

### Sputum Specimen Collection

Sputum smears and culture conversion are frequently used to evaluate treatment response in pulmonary tuberculosis patients. High-quality specimens are vital for the laboratory diagnosis of TB, and sputum is the most frequent specimen collected for TB testing. Sputum is a respiratory secretion originating from deep within the lungs. Patients should be instructed on the difference between sputum and 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 specimens, and bronchoscopy may be considered for patients who are unable to produce sputum. Specimens should be collected in containers that are sterile, clear, plastic, and leak-proof; for example, 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.

### TB Testing for Initial Diagnosis

For initial diagnosis of pulmonary TB, collect a series of three sputum specimens, 8-24 hours apart, at least one of which is an early morning specimen.<sup>3</sup> Optimally, specimens should be collected before drug therapy is started, as even a few days of treatment may inhibit growth and prevent isolation of *M. tuberculosis* complex (MTBC). Certain commercial nucleic acid amplification (NAA) tests cannot be performed if patients have been on anti-tuberculous therapy for seven or more days.<sup>4</sup>

Samples submitted for the initial diagnosis of TB should be tested by both concentrated smear and culture. Reports of AFB smears 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 for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management and TB control activities.<sup>5</sup>

### TB Testing for Release from Isolation

A patient may be released from isolation if the following criteria are met:

1. Have received standard multidrug anti-TB therapy for at least two weeks if original AFB smear was positive OR on therapy for five to seven days if original AFB smear was negative.
2. Demonstrated adherence to treatment (DOT).
3. Demonstrated clinical improvement such as a decrease in symptom severity, radiographic findings indicating improvement, or other medical determination of improvement
4. Have three consecutive negative AFB smears collected at least eight hours apart with at least one early morning specimen.
5. Have no risk factors for drug resistance.

For patients with positive AFB sputum smears at diagnosis, weekly sputum collection should be done until three consecutive specimens are negative.<sup>3</sup> The following guidelines should be used:

1. Patients with **cavities and/or sputum smears that are 3+ (moderate) or 4 (heavy)**: Wait until after one to two weeks of treatment to collect first follow-up sputum. If the first sputum is negative,

obtain second sputum. If the second is negative, obtain a third. If any are positive, wait one week and start the process over.

2. Patients **without cavities and/or with sputum smears that are 1+ (rare) or 2+ (few)**: A single sputum specimen can be collected after one week of treatment. If the first sputum is negative, obtain a second sputum specimen. If the second is negative, obtain a third. If any are positive, wait one week and start the process over.
3. Once three consecutive smear-negative specimens are collected, the patient can be considered for release from isolation (see above for additional release from isolation criteria). Sputum should then be collected monthly (in sets of three) until conversion to culture negative is documented.

### **Culture Conversion to Monitor the Course of Treatment**

For patients whose sputum cultures are positive before treatment, the best way to measure the effectiveness of therapy is to obtain specimens for culture at least monthly until the cultures convert to negative.<sup>6</sup> Conversion is documented by at least two, preferable three, consecutive negative cultures.

Patients with multidrug-resistant tuberculosis (MDR-TB) should have cultures performed monthly for the entire course of treatment. In some cases, a patient may not be able to produce a sputum specimen after two months of treatment. Every effort should be made to obtain sputum, including induced sputum. However, for patients who cease to produce sputum before conversion, clinical judgment such as symptom resolution and chest x-ray improvement will need to be used.

Patients whose cultures have not become negative or whose symptoms do not resolve despite three months of therapy should be reevaluated for potential drug-resistant disease, as well as for potential failure to adhere to the regimen.

### **References**

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Adapted from APHL, *Guidelines for Submission of Sputum Specimens for TB Testing, 2010*.  
[http://www.aphl.org/mrc/documents/mr\\_2010april\\_tb-sputum-collection-guidelines.pdf](http://www.aphl.org/mrc/documents/mr_2010april_tb-sputum-collection-guidelines.pdf)



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