American Thoracic Society Workshop

MEDICAL SECTION OF THE AMERICAN LUNG ASSOCIATION

Rapid Diagnostic Tests for Tuberculosis

What is the Appropriate Use?

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Recent technologic developments have introduced a number of improvements in the ability of clinical laboratories to cultivate and identify *Mycobacterium tuberculosis* complex more quickly than previously. These developments include more rapid detection of growth and tests to identify RNA or DNA of *M. tuberculosis* complex directly in clinical samples. United States Food and Drug Administration (FDA) panels have recently recommended approval of two direct amplification tests (DAT), the Gen-Probe® MTD (San Diego, CA) and the AMPLICOR® *M. tuberculosis* test (Roche Diagnostic Systems, Inc., Branchburg, NJ). The FDA has approved the MTD for identification of *M. tuberculosis* complex in respiratory specimens that are smear-positive for acid-fast bacilli (AFB). In addition, the specimen must be from a patient who has not received antituberculous medication for seven *or* more days or within the last 12 months. From the data reviewed by the FDA, the specificity (100%) and sensitivity (95%/96% in the two studies) of these two tests in AFB smear-positive specimens were found to be comparable to the Accuprobe (Gen-Probe) for identification of *M. tuberculosis* complex in culture, with the advantage that the DAT results are available much sooner. The DAT are significantly more sensitive than the AFB smear. However, in AFB smear-negative samples, the specificity, sensitivity, and positive predictive value were 96/99%, 48/53% and 24/58%, respectively, in the two studies. For some results, the Gen-Probe assay had the higher value and for others, the Roche assay was higher. The DAT result, particularly when discordant with the AFB smear, must be used in conjunction with clinical assessment. While both the MTD and the AMPLICOR® *M. tuberculosis* test have undergone extensive testing in clinical laboratories, neither test has been examined for its utility in routine clinical use or public health settings in the United States. An American Thoracic Society Workshop was convened to examine the data and technology available to date, to develop a consensus addressing the appropriate use of the rapid diagnostic tests (in particular, DAT's) for tuberculosis, and to identify future research needs and directions. The consensus among three focus groups, clinical, laboratory, and public health, was that, while these tests are a major improvement over standard techniques, there is currently insufficient information on their clinical and public health utility. When the AFB smear and DAT are both positive, the diagnosis of tuberculosis can be considered to be established. Furthermore, when the AFB smear is negative and the DAT is also negative, it is unlikely that *M. tuberculosis* will be grown from that sample. When there is discordance between the AFB smear and the DAT, additional consideration must be given to the overall clinical picture and repeat testing should be done. It is recommended that the currently available DAT's always be performed in conjunction with microscopy and culture, and each test result must be interpreted within the overall clinical setting in which it is used.

The major difference between tuberculosis and other mycobacterial infections is the fact that ***Mycobacterium tuberculosis*** is transmitted from person to person. For this reason, it is particularly important to diagnose tuberculosis as early as possible. In addition, tuberculosis is a complex disease with extensive social and legal ramifications. Therefore, recent technologic developments making it possible to diagnose tuberculosis more rapidly are of great importance. A workshop was convened as a program of the American Thoracic Society Assembly on Microbiology, Tuberculosis, and Pulmonary Infection to review the status of nucleic acid amplification tests for the rapid diagnosis of tuberculosis, the clinical trials carried out to evaluate them, the role of the U.S. Food and Drug Administration (FDA) in the process, economic considerations of the tests, and the development of guidelines for health practitioners and public health officials to use in considering the application of these

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tests for diagnosis and management of patients suspected of having tuberculosis. An additional goal was to identify the directions and criteria for future research. The workshop, held in San Diego, California, in February 1996, brought together 120 participants. Data were presented in plenary sessions. Discussions took place initially in three focus groups, laboratory, clinical, and public health, followed by a general discussion. The following is a description of the proceedings of the workshop and the current situation of direct amplification tests (DAT) for tuberculosis.

PROCEEDINGS

Direct Amplification Tests (DAT)

Introduction. The unexpected re-emergence of tuberculosis in industrialized countries and recent outbreaks of multidrug-resistant tuberculosis, as well as the dramatically enhanced vulnerability of human immunodeficiency virus (HIV)-positive individuals to tubercle bacilli, underscore the urgent need for rapid and accurate detection of *M. tuberculosis.*

Methods such as radiometric BACTEC technique and the commercially available DNA probes have considerably reduced turnaround time. In addition, several new formulas for nonradiometric liquid media, i.e., Septi-Check AFB (Becton Dickinson, Sparks, MD), BBL Mycobacteria Growth Indicator Tube (Becton Dickinson), ESP Myco (Difco Laboratories, Detroit, MI), MB/BacT (Organon Teknika, Durham, NC), and BACTEC 9000MB (Becton Dickinson) are aiming for shorter turnaround times of growth detection. The latest advances in tuberculosis diagnostics allow direct detection of *M. tuberculosis* complex in clinical specimens by molecular biological methods. Two DAT have undergone extensive clinical trials and investigations and were discussed in great detail.

