



Report of an Expert Consultation on the Uses of Nucleic Acid Amplification Tests for the Diagnosis of Tuberculosis

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Background

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 institutions for the diagnosis of TB, because NAA tests can rapidly and reliably detect *Mycobacterium tuberculosis* bacteria directly in a specimen one or more weeks earlier than culture. Earlier laboratory confirmation of TB can lead to earlier treatment initiation, better patient care and outcomes, greater opportunities to interrupt transmission, and improved public health interventions.

Two NAA tests are approved for use in the United States by the Food and Drug Administration (FDA). The Enhanced Amplified Mycobacterium Tuberculosis Direct Test (E-MTD, Gen-Probe, San Diego, California) is approved for detection of *M. tuberculosis* complex bacteria in acid-fast bacilli (AFB) smear-positive and smear-negative respiratory specimens from patients suspected of having TB. The E-MTD test combines isothermal transcription-mediated amplification of a portion of the 16S rRNA with a detection method that uses a hybridization probe specific for *M. tuberculosis* complex bacteria. The MTD test displays a sensitivity of >95% for detecting *M. tuberculosis* bacteria in respiratory specimens from AFB-smear positive TB suspects and 75% to 90% for detecting *M. tuberculosis* bacteria in respiratory specimens from AFB-smear negative TB suspects. The Amplicor Mycobacterium Tuberculosis Test (Amplicor, Roche Diagnostics) is approved for the detection of *M. tuberculosis* complex bacteria in AFB smear-positive respiratory specimens from patients suspected of having TB. This test uses the polymerase chain reaction (PCR) to amplify a portion of the 16S rRNA

gene that contains a sequence that hybridizes with an oligonucleotide probe specific for *M. tuberculosis* complex bacteria. The Amplicor test displays a sensitivity of >95% for detecting *M. tuberculosis* bacteria in respiratory specimens from AFB-smear positive TB suspects and a sensitivity of 60% to 70% for detecting *M. tuberculosis* bacteria in respiratory specimens from AFB-smear negative TB suspects.

In response to a request from the Advisory Council for the Elimination of Tuberculosis (ACET), the Association of Public Health Laboratories (APHL) and CDC convened an expert panel to evaluate the evidence and propose new guidelines for the use of NAA tests for the diagnosis of TB in the United States. The panel 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 Regional Training and Medical Consultation Centers, APHL, and CDC. Meeting on June 13, 2008, the panel reviewed available publications and guidelines to discuss applications of NAA testing for TB diagnosis and control and to propose recommendations.

General considerations

1. Optimum patient care and public health must be cornerstones of the recommendations.
2. Many TB suspects are seen initially by less experienced clinicians who may delay specific treatment until laboratory results confirm the diagnosis. On the assumption that, generally, knowledge and skills for TB diagnosis will remain stable or deteriorate, the laboratory will play an increasingly critical role in reducing delays in the initiation of TB treatment.
3. NAA testing has significant potential added value for clinicians and TB control officials.
 - a. Earlier diagnosis leads to earlier initiation of treatment, a reduced period of infectiousness, and improved patient outcomes.
 - b. Earlier notification of TB cases to public health authorities should permit public health interventions sooner and may engage a TB expert sooner in the care of the TB patient.
 - c. Earlier detection of *M. tuberculosis* bacteria in sputum specimens can facilitate earlier infection control (respiratory isolation) decisions.
 - d. Earlier differentiation of AFB-smear positive specimens containing *M. tuberculosis* from those containing other mycobacteria can eliminate unnecessary contact investigations.
 - e. Prompt confirmation of tuberculosis may help avoid inappropriate empirical use of fluoroquinolones as monotherapy of pneumonias, a practice which is suspected to lead to development of tuberculosis resistant to fluoroquinolones.
4. NAA tests have limitations, and caution must be taken when interpreting NAA test results.
 - a. The FDA-approved NAA tests for TB have slightly less sensitivity than culture-isolation methods, and the 15% to 20% of U.S. TB cases that are reported with negative culture results may also have negative NAA test results. Thus, a negative NAA test result does not exclude the diagnosis of TB.
 - b. Sputum specimens (up to 20% in some studies) may contain inhibitors that prevent or reduce amplification to cause false-negative NAA test results, although inhibitors rarely cause false-negative NAA test results in smear-positive specimens (<3% of samples).
 - c. Several sporadic or systematic errors can cause false-positive NAA test results.
5. Costs and funding issues
 - a. Only FDA-approved or laboratory-validated analyte specific reagent (ASR) tests are eligible for Medicare or Medicaid reimbursement.
 - b. The FDA-approved tests are
 - i. a substantial added cost to the laboratory;
 - ii. additional tests that augment but do not replace any current laboratory test;
 - iii. labor intensive, technically demanding, and not suitable for automation; and
 - iv. susceptible to end-product contamination and amplification inhibition.

