Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis

Guidelines for the use of nucleic acid amplification (NAA) tests for the diagnosis of tuberculosis (TB) were published in 1996 (*1*) and updated in 2000 (*2*). Since then, NAA testing has become a routine procedure in many settings because NAA tests can reliably detect *Mycobacterium tuberculosis* bacteria in specimens 1 or more weeks earlier than culture (*3*). Earlier laboratory confirmation of TB can lead to earlier treatment initiation, improved patient outcomes, increased opportunities to interrupt transmission, and more effective public health interventions (*4*,*5*). Because of the increasing use of NAA tests and the potential impact on patient care and public health, in June 2008, CDC and the Association of Public Health Laboratories (APHL) convened a panel of clinicians, laboratorians, and TB control officials to assess existing guidelines (*1*,*2*) and make recommendations for using NAA tests for laboratory confirmation of TB. On the basis of the panel's report and consultations with the Advisory Council for the Elimination of TB (ACET),* CDC recommends 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 or TB control activities, such as contact investigations. These guidelines update the previously published guidelines (*1*,*2*).

Background

Conventional tests for laboratory confirmation of TB include acid-fast bacilli (AFB) smear microscopy, which can produce results in 24 hours, and culture, which requires 2--6 weeks to produce results (*5*,*6*). Although rapid and inexpensive, AFB smear microscopy is limited by its poor sensitivity (45%--80% with culture-confirmed pulmonary TB cases) and its poor positive predictive value (50%--80%) for TB in settings in which nontuberculous mycobacteria are commonly isolated (*3*,*6*,*7*).

NAA tests can provide results within 24--48 hours. The Amplified *Mycobacterium tuberculosis* Direct Test (MTD, Gen-Probe, San Diego, California) was approved by the Food and Drug Administration (FDA) in 1995 for use with AFB smear-positive respiratory specimens, and in a supplement application, an enhanced MTD test was approved in 1999 for use with AFB smear-negative respiratory specimens from patients suspected to have TB. In addition, the Amplicor *Mycobacterium tuberculosis* Test (Amplicor, Roche Diagnostics, Basel, Switzerland) was approved by FDA in 1996 for use with AFB smear-positive respiratory specimens from patients suspected to have TB. NAA tests for TB that have not been FDA-approved also have been used clinically (e.g., NAA tests based on analyte specific reagents, often called "home-brew" or "in-house" tests) (*8*,*9*). Compared with AFB smear microscopy, the added value of NAA testing lies in its 1) greater positive

predictive value (>95%) with AFB smear-positive specimens in settings in which nontuberculous mycobacteria are common and 2) ability to confirm rapidly the presence of *M. tuberculosis* in 50%--80% of AFB smear-negative, culture-positive specimens (*3,7--9*). Compared with culture, NAA tests can detect the presence of *M. tuberculosis* bacteria in a specimen weeks earlier than culture for 80%--90% of patients suspected to have pulmonary TB whose TB is ultimately confirmed by culture (*3,8,9*). These advantages can impact patient care and TB control efforts, such as by avoiding unnecessary contact investigations or respiratory isolation for patients whose AFB smear-positive specimens do not contain *M. tuberculosis*.

Despite being commercially available for more than a decade (*1*), NAA tests for TB have not been widely used in the United States largely because of 1) an uncertainty as to whether NAA test results influence case-management decisions or TB control activities; 2) a lack of information on the overall cost-effectiveness of NAA testing for TB; and 3) a lack of demand from clinicians and public health authorities. However, recent studies showed that 1) clinicians already rely on the NAA test result as the deciding factor for the initiation of therapy for 20%--50% of TB cases in settings where NAA testing is a routine practice (*4,7*) and 2) overall cost savings can be achieved by using NAA test results for prioritizing contact investigations, making decisions regarding respiratory isolation, or reducing nonindicated TB treatment (*4,7*).

