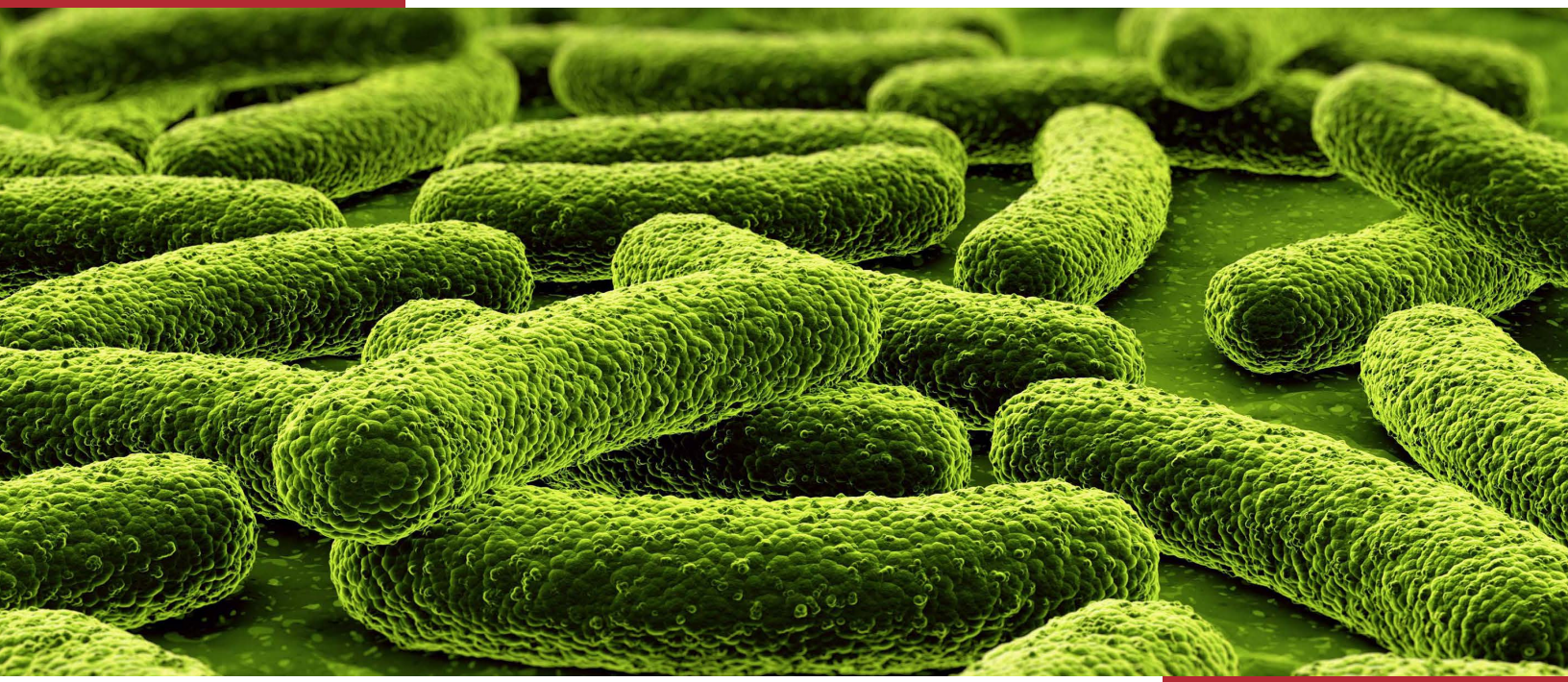
Guidelines for Submission of Sputum Specimens for Tuberculosis Testing



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APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

A key role of the public health laboratory is to provide specialized testing for low incidence, high risk diseases such as tuberculosis (TB). This includes testing of patient samples to identify *Mycobacterium tuberculosis*, perform drug susceptibilities for treatment guidance and identify clusters of active disease transmission through genotyping.[1]

Background

Public health laboratories throughout the US perform diagnostic and reference services in support of the National Plan for Elimination of TB. The level of service provided and frequency these services are provided vary among states. This guidance may be used by laboratories to assist in the development of institutional policies to ensure proper collection and submission of sputum specimens for TB testing.

In 2015, the Association of Public Health Laboratories (APHL) and the US Centers for Disease Control and Prevention (CDC) described models of network collaboration in six states.[2] The article recommended that laboratorians and TB control officials work together to design a system to prioritize testing and maximize resources to obtain prompt, reliable test results. Recommended benchmarks were offered to improve laboratory TB services and TB control. The article discussed strategic planning to help jurisdictions select appropriate resources and testing algorithms to serve their population and public health system. Capacity, capability and cost analysis were factors which must be evaluated for the maintenance and improvement of TB services.

Sputum Specimen Collection

The diagnosis of TB, management of patients with the disease, and public health TB control services rely on accurate and timely laboratory test results. Laboratory services are an essential component of effective TB control, providing key information to clinicians and public health agencies.

