   | Laboratory  | Turnaround Time   |
|--|---|---|
|  |   | drugs should be available within 30 days from specimen receipt in the laboratory, but this is dependent on many factors (for example, growth rate, presence of a pure culture). |
| Nucleic acid amplification (NAA) test  | Idaho Bureau of Laboratories may send samples, on a case-by-case basis, to National Jewish Medical and Research Center  | Within 48 hours from laboratory receipt of specimen   |
| <b>Treatment Monitoring</b>  |   |   |
| 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 local clinical laboratories   | Usually available same day  |
| Uric acid  | Available at most local clinical laboratories   | Usually available same day  |
| Complete blood count (CBC) and platelets   | Available at most local clinical laboratories   | Usually available same day  |
| Kidney function  | Available at most local clinical laboratories   | Usually available same day  |
| <b>Epidemiologic Monitoring</b>  |   |   |
| Genotyping   | Idaho Bureau of Laboratories sends all MTB isolates to the California State Public Health Laboratory for spoligotyping and MIRU typing; RFLP will also be done by special request if cluster of cases is suspected. | 2 to 4 weeks from specimen receipt  |

Laboratories should report positive smears or positives 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.<sup>3</sup>



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

For a list of all of the services available, see the Idaho Investigative Guidelines, Section IV, Laboratory Protocols, which is on the Learning Management System in the “Investigative Guidelines” Team Room and also in the Investigative Guidelines binder.

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# Genotyping

Genotyping is a useful tool for studying the pathogenesis, epidemiology, and transmission of *Mycobacterium tuberculosis*. *M. tuberculosis* genotyping refers to laboratory procedures developed to identify *M. tuberculosis* isolates that are identical in specific parts of the genome (of similar strain types).

Genotyping is based on an analysis of deoxyribonucleic acid (DNA). Mycobacteria reproduce by binary fission, which means that in almost all cases each new bacillus has identified DNA, just as human identical twins are genetically identical to each other. However, changes in the DNA occur spontaneously at low frequency. Over time, these changes, known as DNA mutations, have accumulated to produce the diversity of *M. tuberculosis* strains currently circulating in the world.

The diversity of strain provides a means to identify instances of recent transmission of TB as well as the chains of transmission that occur among persons with TB. This diversity also helps to elucidate the patterns and dynamics of TB transmission. When a person with TB improves but then becomes ill again, this diversity can differentiate reactivation with the same strain of *M. tuberculosis* from reinfection with a different strain. Genotyping can also be used to identify false-positive cultures.

Advances in DNA analytic methods have made it possible for TB programs to obtain rapid and reliable genotyping results. These advances include the following:

- The determination of the complete DNA sequence of *M. tuberculosis* in 1998
- The development of IS6110-based restriction fragment length polymorphism (RFLP) genotyping, which provided a discriminatory typing method and led to a standardized system for genotyping *M. tuberculosis* isolates
- The development of two new methods, spoligotyping and mycobacterial interspersed repetitive units (MIRU) analysis, which are based on polymerase chain reaction (PCR) and provide much more rapid results than RFLP analysis<sup>4</sup>

Confirmation of suspected transmission via normal surveillance data collection and epidemiologic investigations has been enhanced by the addition of information from genotyping. A potential outbreak should be suspected whenever there is more than one case of tuberculosis (TB) whose isolate has the same genotype (genotype cluster). Further investigation that includes a review of surveillance data, chart review, and re-interview of TB cases may refute or confirm the epidemiologic connection between more than one TB case. In some instances, a genotype cluster reflects a false-positive culture that may be a result of laboratory cross-contamination. Routine review of genotyping data along with epidemiologic, clinical, and laboratory data may identify patients who are wrongly classified as TB patients and should be further investigated.

The Idaho TB Program reviews genotyping data to check for any matches. Upon identification of a match, the Idaho TB Program Manager notifies the local public health districts managing the cases to discuss what further steps should be taken.



For more information on genotyping, see the National Tuberculosis Controllers Association/ Centers for Disease Control and Prevention (CDC) Advisory Group on Tuberculosis Genotyping's *Guide to the Application of Genotyping to Tuberculosis Prevention and Control* (2004) at <http://www.cdc.gov/tb/genotyping/manual.htm> .



All *M. tuberculosis* cultures originating at the Idaho Public Health Laboratory are submitted to a national genotyping laboratory for genotyping analysis.

After the Idaho Genotyping Plan is completed, more detailed instructions and information will be provided.

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## 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, so 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 conditions that inhibit the growth of contaminating organisms, since most specimens will contain bacteria other than mycobacteria.<sup>5</sup> Send specimens for AFB smear, culture, and drug susceptibilities.

Refer to Table 3 to review the methods used to collect various specimens and the type of specimens obtained for pulmonary TB.



During 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 <http://www.cdc.gov/mmwr/pdf/rr/rr5417.pdf>.