In response to the increasing demand for NAA testing for TB and recognition of the importance of prompt laboratory results in TB diagnosis and control, ACET requested that APHL and CDC convene a panel to evaluate the available information (e.g., current practices, existing guidelines, and publications) and to propose new guidelines for the use of NAA tests for TB diagnosis. The panel met in June 2008 and included TB clinicians; TB control officials; laboratory directors or supervisors from small, medium, and large public health laboratories, hospital laboratories, and commercial laboratories; and representatives from the TB Regional Training and Medical Consultation Centers, ACET, APHL, and CDC. In brief, the panel recommended[†] that NAA testing become a standard practice in the United States to aid in the initial diagnosis of patients suspected to have TB, rather than just being a reasonable approach, as suggested in previously published guidelines (*1,2*). On the basis of the panel's report and consultations with ACET, CDC developed revised guidelines.

Updated Recommendation

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. The following testing and interpretation algorithm is proposed.

Revised 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*).

Cautions

Culture remains the gold standard for laboratory confirmation of TB and is required for isolating bacteria for drug-susceptibility testing and genotyping. In accordance with current recommendations (*6*), sufficient numbers and portions of specimens should always be reserved for culturing. Nonetheless, NAA testing should become standard practice for patients suspected to have TB, and all clinicians and public health TB programs should have access to NAA testing for TB to shorten the time needed to diagnose TB from 1--2 weeks to 1--2 days (*3*). More rapid laboratory results should lead to earlier treatment initiation, improved patient outcomes, and increased opportunities to interrupt transmission (*4*,5). Rapid laboratory confirmation of TB also can help reduce inappropriate use of fluoroquinolones as empiric monotherapy of pneumonias, a practice which is suspected to lead to development of fluoroquinolone-resistant *M. tuberculosis* and delays in initiating appropriate anti-TB therapy (*10*).

To maximize benefits of NAA testing, the interval from specimen collection to communication of the laboratory report to the treating clinician should be as brief as possible. NAA test results should be available within 48 hours of specimen collection. Laboratorians should treat an initial positive NAA test result as a critical test value, immediately report the result to the clinician and public health authorities, and be available for consultation regarding test interpretation and the possible need for additional testing.

Although NAA testing is recommended to aid in the initial diagnosis of persons suspected to have TB, the currently available NAA tests should not be ordered routinely when the clinical suspicion of TB is low, because the positive predictive value of the NAA test is <50% for such cases (*8*). Clinicians, laboratorians, and TB control officials should be aware of the appropriate uses of NAA tests.

Clinicians should interpret all laboratory results on the basis of the clinical situation. A single negative NAA test result should not be used as a definitive result to exclude TB, especially when the clinical suspicion of TB is moderate to high. Rather, the negative NAA test result should be used as additional information in making clinical decisions, to expedite testing for an alternative diagnosis, or to prevent unnecessary TB treatment. Consultation with a TB expert should be considered if the clinician is not experienced in the interpretation of NAA tests or the diagnosis and treatment of TB.

Although FDA-approved NAA tests for TB are eligible for Medicare or Medicaid reimbursement, the costs of adding NAA testing to the routine testing of respiratory specimens from patients suspected to have TB might be considerable (e.g., operating costs exceed $100 per MTD test) (*8*). However, NAA testing has the potential to provide overall cost savings to the treatment center and TB control program through reduced costs for isolation, reduced costs of contact investigations of persons who

do not have TB, and increased opportunities to prevent transmission. Within the parameters of these guidelines, each TB control or treatment program should evaluate the overall costs and benefits of NAA testing in deciding the value and optimal use of the test in their setting.

Because the testing algorithm includes NAA testing of AFB smear-negative specimens, laboratories must use an FDA-approved test for such specimens or a test produced and validated in accordance with applicable FDA and Clinical Laboratory Improvement Amendments (CLIA) regulations.[§] However, the performance of in-house tests or FDA-approved tests used for nonapproved indications (off-label use) is variable (*8*,*9*), and insufficient information is available to provide recommendations on the use of such tests for the diagnosis of TB. Their use should be guided by the clinical context, and the results of such tests should be interpreted on the basis of performance in the local laboratory and in validation studies.