Quality specimens are vital for the laboratory diagnosis of TB. Sputum, a respiratory secretion originating from deep within the lungs, is the most frequent specimen collected for TB testing. Patients should be instructed on the difference between sputum, saliva or nasopharyngeal secretions and the necessity for a deep, productive cough. Sputum specimens are preferably collected under the direction of a trained health care professional. Sputum induction with hypertonic saline may be necessary to obtain quality specimens when a patient is unable to produce sputum spontaneously and bronchoscopy may be considered for patients who are unable to produce sputum at all.

Specimens should be collected in containers that are sterile, clear, plastic and leak-proof such as a 50-ml screw-cap centrifuge tube. Sputum collection devices and wide-mouth sterile collection containers are commercially available. It is recommended that specimens be delivered to the public health laboratory within 24 hours of collection. Samples that cannot immediately be transported to the laboratory should be refrigerated to reduce growth of contaminating endogenous respiratory organisms. More detailed instructions for collection of spontaneous and induced sputum specimens are included in Appendix A.

TB Testing for Initial Diagnosis

For initial diagnosis of pulmonary TB, collect a series of three sputum specimens 8-24 hours apart, with at least one obtained as an early morning specimen.[3] Optimally, specimens should be collected before drug therapy is started, since a few days of treatment may inhibit growth and prevent isolation of *M. tuberculosis* complex. Certain commercial nucleic acid amplification (NAA) tests cannot be performed if patients have been on anti-tuberculous therapy for three days or more when using the Cepheid Xpert MTB/RIF® nucleic acid amplification test (Xpert MTB/RIF®) or seven days or more with the Hologic Amplified MTD Test.[4,5] Samples submitted for the initial diagnosis of TB should be tested by both concentrated smear and culture. Reports of AFB smear results should be made to the submitting agency within 24 hours. Cultures should be held for a period of at least six weeks before being reported as negative. It is recommended that NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB where a diagnosis of TB is being considered but has not yet been established, and where the test result would alter case management and TB control activities.[6]

TB Testing for Release from Airborne Isolation Infection (AII)

Several criteria have been used to determine when a patient with suspected TB may be released from hospitalized airborne isolation:

1) an alternative diagnosis is established that explains the clinical presentation,
2) a multi-drug course of chemotherapy has been administered for a minimum of two weeks and there is clinical evidence of improvement such as a decrease in symptom severity, radiographic findings indicating improvement, or other medical determination of improvement, and
3) the patient's sputum or bronchial secretions are free of acid fast bacilli as determined by three consecutive negative smear results.[3]

Specimens to be tested for patient release from hospitalized airborne isolation should be collected at 8-24 hour intervals, with at least one obtained as an early morning specimen. It is not necessary to perform cultures on these specimens unless multi-drug resistant *M. tuberculosis* (MDR-TB) is present.

In February 2015, the US Food and Drug Administration approved the use of the Xpert MTB/RIF® as an alternative to the examination of three initial sputum smears to aid in the decision to discontinue AII for patients with a high suspicion of pulmonary TB.[7] However, Xpert MTB/RIF® should not be used as an alternative for the three negative smears following two weeks of treatment as it can detect dead and live bacteria. According to this change, negative results using the Xpert MTB/RIF® on either one or two sputum specimens may be used in making decisions to discontinue AII. The National Tuberculosis Controller's Association (NTCA) and APHL have created a consensus statement on the use of Xpert MTB/RIF® for this purpose.[8] A decision to remove a patient with a negative Xpert MTB/RIF® result from AII must consider the patient's clinical presentation and the possible risk of transmission of TB from an infectious patient to others. Such a decision should not be based on sputum test results alone. It has been suggested that use of Xpert MTB/RIF® could provide cost savings to the health care system by reducing the time a patient is in isolation.[9]

TB Testing to Monitor the Course of Treatment

For patients whose sputum cultures are positive before treatment, the best method to measure the effectiveness of therapy is to obtain specimens for culture at least monthly until cultures convert to negative.[10] If a sputum culture becomes contaminated with non-acid fast bacteria, laboratories should request submission of a new sputum specimen to avoid gaps in patient monitoring. Patients with MDR-TB should have cultures performed monthly for the entire course of treatment. Patients whose cultures have not become negative or whose symptoms do not resolve despite three months of therapy should be re-evaluated for potential drug-resistant disease, as well as for potential failure to adhere to the treatment regimen. Laboratories should consider consultation with their TB Centers of Excellence for Training, Education and Medical Consultation.

TB Testing and Follow-up for Drug Resistance

Patients with MDR-TB should have cultures performed monthly for the entire course of treatment. Second-line drug susceptibility testing should be considered for patients who:

- have had prior therapy
- are contacts of patients with drug resistant TB
- have demonstrated resistance to rifampin or to other first-line drugs, or
- have positive cultures after three or more months of treatment.[10]

If drug susceptibility test results show resistance to rifampin or any other first line drugs, or if the patient remains symptomatic or smear/culture positive after three months, a tuberculosis medical expert should be consulted. Consider consultation with the TB Centers of Excellence for Training, Education and Medical Consultation.