- c. NAA tests can provide substantial savings
 - i. for the patient (earlier diagnosis, improved outcomes, reduced health-care costs);
 - ii. for the health care provider (definitive diagnosis earlier, focused diagnostic testing, optimum patient care);
 - iii. for the hospital (less potential for nosocomial transmission, briefer period of respiratory isolation if TB is excluded); and
 - iv. for the public health program (interrupt transmission earlier, abbreviated period for transmission, focused contact investigations).
6. Turnaround time (TAT) must be as brief as possible to maximize benefits of NAA testing. The key TAT is the interval from specimen collection to time that the laboratory report is communicated to the health care provider.
7. Good communication between laboratorians, health care providers, and public health officials is critical to optimizing the benefits of NAA testing. Standard language or statements to include in laboratory reports of NAA test results are needed.
8. Education of laboratorians, clinicians, health care providers, TB controllers, and policy makers on the appropriate use and interpretation of NAA tests for the diagnosis of TB will be essential.
9. For general medical care and public health systems, a single, simple algorithm for NAA testing is preferred. Some special circumstances, such as infection control and inpatient medical care, may require separate algorithms.
10. There are only two FDA-approved tests which may be impractical for use by laboratories with a small volume of testing.
11. Analyte Specific Reagent (ASR) tests for detecting *M. tuberculosis* bacteria (e.g., real-time PCR assays) with excellent sensitivity, specificity, speed, and ease-of-use have been recently independently developed, validated, and implemented in a variety of laboratories.
 - a. Home-brew tests themselves are not regulated by the FDA, but the components (ASRs) that compose them may be regulated if purchased from a manufacturer. ASRs used in home-brew tests for TB are classified as Class III medical devices by the FDA (21 CFR 864.4020) and must be cleared or approved by FDA before they can be marketed in the United States (21 CFR 864.4020)
 - b. Laboratories developing and performing home-brew tests as a diagnostic service are required to meet CLIA high-complexity certification requirements, to comply with CLIA quality control requirements, and to establish the performance of the in-house test following CLIA regulations.
 - c. The ordering of home-brew tests that are developed using ASRs is limited under section 520(e) of the Federal Food, Drug and Cosmetic Act to physicians and other persons authorized by applicable State law to order such tests.
 - d. The laboratory that develops and performs a home-brew test using an ASR is required to inform the ordering person of the test result by appending to the test report the statement: "This test was developed and its performance characteristics determined by (Laboratory Name)." It has not been cleared or approved by the U.S. Food and Drug Administration." (21 CFR 809.30(e))
12. Cost efficiency, rapid turnaround time, and expertise could be enhanced by establishing high-volume regional laboratories offering molecular tests.
13. Sufficient information is available for making recommendations for respiratory specimens.
14. Further research is needed before specific recommendations can be made on the use of NAA testing in the diagnosis of TB in children who cannot produce sputum and in the diagnosis of extrapulmonary TB, although there is much anecdotal evidence of the utility of such testing in individual cases.

Research needs

1. Conduct operational, translational, and implementation research for developing, evaluating, and selecting the most effective and efficient NAA testing algorithms for routine use and for specific scenarios.
2. Develop and evaluate tests suitable for use with non-respiratory specimens (e.g., cerebrospinal fluid, gastric aspirates, or biopsies).
3. Develop and evaluate tests that will enhance the diagnosis of TB in children.
4. Develop and evaluate optimal specimen collection, transport, and processing methods.
5. Determine the influences of specimen quality and quantity on NAA test performance.
6. Characterize the ability of NAA tests to detect *M. tuberculosis* bacteria in mixed infections, specimens, and cultures.
7. Develop, evaluate, and deploy NAA tests with improved performance and ease-of-use and features that include
 - a. point-of-care testing, turnaround times of <2 hours
 - b. automatable, minimal hands-on time
 - c. minimal specimen processing required
 - d. closed system to minimize end-product contamination concerns
 - e. internal controls for inhibitors
 - f. increased sensitivity, perhaps through using target capture technologies
 - g. quality-assured reagents for use in the test reactions and for use as controls
8. Conduct regulatory quality trials for any new NAA test aimed at obtaining FDA approval.