TABLE 3: SPECIMEN COLLECTION METHODS AND TYPES FOR PULMONARY TUBERCULOSIS

| Pulmonary Tuberculosis  |   |
|---|---|
| Collection Method   | Specimen Type   |
| <p><b>Spontaneous sputum collection</b> occurs when the patient can cough up sputum without extra assistance.</p>   | <ul style="list-style-type: none"> <li>▪ 5–10 ml sputa from deep in the lung</li> </ul>   |
| <p><b>Induced sputum collection</b> should be considered if a patient needs assistance in bringing up sputum.*</p>  | <ul style="list-style-type: none"> <li>▪ 5–10 ml sputa from deep in the lung</li> </ul>   |
| <p><b>Gastric aspirates</b> can be submitted for the diagnosis of pulmonary tuberculosis (TB) in young children who cannot produce sputum.</p>  | <ul style="list-style-type: none"> <li>▪ 50 ml of gastric contents</li> </ul>   |
| <p><b>Bronchoscopy</b> can be used in the following situations:</p> <ul style="list-style-type: none"> <li>▪ If a patient cannot produce sputum by the above three methods<sup>6</sup> or</li> <li>▪ If a patient has a substantial risk of drug-resistant TB and has initial routine studies that are negative<sup>7</sup> or</li> <li>▪ In a patient in whom there is suspicion of endobronchial TB<sup>8</sup> or</li> <li>▪ If a variety of clinical specimens for the diagnosis of pulmonary TB or other possible diseases needs to be obtained</li> </ul> | <ul style="list-style-type: none"> <li>▪ Bronchial washings</li> <li>▪ Bronchoalveolar lavage</li> <li>▪ Transbronchial biopsy</li> </ul> |
| <p>* It is important to specify if the sputum is induced or note because induced sputum is “more watery” and appears to be just saliva. Some laboratories may throw induced sputum out and report it as an inadequate specimen.</p>   |   |

Refer to Table 4 for collection methods and specimen types for extrapulmonary TB.

TABLE 4: 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. | <b>Examples of tissues (biopsy)*</b> <ul style="list-style-type: none"> <li>▪ Lymph node</li> <li>▪ Pleural</li> <li>▪ Bone/joint</li> <li>▪ Kidney</li> <li>▪ Peritoneal</li> <li>▪ Pericardial</li> </ul> | <b>Examples of fluids</b> <ul style="list-style-type: none"> <li>▪ Pleural</li> <li>▪ Cerebrospinal</li> <li>▪ Blood</li> <li>▪ Urine</li> <li>▪ Synovial</li> <li>▪ Peritoneal</li> <li>▪ Pericardial</li> </ul> |
| * Do not place specimens in formalin.   |   |   |

## 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.<sup>2</sup>
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.)
3. Review with the patient how to collect sputum.
4. Make sure the specimen container and laboratory requisition are filled out completely before shipping.
  - a. On the specimen container, record the patient name and the date filled out completely before shipping.

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



- b. Use the Idaho Bureau of Laboratories specimen submission form if the sample is to be shipped to the Idaho state public health laboratory; the form is available at

[http://www.healthandwelfare.idaho.gov/portal/alias\\_Rainbow/lang\\_en-US/tabID\\_3531/DesktopDefault.aspx](http://www.healthandwelfare.idaho.gov/portal/alias_Rainbow/lang_en-US/tabID_3531/DesktopDefault.aspx).

- c. 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."



5. Make sure the specimen and laboratory requisition are packaged into appropriate shipping containers, per laboratory instructions. (See the Specimen Shipment subtopic below.)
6. 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.
7. Do not keep specimens to send all three on the same day.
8. Use the most rapid transport to the laboratory: yourself, courier, or 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 15 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 8 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* at

[http://www.nationaltbcenter.edu/products/product\\_details.cfm?productID=ONL-06](http://www.nationaltbcenter.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.

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# Specimen Shipment

There are three main categories of transportation methods: medical couriers, ground transportation, and air transportation. Category B Infectious Substances (raw diagnostic specimens such as sputum, blood, or tissue) can be mailed through the U.S. Postal Service, shipped by private carrier (e.g., Federal Express, Airborne Express, etc.), or transported by a medical courier. Pure mycobacterial cultures (or culture isolates suspected of being mycobacteria) are Category A Infectious Substances and can be only be transported by a medical courier or shipped by private carrier as dangerous goods. Category A Infectious Substances cannot be mailed through the U.S. Postal Service. Each category requires different packaging requirements to provide increased levels of protection against leaks and contamination.

Shipment of dangerous goods by USPS is regulated by the U.S. Department of Transportation. Specific shipping instructions from CDC can be found in the publication by the U.S. 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 <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
- U.S. Department of Transportation: 49 CFR, Parts 171–180 (August 14, 2002) [http://www.access.gpo.gov/nara/cfr/waisidx\\_04/49cfrv2\\_04.html](http://www.access.gpo.gov/nara/cfr/waisidx_04/49cfrv2_04.html)
- The Department of Labor, Occupational Safety and Health Administration (OSHA): 29 CFR 1910.1030<sup>9</sup>

For shipments by private carriers, follow International Air Transportation Association (IATA) instructions. *M. 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.<sup>10</sup>

Refer to the shipping regulations that are listed under Resources at the end of this section. Personnel who handle, package, and ship infectious materials must be trained in these procedures.