Gen-Probe@ Amplified Mycobacterium tuberculosis Direct Test (MTD). Gen-Probe Incorporated (San Diego, CA) has developed an isothermal transcription-mediated amplification system which follows three main steps: (I) sample preparation releases rRNA; (2) transcription-mediated amplification produces RNA amplicon; and (3) hybridization protection assay detects RNA amplicon. In December 1995, the FDA approved this kit for direct detection of tubercle bacilli in acid-fast bacilli (AFB) smear-positive respiratory samples. In most published studies, sputum specimens were decontaminated with *N*-acetyl-L-cysteine/NaOH; however, alternative decontamination procedures such as sodium dodecyl (lauryl) sulfate/NaOH demonstrated excellent test performance as well (1). Furthermore, the use of 500 μl instead of the recommended 50 μl of the processed sediment improved the overall performance (2), although it should be noted that the specimens tested with 500 μl were not the same specimens tested with 50 μl from the first study. In analyzing the diagnostic performance of this test kit during a two-year period for respiratory specimens and a one-year period for nonrespiratory specimens, no statistically significant difference in sensitivity and specificity for nonrespiratory (93.1% and 97.7%, respectively) compared with respiratory specimens (86.6% and 96.4%, respectively) was found, and repeating all tests which yielded a result between 30,000 and 200,000 RLU would have helped to reduce the number of false positives (3). To shorten the turnaround time, an amplification time of 30 min instead of 120 min is under clinical evaluation.

AMPLICOR® Mycobacterium tuberculosis Test and COBAS AMPLICOR®. The Roche AMPLICOR® ***M. tuberculosis*** test kit follows four main steps: (*1*) specimen preparation; (2) DNA polymerase chain reaction (PCR) amplification; (3) hybridization; and (4) detection. False-positive results due to carryover contamination are prevented by the incorporation of dUTP coupled with uracil-N-glycosylase restriction. Once the specimen has been received in the laboratory and *N*-acetyl-L-cysteine/NaOH decontamination has been performed, the results are available in approximately 6.5 hours. In December of 1996, this kit was approved by the FDA.

The minimum number of ***M. tuberculosis*** colony-forming units (CFU) detectable in clinical sputum specimens by the AMPLICOR® ***M. tuberculosis*** test was estimated by performing the test on duplicate samples of quantitatively cultured, serial dilutions of sputum (4). Positive PCR test results were obtained for all samples that contained at least 42 CFU. The detection limits of the PCR assay for decontaminated and nondecontaminated specimens were equivalent, even though the number of CFU cultured from decontaminated samples was only 11 to 20% of the number cultured from nondecontaminated specimens.

The performance of the AMPLICOR® *M. tuberculosis* test and that of conventional culturing were compared (5). Of 662 respiratory specimens, four of five PCR-positive, culture-negative results were found to be "truly positive" by other criteria. The remaining specimen was positive when retested by PCR for a sequence target distinct from the 16S rRNA gene, the current AMPLICOR® *M. tuberculosis* test target, suggesting the presence of dead or nonculturable bacteria or a subclinical infection. Seventy of the 662 clinical specimens were nontuberculous mycobacteria (NTM), all of which tested negative by the AMPLICOR® ***M. tuberculosis*** test. Seven PCR-negative, AFB smear-negative specimens proved to be culture-positive. AMPLICOR® *M. tuberculosis* test provided a final clinical sensitivity and specificity of 91.9% and 99.8%, respectively, compared to 95.3% and 100%, respectively, for culture.

In Europe, COBAS AMPLICOR®, which automates amplification and detection, simplifies laboratory setup, and decreases hands-on labor, is under clinical evaluation. Preliminary results, presented at the first European Meeting on Diagnostic PCR held in Amsterdam on October 12-13, 1995, demonstrated excellent correlation between the manual AMPLICOR® and the automated COBAS AMPLICOR®.

Strand Displacement Amplification (SDA)/Becton Dickinson ProbeTec System. SDA is an isothermal, ***in vitro*** DNA amplification technique that is based upon the ability of (I) the restriction enzyme, *Hinc*II, to nick the unmodified strand of a hemiphosphorothioate form of its recognition site; and (2) an exonuclease-deficient form of the large fragment of *Escherichia coli* DNA polymerase I (exo⁻ Klenow polymerase) to initiate replication at the nick and displace the downstream nontemplate strand (6, 7). Primers containing sequence recognition sites for the nicking restriction enzyme bind to opposite strands of target DNA at positions flanking the sequence to be amplified. The target fragment is exponentially amplified by coupling sense and antisense reactions in which strands displaced from the sense reaction serve as a target for the antisense reaction and vice versa (6).