Molecular Drug Susceptibility Tests (DSTs)

The expert panel also addressed available information on molecular tests for determining drug susceptibilities of *M. tuberculosis* bacteria directly from clinical specimens. Two well-characterized, CE-marked molecular DST kits are commercially available in Europe and elsewhere. (The CE marking, "Conformite Européenne," certifies that a product has met European Union requirements.) The World Health Organization has recommended the use of these tests as screening tests for multidrug-resistant (MDR) TB. Although several laboratories in the U.S. offer molecular DSTs as ASR tests, none have been approved by the FDA.

The expert panel considers molecular DSTs to be an urgent public health and diagnostic need, because extensively drug-resistant (XDR) TB and MDR TB are becoming more prevalent globally. Such molecular DSTs should be available to all U.S. TB programs. The expert panel endorses the recommendations under consideration in the proposed American Thoracic Society 'Diagnostic Standards and Classification of Tuberculosis in Adults and Children' which supports the use of molecular methods for detection of drug resistance directly in AFB-smear positive sputum sediments for TB patients who have factors predictive of drug resistance.

Communication Plan for New Recommendations

The panel recommends disseminating the recommendations in multiple media, in order to reach clinicians, TB control officials, laboratorians, governmental organizations, regulatory agencies, policy makers, and other TB partners. This includes publication in more than one journal, use of electronic mail lists, and direct distribution to key stakeholders. Draft recommendations will be presented to ACET for their contributions and endorsement. The new guidelines will supersede the CDC 'Guidelines for the use of nucleic acid amplification (NAA) tests in the diagnosis of tuberculosis' (MMWR 2000;49:593-4.)

General Recommendations of the Expert Panel

1. All U.S. clinicians and public health TB programs should have access to molecular tests to aid in the diagnosis of TB. NAA testing for TB should become standard practice for TB suspects.
2. NAA testing should be performed on a respiratory specimen from each patient with signs and symptoms of active pulmonary TB disease for whom a diagnosis of TB is being considered (i.e., TB suspect), but has not been established.
 - a. NAA testing does not replace the need for AFB smear and culture. All current guidelines and recommendations for culture-based testing should remain in effect, especially recommended turn around times for culture and DST.
 - b. A single positive NAA test result can support the diagnosis of TB in a patient for whom there is a reasonable index of suspicion. This result should trigger reporting to public health officials, initiation of treatment if not already started, and intensified efforts to obtain an isolate for drug susceptibility testing.
 - c. In a patient with little suspicion of having active TB, a single positive NAA test result should be viewed with suspicion (i.e., a possible false-positive result) and interpreted in the same way as a single culture-positive result, i.e., by correlating the results with other diagnostic findings.
 - d. A single negative NAA test result should never be used as a definitive test to exclude TB, especially in suspects with a moderate to high clinical suspicion of TB. Rather, the negative NAA test result should be used as additional information to aid in making clinical decisions to expedite a work-up for an alternative diagnosis or to prevent unnecessary use of TB treatment in suspects with a low clinical suspicion.
 - e. Specimens may contain inhibitors. Testing for inhibitors should be considered for specimens that are AFB-smear positive and NAA test-negative. Each laboratory should establish the rate of inhibition to determine if routine testing for inhibitors is necessary. If inhibition testing is not performed on NAA test-negative specimens, it should be noted on the laboratory report.
 - f. If the clinician is inexperienced with the diagnosis and treatment TB, consultation with a TB expert should be obtained with respect to the interpretation of NAA test results in the context of other diagnostic evidence.
3. It is recommended that the appropriate work group consider amending the guidelines for ‘Controlling the Transmission of TB in Health Care Settings 2005’ section on ‘Suspected TB Disease’ to: *‘For patients placed under airborne precautions because of suspected infectious TB disease of the lungs, airway, or larynx, airborne precautions can be discontinued when infectious TB disease is considered unlikely and either 1) another diagnosis is made that explains the clinical syndrome, 2) the patient has three negative AFB sputum smear results,’* or 3) the patient has a sputum specimen that has a negative NAA test result and two additional sputum specimens that are AFB-smear negative. *‘Each of the three consecutive sputum specimens should be collected in 8–24-hour intervals, and at least one specimen should be an early morning specimen because respiratory secretions pool overnight. Generally, this method will allow patients with negative sputum smear results to be released from airborne precautions in 2 days.’*

Note: this recommendation does not apply to patients with a suspicion for TB that is high enough to start TB medication. For these patients, release from isolation requires clinical response to treatment, usually four to seven days of treatment in addition to three negative specimens by sputum AFB smears or NAA testing as outlined above.
4. NAA testing should be treated as a priority test.
 - a. Health care providers should be provided with clear instructions for the collection of quality specimens and encouraged to collect an adequate volume (5-10ml).

