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The Commonwealth of Massachusetts
Executive Office of Health and Human Services
Department of Public Health
William A. Hinton State Laboratory Institute
Bureau of Infectious Disease Prevention,
Response and Services
305 South Street, Jamaica Plain, MA 02130

Division of Tuberculosis Prevention and Control
(617) 983-6970

M E M O R A N D U M

To: Massachusetts Clinicians and Microbiology Laboratory Directors

From: John Bernardo, MD, Medical Officer, TB Division
Linda Han, MD, MPH, Acting Director, Hinton State Laboratory Institute
Paul Elvin, Supervisor, Mycobacteriology Laboratory, Hinton State Laboratory Institute

Subject: Molecular testing for *M. tuberculosis* in Respiratory Secretions

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In January 2009, CDC published *Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis*, a revision of their previous statement published in 2000: [[http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm?s_cid=mm5801a3_e.](http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm?s_cid=mm5801a3_e)]

Nucleic acid amplification (NAA) tests for *M. tuberculosis* can be performed directly on sputum and other respiratory specimens. It can allow for the diagnosis of pulmonary tuberculosis as early as 24 hours from specimen receipt, in a moderate-to-high-risk patient (*i.e.*, a true *TB Suspect*) who has received <7days anti-tuberculous treatment. Compared to culture, NAA tests are highly sensitive [>95% in AFB-smear positive TB suspects; 50% to 90% in AFB-smear negative suspects] and highly specific [approaching 100%] for *M. tuberculosis*.

Recommendations of the revised *Guidelines* are based on two principles:

- *NAA testing for TB in the United States should become standard practice, **but only for moderate to high suspicion TB suspects and***
- *NAA testing should 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 or TB control activities.***

Key elements of the attached Testing and Interpretation Algorithm include:

- A patient with a sputum specimen that is AFB smear-positive and NAA test-positive should be presumed to have TB (PPV>95%).
- A single negative NAA test result does not definitively exclude TB, especially in persons with a moderate-to-high clinical suspicion of TB.
- However, a negative NAAT and two independent AFB smear-negative sputum samples (taken at least 8 hr apart, with at least 1 early morning sample) may be used to determine whether patients may be removed from respiratory isolation, provided (1) that clinical suspicion for *infectious* TB is low, and (2) if the patient has been started on multidrug treatment, that there is clinical improvement.

NAA Testing at the Massachusetts State Laboratory in Jamaica Plain

- The Massachusetts State Laboratory currently performs NAA testing (GenProbe *E-MTD*[®]) on the first AFB smear-positive respiratory specimen received from each patient. The Laboratory will also perform the test on smear-negative respiratory specimens, upon request by the clinician.
- Clinicians should request NAA testing **only for patients in whom the clinical suspicion for pulmonary TB is moderate-to-high.**
- NAA testing can be performed **only** on sputum (spontaneous or induced) or other respiratory secretions, such as tracheal aspirates, bronchial aspirates, and bronchioalveolar lavage. It can not be performed on other fluids/secretions or on tissue.
- All specimens tested by NAA will also be tested by conventional mycobacterial culture methods.
- NAA testing is performed at the State Laboratory 5 days a week with a turnaround time of 1-6 days from receipt of the specimen.
- There is no additional fee associated with NAA testing at the State Laboratory.
- The name and contact information of the person to whom the result is to be reported should be indicated clearly on the test requisition. The laboratory will treat an initial positive NAA test result as a *critical test value* and immediately report the result to the clinician and to the appropriate public health agency
- Questions regarding specimen submission and test interpretation may be directed to the Mycobacteriology Laboratory at 617-983-6381.

Testing and Interpretation Algorithm*

1. Routinely collect respiratory specimens (e.g., sputum), process (liquefy, decontaminate, and concentrate), and test by AFB smear microscopy and culture as previously recommended (6). Specimen collection and microbiologic testing should not be delayed to await NAA test results.
2. At least one specimen, preferably the first diagnostic specimen, from each patient to be tested by NAA should be processed, suspended in a sufficient volume of buffer to ensure adequate sample volume for all planned tests (e.g., microscopy, culture, and NAA), and tested using an NAA test for TB. NAA testing should be performed in accordance with the manufacturer's instructions or a validated standard operating procedure.
3. Interpret NAA test results in correlation with the AFB smear results.
 - a. 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 (8).
 - b. 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 NAA result. A patient can be presumed to have TB, pending culture results, if two or more specimens are NAA positive.
 - c. If the NAA result is negative and the AFB smear result is positive, a test for inhibitors should be performed and an additional specimen should be tested with NAA. Sputum specimens (3%--7%) might contain inhibitors that prevent or reduce amplification and cause false-negative NAA results (8,9).
 - i. If inhibitors are detected, the NAA test is of no diagnostic help for this specimen. Use clinical judgment to determine whether to begin anti-TB treatment while awaiting results of culture and additional diagnostic testing.
 - ii. If inhibitors are not detected, 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 and has no inhibitors detected.
 - d. 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 (8,9).

* From CDC. MMWR, January 16, 2009 / 58(01);7-10 (see above)