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APPENDIX A: SPUTUM COLLECTION FOR TUBERCULOSIS*

Purpose

To obtain sputum specimens for AFB smear microscopy and culture from a patient with suspected pulmonary tuberculosis.

Materials and Equipment Required

1. Sterile, filtered water or normal saline (150-250 mL)
2. N95 mask (particulate respirator) for AFB
3. Gloves
4. Box of tissues
5. Sterile specimen container approved by the laboratory for sputum collection and transport
6. ALSO, for Nebulized Sputum Induction:

   a. A hand-held nebulizer with mouthpiece and 15 mL vial of 3% saline. Note: A mask may be used if a patient absolutely cannot use the mouthpiece.

   b. Patients who are unable to protect their airway or are at risk for aspiration should be NPO for 3 hours prior to the induction procedure to reduce the risk of vomiting and aspiration.

Procedure

Ensure that the patient is outdoors or placed in an airborne isolation room or negative-pressure sputum collection booth with the door shut. The air in the negative-pressure room or booth should be drawn out of the space and vented outside of the building.

PREPARATION (FOR SPONTANEOUS OR INDUCED SPUTUM)

1. Instruct the patient to gently brush his/her teeth, gingival margins, tongue and buccal surfaces using sterile, filtered water or normal saline to rinse
2. Do not use toothpaste, commercial mouth wash preparations, nose drops, or any medications containing alcohol, or oil. Instruct the patient to avoid taking oral antibiotics immediately before the sputum collection procedure.
3. Instruct the patient to gargle several times with sterile, filtered water or normal saline after brushing. Do not use tap water or bottled water, as it may contain non-tuberculous mycobacteria that may alter findings.

SPONTANEOUSLY PRODUCED SPUTUM COLLECTION

1. Observe standard precautions at all times.
   Note: *N95 masks must be worn by healthcare personnel for AFB cough-producing procedures.*
2. Coach the patient and supervise the first sputum collection, at a minimum, in order to obtain a good quality sputum sample that represents secretions from the lower respiratory tract.
   Note: *The patient should understand that sputum is material that is brought up from the lungs and that nasal secretions and saliva or spit are not acceptable.*
3. Instruct the patient to inhale deeply, as far as possible, and then exhale slowly three times.
4. After the third breath, direct the patient to inhale completely and try to cough hard to produce sputum from deep in the lungs. The patient may feel a rattle or tickle as the sputum moves up from the lungs into the throat.
5. Instruct the patient to expectorate the sputum into a sterile specimen container.

6. When there is at least 5 mL (1 teaspoon) of sputum, replace the lid on the container and tighten it so it does not leak. Apply Parafilm to the outer edges of the sterile specimen container to further protect the container from leaking during shipment and processing.
   Note: *High-quality sputum is required for smear, culture and NAA testing. For AFB NAA testing alone, a minimum of 1 mL of raw sputum (or 0.5 mL of sputum sediment) is needed. It is preferred to collect 5-10 mL of raw sputum.*
7. If the patient is in a negative air pressure room or booth, ask the patient remain in the booth or room until cleared to leave.
8. Label the specimen with time and date of its collection and place it in a specimen bag. Attach a laboratory request form, if applicable.
9. Document the procedure in the appropriate flow sheet or medical record.
   Note: *Documentation also is required for unsuccessful procedures.*

NEBULIZED SPUTUM INDUCTION AND COLLECTION

1. Observe standard precautions at all times.
   Note: *N95 masks must be worn by healthcare personnel for AFB cough-producing procedures.*
2. Place approximately 5 mL of 3% saline into the hand-held nebulizer. Set the flow at 6-8 L/min and nebulize saline for 7-10 minutes or until sputum is expectorated. The maximum nebulization time is 20 minutes.
   Note: *More saline may be added to the nebulizer if more than 10 minutes is needed to produce an adequate cough.*
3. Ask the patient to inhale the nebulized 3% saline deeply 2-3 times followed by a vigorous cough. This will assist in expectorating quality sputum. Collect the sputum into a sterile specimen container.
   Note: *Coaching the patient is very important in order to get quality results in a timely manner.*
   Note: *High-quality sputum is required for smear, culture and NAA testing. For AFB NAA testing alone, a minimum of 1 mL of raw sputum (or 0.5 mL of sputum sediment) is needed. It is preferred to collect 5-10 mL of raw sputum.*
4. Label the specimen with time and date of its collection and place it in a specimen bag. Attach a laboratory request form, if applicable.
5. Document the procedure in the appropriate flow sheet or medical record.
   Note: *Documentation also is required for unsuccessful procedures.*

*Adapted from *Controlling TB in Correctional Facilities*. Atlanta, GA, 1995.

Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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