Target DNA is heat-denatured in the presence of all reagents except exo-Klenow and *Hinc*II. Amplification then proceeds at 37" C after cooling and addition of the enzymes (6). An improved and simplified version of the SDA protocol that has been applied to ***M. tuberculosis*** replaces the target restriction step with a novel method of generating amplifiable fragments (7). Rather than using a restriction enzyme to cleave the target sample prior to amplification, this new method exploits the strand displacement activity of exo⁻ Klenow to generate target DNA copies with defined 5' and 3' ends. The new target generation process occurs at a single

temperature. Greater than 10^7-fold amplification of a genomic sequence from *M. tuberculosis* is achieved in two hours at 37" C with as much as 10 μg of human DNA per 50 μl reaction. Advantages of SDA include (*1*) its operation at a single temperature, which obviates the need for a thermal cycler; (2) its requirement for fewer enzymes; (3) its simpler mechanism; and (4) its resistance to contaminating ribonuclease activity. Disadvantages include (*1*) its low operating temperature, which decreases stringency and increases background reactions; and (2) its inability to efficiently amplify long target sequences (6).

The ProbeTec System contains three elements: conventional sample preparation, the ProbeTec instrument using robotic specimen handling, and a luminometer reading a DNA probe microwell assay. It provides three answers per specimen: (*1*) ***M. tuberculosis*** complex; (2) genus or a selected NTM species; and (3) internal control for sample inhibition and system performance. The robotic platform provides containment through a disposable decontamination-amplification device and chemical decontamination through the use of uracil DNA glycosylase.

No clinical data on the ProbeTec System had been reported at the time the workshop was held.

Status of other tests. Kathleen D. Eisenach, from J. L. McClellan Memorial Veterans Administration Hospital in Little Rock, Arkansas, offered information on the status of other tests. Molecular amplification assays must demonstrate equivalent or greater sensitivity and specificity than the standard AFB smear and culture. The insertion sequence, *IS*6110, has been used as a target for PCR amplification of ***M. tuberculosis*** DNA for over six years. Interest in *IS*6110 PCR has been rooted in a need for greater sensitivity of currently available diagnostic methods for the diagnosis of extrapulmonary tuberculosis, childhood tuberculosis, and paucibacillary disease. *IS*6110-based PCR can (*1*) detect 1 to 10 tubercle bacilli in broth cultures, (2) distinguish ***M. tuberculosis*** from NTM, and (3) provide results within one day. Overall sensitivity and specificity in patient sputum specimens has ranged from 83 to 100% and 98 to 100%, respectively. In studies where most samples were heavy AFB smear-positive, the sensitivity was 100%. Detection of ***M. tuberculosis*** in formalin-fixed, paraffin-embedded tissue by *IS*6110 PCR has proven successful, and can detect as few as nine ***M. tuberculosis*** organisms per 5-μm section (8).

High performance liquid chromatography (HPLC) with fluorescence detection appears to require less mycobacterial growth than conventional HPLC with ultraviolet detection (9, 10). The extraction of mycolic acids from cultures with less biomass (i.e., with growth indices as low as 50 in the BACTEC TB system, or even AFB smear-positive sputum samples) can be tested. Additionally, there is a need to monitor bacterial clearance from the sputum of patients on therapy. Molecular-based tests that indicate the presence of viable tubercle bacilli are important when monitoring the patient's response to therapy. A test for the detection of alpha antigen (85B protein), a major secretory protein of mycobacteria, has been developed and may be a good indicator for viable ***M. tuberculosis (11).***

Evaluation by FDA and Product Labeling

The performance of the DAT has been critically evaluated by the FDA staff and Advisory Panel. Sharon L. Hansen and Mark J. Goldberger of the FDA presented the rationale for the FDA's decision to approve the Gen-Probe@ MTD for limited use. The diagnostic tests are considered devices and the two DAT devices for tuberculosis submitted for approval to date were submitted for premarket approval (PMA). New devices (or diagnostic tests) that are intended to replace devices which existed prior to 1976 may be submitted for premarket notification (510[k]). Since there was no pre-existing approved diagnostic test for ***M. tuberculosis-complex*** genetic material, the PMA process was used. For both the approved test kit (Gen-Probe@ MTD) and the other device recommended for approval by an FDA Advisory Panel (Roche's AMPLICOR® ***M. tuberculosis*** test), the FDA accepted the recommendation of its advisory panel and limited the claims of the manufacturers for broad diagnostic testing to the sole indication the FDA was willing to consider for approval at this time-use in AFB smear-positive respiratory tract specimens from patients who (*1*) have not been on antituberculosis medication for seven or more days; or (2) have not been treated for tuberculosis within the last twelve months.