Anyone who will be packaging and shipping infectious substances must be certified. For packaging and shipping recommendations and training information, call Jay Conn at the IBL at (208) 334-2234 x226.



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

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# Resources and References

## Resources for Laboratory Services

Detailed descriptions of recommended laboratory tests; recommendations for their correct use; and methods for collecting, handling, and transporting specimens have been published. (For easy access to references, hyperlinks are provided for online references in the list below.)

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]) 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]) at <http://www.cdc.gov/tb/pubs/PDF/1376.pdf>
- Idaho Public Health Laboratory. [http://www.healthandwelfare.idaho.gov/portal/alias\\_Rainbow/lang\\_en-US/tabID\\_3531/DesktopDefault.aspx](http://www.healthandwelfare.idaho.gov/portal/alias_Rainbow/lang_en-US/tabID_3531/DesktopDefault.aspx)
- National Committee for Clinical Laboratory Standards. *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard* [Document no. M24-A] (Wayne, PA 2003)

## Resources for Specimen Collection and Shipment

- CDC. "Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-care Settings, 2005" (*MMWR* 2005;54[No. RR-17]) at <http://www.cdc.gov/mmwr/pdf/rr/rr5417.pdf>
- CDC. *Public Health Mycobacteriology: A Guide for the Level III Laboratory*
- Francis J. Curry National Tuberculosis Center. *Conducting Sputum Induction Safely* at [http://www.nationaltbcenter.edu/products/product\\_details.cfm?productID=WPT-01](http://www.nationaltbcenter.edu/products/product_details.cfm?productID=WPT-01)
- Francis J. Curry National Tuberculosis Center. *Pediatric TB: A Guide to the Gastric Aspirate (GA) Procedure* at [http://www.nationaltbcenter.edu/products/product\\_details.cfm?productID=ONL-06](http://www.nationaltbcenter.edu/products/product_details.cfm?productID=ONL-06)
- International Air Transport Association (IATA). *Dangerous Goods Regulations ED 43* at <http://www.iata.org/index.htm>
- Idaho Public Health Laboratory. *MTPHL Laboratory Services Manual* at <http://www.dphhs.mt.gov/PHSD/Lab/Clinical/pdf/clinical-lab-manual2006.pdf>
- National Jewish Medical and Research Center. *How to Mail Specimens and Cultures to the National Jewish Mycobacteriology Laboratory* (March 2005)

- National Jewish Medical and Research Center. *Instructions (for Patients) for Collecting and Mailing Sputum Specimens*
- National Tuberculosis Controllers Association-National Tuberculosis Nurse Consultant Coalition. *Tuberculosis Nursing: A Comprehensive Guide to Patient Care* (Atlanta, GA. 1997):39–42
- U.S. Department of Transportation. Hazardous Materials: Revision to standards for infectious substances. Part III 49 CFR part 171. Federal Register (August 14, 2002)
- USPS. *Mailing Standards of the United States Postal Service: Domestic Mail Manual* at <http://pe.usps.com/>

## References

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- <sup>1</sup> 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.
  - <sup>2</sup> Association of Public Health Laboratories. The Future of TB Laboratory Services: A framework for integration / collaboration / leadership. 2004.
  - <sup>3</sup> CDC. Diagnostic microbiology; in Chapter 5: Diagnosis of TB. Core Curriculum (2000). Revised November 2001.
  - <sup>4</sup> National Tuberculosis Controllers Association/ Centers for Disease Control and Prevention (CDC) Advisory Group on Tuberculosis Genotyping's *Guide to the Application of Genotyping to Tuberculosis Prevention and Control* [DTBE Web site]. Available at: <http://www.cdc.gov/tb/genotyping/manual.htm>. Accessed February 7, 2007.
  - <sup>5</sup> CDC, ATS, IDSA. Diagnostic standards and classification of tuberculosis in adults and children, 1999. *Am J Respir Crit Care Med*, (2000) Vol. 161:1376-1395.
  - <sup>6</sup> Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1<sup>st</sup> ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
  - <sup>7</sup> Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1<sup>st</sup> ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
  - <sup>8</sup> Iseman, MD. *A Clinician's Guide to Tuberculosis, 2000*. 1<sup>st</sup> ed. Philadelphia, PA: Williams & Wilkins; 2000:135–136.
  - <sup>9</sup> National Jewish Medical and Research Center. *How to Mail Specimens and Cultures to the National Jewish Mycobacteriology Laboratory* (March 2005):2.
  - <sup>10</sup> National Jewish Medical and Research Center. *How to Mail Specimens and Cultures to the National Jewish Mycobacteriology Laboratory* (March 2005):5–7.