For procedural and economic reasons, NAA testing might be impractical in laboratories with a small volume of testing. Referral of samples for NAA testing to high-volume laboratories might be preferable to improve cost-efficiency, proficiency, and turnaround times. The New York and Florida Fast Track Programs are successful NAA testing services that could serve as models for a regional service (*5*).

Information is limited regarding NAA test performance for nonrespiratory specimens or specimens from patients under treatment (*8*). NAA results often remain positive after culture results become negative during therapy. Further research is needed before specific recommendations can be made on the use of NAA testing in the diagnosis of extrapulmonary TB and TB in children who cannot produce sputum; however, evidence exists for the utility of such testing in individual cases (*8*).

These guidelines do not address the use of molecular tests for detecting drug resistance, which is an urgent public health and diagnostic need. No molecular drug-susceptibility tests (DSTs) have been approved by FDA for use in the United States, although well-characterized molecular DSTs are commercially available in Europe and elsewhere.[¶] Nonetheless, a proposed revision of the Diagnostic Standards and Classification of Tuberculosis in Adults and Children (*6*) is likely to support the use of molecular DSTs for AFB smear-positive sputum sediments from TB patients who are suspected to have drug-resistant disease or who are from a region or population with a high prevalence of drug resistance.

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References

1. CDC. Nucleic acid amplification tests for tuberculosis. MMWR 1996;45:950--1.
2. CDC. Update: nucleic acid amplification tests for tuberculosis. MMWR 2000;49:593--4.
3. Moore DF, Guzman JA, Mikhail LT. Reduction in turnaround time for laboratory diagnosis of pulmonary tuberculosis by routine use of a nucleic acid amplification test. Diagn Microbiol Infect Dis 2005;52:247--54.
4. Taegtmeyer M, Beeching NJ, Scott J, et al. Clinical impact of nucleic acid amplification tests on the diagnosis and management of tuberculosis in a British hospital. Thorax 2008;63:317--21.
5. CDC. National plan for reliable tuberculosis laboratory services using a systems approach: recommendations from CDC and the Association of Public Health Laboratories Task Force

on Tuberculosis Laboratory Services. MMWR 2005;54(No. RR-6):1--12.
6. American Thoracic Society; CDC; Council of the Infectious Disease Society of America. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med 2000;161:1376--95.
7. Guerra RL, Hooper NM, Baker JF, et al. Use of the Amplified *Mycobacterium tuberculosis* Direct Test in a public health laboratory: test performance and impact on clinical care. Chest 2007;132:946--51.
8. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess 2007;11:1--196.
9. Flores LL, Pai M, Colford JM Jr, Riley LW. In-house nucleic acid amplification tests for the detection of *Mycobacterium tuberculosis* in sputum specimens: meta-analysis and meta-regression. BMC Microbiol 2005;5:55.
10. Wang Y, Hsueh P-R, Jan I-S, et al. Empirical treatment with a fluoroquinolone delays the treatment for tuberculosis and is associated with a poor prognosis in endemic areas. Thorax 2006;61:903--8.

* Additional information regarding ACET is available at http://www.cdc.gov/maso/facm/facmacet.htm.
† The full report and recommendations of the panel (released in December 2008) are available at http://www.cdc.gov/tb/amplification_tests/amplification_tests.pdf.
§ Information on ASR regulations (21 CFR 809.10(e), 809.30, and 864.4020) is available at http://www.fda.gov/cdrh/oivd/guidance/1590.html. Information on the Clinical Laboratory Improvement Amendments (42 CFR 493) is available at http://wwwn.cdc.gov/clia/regs/toc.aspx.
¶ Additional information available at http://www.who.int/tb/features_archive/expert_group_report_june08.pdf.

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