



STATE OF CONNECTICUT

DEPARTMENT OF PUBLIC HEALTH

Guidelines for Nucleic Acid Amplification Testing for *M. tuberculosis* at the Connecticut Department of Public Health Laboratory

Nucleic acid amplification (NAA) testing for *M. tuberculosis* allows for the rapid identification of tuberculosis (TB) through the detection of *Mycobacterium tuberculosis* complex (MTBC) genetic material directly in clinical specimens. While MTBC may take weeks to grow when using conventional culture techniques, NAA testing results are generally available within 24–48 hours after the specimen is received in the laboratory.

In January 2009, the Centers for Disease Control and Prevention (CDC) made recommendations on the use of NAA tests for the diagnosis of TB (1).

Two main highlights from those recommendations include the following:

- NAA testing should become standard practice for moderate to high TB suspects.
- NAA testing should be performed on at least one respiratory specimen from each patient with signs/symptoms of pulmonary TB but for whom the diagnosis has not been established AND for whom the test result would change case management or TB control activities.

NAA testing for MTBC is performed at the Connecticut Department of Public Health (CTDPH) Laboratory using the Cepheid® Xpert MTB/RIF test. The Food and Drug Administration has permitted marketing of the Xpert MTB/RIF assay and the CTDPH laboratory has verified its performance specifications for the detection of MTBC DNA in sputum, bronchoalveolar lavage (BAL) and bronchial wash specimens that are either acid-fast bacilli (AFB) smear positive or negative and, in these specimens where MTBC is detected, for the detection of the rifampin (RIF)-resistance associated mutations of the *rpoB* gene.

In October 2013, the Centers for Disease Control and Prevention made recommendations for incorporation of the Xpert MTB/RIF assay into tuberculosis diagnostic algorithms (2).

In addition to a summary of the use of NAA for the detection of MTBC, the document includes information on practical considerations for use of the Xpert MTB/RIF assay for detection of mutations associated with rifampin resistance and considerations for infection control.



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Intended Use

- The Xpert MTB/RIF test is intended as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings. It is intended for use with specimens from patients for whom there is clinical suspicion of tuberculosis and at the time of specimen collection have received no antituberculosis therapy or less than 3 days of therapy. NAA testing should not be ordered routinely when clinical suspicion is low, because the positive predictive value of NAA tests (the likelihood that the patient has tuberculosis when the test is positive) is low for such cases.
- NAA testing is not indicated for use in determining bacteriologic cure or to monitor response to antituberculous therapy.

CTDPH Laboratory Specimen Submission Policy & Protocol for NAA Testing

- NAA testing will only be performed on raw unprocessed sputum, bronchoalveolar lavage (BAL) and bronchial wash specimens.
- NAA testing will be automatically performed on the first specimen received for each patient found to be AFB SMEAR POSITIVE by the CTDPH laboratory.
- NAA testing will be performed on specimens found to be AFB SMEAR NEGATIVE by the CTDPH laboratory ONLY ON REQUEST by the submitter or other authorized provider. To request that NAA testing be performed, regardless of AFB smear results, include a *Mycobacterium tuberculosis* complex Nucleic Acid Amplification (NAA) Test Requisition, along with a Clinical Test Requisition [Select AFB Clinical Specimen (Mycobacteria Smear & Culture)] when submitting the specimen. NAA test requests received later than 7 calendar days after receipt of the specimen in the laboratory will not be accepted.
- When requested at submission, NAA testing will generally be performed within two business days of specimen receipt in the laboratory.
- Routine mycobacteria smear and culture testing will always be performed on specimens received for NAA testing.
- The Xpert MTB/RIF Assay does not provide confirmation of rifampin susceptibility. For specimens where RIF resistance is detected, the specimen will be submitted to the CDC for testing to confirm the presence of RIF-resistance associated mutations of the *rpoB* gene and to perform conventional drug susceptibility testing.
- A percentage of sputum specimens (3%--7%) may contain inhibitors that prevent or reduce amplification of the NAA test. Each Xpert MTB/RIF test includes an internal inhibition control to determine if the test specimen contains substances that are inhibiting a positive test when MTBC is present in the specimen. If after repeat testing inhibitors are detected in the specimen, the presence or absence of MTBC DNA



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cannot be determined and the test is reported as invalid (See Interpretation of Test Results).

- Results from NAA testing will be reported to the submitter and other authorized providers by phone and fax/mail.

Interpretation of Test Results

Detection of MTBC

The Xpert MTB/RIF assay does not differentiate between the species of the MTB-complex (i.e., *M. tuberculosis*, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. canettii*, *M. microti*, *M. caprae*, *M. pinnipedi*, *M. mungi*, and *M. orygis*).

NAA test results should be interpreted in conjunction with the AFB smear results (1).

- If the **NAA result is positive and the AFB smear result is positive**, presume the patient has TB and begin anti-TB treatment while awaiting culture results. The positive predictive value of FDA-approved NAA tests for TB is >95% in AFB smear-positive cases.
- If the **NAA result is positive and the AFB smear result is negative**, use clinical judgment whether to begin anti-TB treatment while awaiting culture results and determine if additional diagnostic testing is needed. Consider testing an additional specimen using NAA to confirm the initial NAA result. A patient can be presumed to have TB, pending culture results, if two or more specimens are NAA positive.
- If the **NAA result is negative and the AFB smear result is positive**, use clinical judgment to determine whether to begin anti-TB treatment while awaiting culture results and determine if additional diagnostic testing is needed. A patient can be presumed to have an infection with nontuberculous mycobacteria if a second specimen is smear positive and NAA negative.
- If the **NAA result is negative and the AFB smear result is negative**, use clinical judgment to determine whether to begin anti-TB treatment while awaiting results of culture and additional diagnostic tests. Currently available NAA tests are not sufficiently sensitive (detecting 50–80% of AFB smear-negative, culture-positive pulmonary TB cases) to exclude the diagnosis of TB in AFB smear-negative patients suspected to have TB.
- If **inhibitors are detected**, the presence or absence of MTBC DNA in the specimen cannot be determined. Another sample can be submitted for NAA testing if indicated. Use clinical judgment to determine whether to begin anti-TB treatment while awaiting results of culture and additional diagnostic testing.

Detection of Rifampin-resistance Associated Mutations

- The Xpert MTB/RIF assay provides a RIF result **ONLY** when MTBC DNA is **DETECTED**



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- If RIF Resistance is DETECTED, a mutation in the *rpoB* gene is detected indicating possible RIF resistance. Additional confirmatory testing will be performed (see CTDPH Laboratory Specimen Submission Policy & Protocol for NAA Testing).
- If RIF Resistance is NOT DETECTED, a mutation in the *rpoB* gene has not been detected. The MTBC detected is probably RIF susceptible.
- If RIF Resistance is INDETERMINATE, the presence of a mutation in the *rpoB* gene cannot be accurately determined.

For additional questions, contact the DPH Tuberculosis Control Program at (860) 509-7722 or the DPH Public Health Laboratory at (860) 509-8573.

References

1. Centers for Disease Control and Prevention. Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis. MMWR, 2009; 58: 7-10.
Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm>.
2. Centers for Disease Control and Prevention. Availability of an Assay for Detecting *Mycobacterium tuberculosis*, Including Rifampin-Resistant Strains, and Considerations for Its Use — United States, 2013. MMWR 2013; 62: 821-824.
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6241a1.htm?s_cid=mm6241a1_e



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