- b. The processed diagnostic specimen must be suspended in sufficient volume to ensure adequate samples for NAA testing, AFB-smear microscopy, and culture.
5. The interval from specimen collection to the time that the laboratory report is communicated to the treating clinician must be as brief as possible. Laboratories and programs should track this performance measure.
 - a. Specimens must be delivered promptly to the laboratory that does the NAA testing.
 - b. Specimens must be tested promptly in the laboratory, preferably on the day received (i.e., without introducing significant delays by batching specimens).
 - c. The results of NAA tests should be available within 48 hours of specimen collection.
 - d. Laboratorians should treat an initial positive NAA test result as a critical test value. They must immediately report NAA test results to the health care provider and be available for consultation as to appropriate test interpretation and possible need for follow-up testing.
 - e. Laboratorians should immediately report initial positive NAA test results to public health authorities for earlier interventions and possibly earlier engagement of a TB expert in the management of the patient.
6. For laboratories that do not have sufficient resources for NAA testing or sufficient test volume for NAA testing without adding delays from batching, specimens for NAA testing should be referred promptly to laboratories that have demonstrated proficiency in the test and can provide timely results (e.g., within 24–48hrs).
7. Laboratories performing NAA testing should participate in a NAA proficiency testing program (e.g., WSLH PT [Wisconsin State Laboratory of Hygiene Proficiency Testing], CAP [College of American Pathologists], or other accredited program).
8. The number and types of NAA tests, commercial sources, FDA-approved tests, and validated ASR tests should be increased.
9. Research is needed to improve specimen processing, referral processes, testing algorithms, NAA test performance and ease-of-use, utility for diagnosing extrapulmonary and pediatric TB, and regulatory quality trials.
10. The expert panel endorses the recommendations under consideration in the proposed revision of the ‘Diagnostic Standards and Classification of Tuberculosis in Adults and Children’ supporting the routine use of molecular methods for detection of drug resistance directly in AFB smear-positive sputum sediments for TB patients who are suspected of having drug-resistant disease or are from a region or population with a high prevalence of drug resistance.

Recommendations to ACET, CDC, DTBE

1. ACET should discuss adopting the expert panel’s guidance and recommendations for the use of NAA testing for the diagnosis of TB.
2. To assist in developing more sources of FDA-approved NAA tests,
 - a. CDC and FDA should encourage manufacturers to develop NAA tests for TB.
 - b. CDC should assist manufacturers with regulatory quality trials of NAA tests.
 - c. CDC and ACET should request that the FDA streamline or facilitate the approval process for the new tests, perhaps by reducing the filing requirements from the Premarket Approval (PMA) process to the less stringent Pre-Market Notification (510K) process.
3. ACET and CDC should encourage the College of American Pathologists (CAP) to add ‘provision of TB NAA testing’ as a checklist question to encourage private and public health laboratories to provide access to NAA testing for TB.
4. ACET and CDC should develop recommendations on the use of molecular tests for the detection of drug-resistant TB in the United States.
5. ACET should develop and promote a research agenda for TB NAA testing.

6. CDC should take the lead in disseminating recommendations of the expert panel through a revision of the current 'Guidelines for the use of nucleic acid amplification (NAA) tests in the diagnosis of tuberculosis' and publications in appropriate venues.
7. Because of the potential for more prompt initiation of therapy and a reduction in TB transmission, CDC should give priority to assisting state and local TB programs to gain access to NAA testing for TB.
8. CDC should explore validating and deploying selected Analyte Specific Reagent (ASR) tests for TB to public health laboratories in a manner similar to that of bioterrorism-related ASR tests. Priority should be given to rapid detection tests, such as a real-time PCR test for TB, and tests for molecular detection of rifampin resistance. Such tests should aim to build on the infrastructure generated by, and the experiences of, the Bioterrorism Preparedness and Response Program.
9. CDC should consider establishing centers of excellence or regional laboratories to provide access to molecular tests for state and local TB public health programs. This should reduce the cost of testing associated with reagent waste, and reduce delays in testing due to batching of samples or to the lack of 7-day a week testing. CDC and partners should develop a clear policy and standard operating procedure for referring specimens to a regional laboratory for NAA testing.
10. CDC should help ensure the availability of proficiency testing programs for TB NAA testing, such as the programs of CAP or WSLH.
11. CDC should develop a broader evidence base to support changes in recommendations and practices and investigate the economic implications of molecular testing.
12. CDC should work with partners to establish an education program for TB control officials, laboratorians, health care providers, and policy makers on the appropriate use of NAA tests for the diagnosis of TB.