There are several reasons for the above action. The performance of both tests was quite good in AFB smear-positive specimens and they appear to be useful in both confirming the diagnosis of tuberculosis and identifying those specimens containing NTM. However, concerns were raised about the use with AFB smear-negative samples, since, even with the relatively high specificity of these tests, the low prevalence of ***M. tuberculosis-complex*** in AFB smear-negative samples will lead to many false-positive DAT results relative to the additional cases of tuberculosis detected. This is reflected in the positive predictive values shown in Table 1. Table 1 shows that the tests performed comparably, reinforcing the view that high sensitivity, specificity, and positive predictive value in AFB smear-positive samples have been achieved. The lower sensitivity and positive predictive value found with both tests when results from all samples were combined (without regard to smear result, the "overall" column in Table 1) appear attributable to the AFB smear-negative samples. The wide differences shown in the table for positive predictive value in the overall and smear-negative columns cannot be used to infer that one of the tests was superior, both because the two tests were studied on different samples and because the confidence intervals for the results would overlap. If one manufacturer sought to claim superior performance for its test, that claim would have to be based on results from a controlled, head-to-head clinical trial. Ideally, one would use these tests in conjunction with the available clinical data on the patient. However, these studies, which were designed several years ago,

TABLE 1

PERFORMANCE OF GEN-PROBE" MTD AND ROCHE AMPLICOR® *M.* tuberculosis TEST DIRECT IN AFB SMEAR-POSITIVE VERSUS SMEAR-NEGATIVE PATIENTS. DATA REVIEWED BY FDA

	Overall (%)	Smear-positive (%)	Smear-negative (%)
Sensitivity	77/80*	95/96*	48/5 3'
Specificity	96/99*	100†	96/99*
PPV	57/85*	100†	24/58*
NPV	99†	86/90*	99†

Definition of abbreviations: PPV = positive predictive value; NPV = negative predictive value.

* For some results, the Cen-Probe assay had the higher value and for others, the Roche assay was higher. The table does not identify which values are associated with either assay. The wide differences shown in the table for positive predictive value in the overall and smear-negative columns cannot be used to infer that one of the tests was superior, both because the two tests were studied on different samples and because the confidence intervals for the results would overlap. If one manufacturer sought to claim superior performance for its test, that claim would have *to* be based on results from a controlled, head-to-head clinical trial.

† Single values indicate the two assays had the same value.

were laboratory based and did not integrate the available clinical information into the decision process. It was recognized by the FDA representatives, other meeting participants and the manufacturers that, in future studies, such data must be properly collected and evaluated in conjunction with the test results.

There were other concerns raised about these studies as well. They utilized culture result as a major endpoint, but it is well recognized that culture-negative tuberculosis may represent 20% of cases (12). One would hope, in fact, that DAT might be particularly useful in this setting, but the trials did not provide that information. The trials also used clinical diagnosis as an endpoint, but the lack of standardized definitions of clinical tuberculosis and methodology in performing this evaluation rendered this approach of limited value. Finally, many of the analyses performed in these studies were on a "per sample" basis. The results of such analyses were affected by some patients in whom many specimens were obtained, although ***post hoc*** analyses restricting each patient to no more than three samples were used. "Per patient" analyses were also performed, although interpretation was limited by the need to consider how to "resolve" situations in which the DAT results differed in different samples from the same patient.

Despite these concerns, there was general agreement that sufficient data were available to begin to assess the role of these tests in the diagnosis of tuberculosis. There was also agreement among all the relative parties of the need for better communication in designing future trials for the use of DAT in diagnosing tuberculosis.

Meanwhile, the labeling for the sole approved product, the Gen-Probe@ MTD assay, permits its use in respiratory specimens that are AFB smear-positive. Although the Roche AMPLICOR® *M. tuberculosis* test has not yet been approved for marketing, it appears that its approved labeling will carry the same limitation.

Economic Issues in the Use of the DAT

Sharon Perry, from the University of California, San Diego School of Medicine (UCSD), presented a cost-effectiveness analysis of the DAT (13). Ms. Perry evaluated five strategies to consider for incorporation of the DAT:

1. Perform AFB smear on all suspected tuberculosis patients (i.e., ignore DAT, treat if positive).
2. Perform DAT on AFB smear-positive specimens for confirmation (i.e., treat if both are positive).
3. Perform DAT on AFB smear-negative specimens (i.e., treat if positive).
4. Perform DAT alone (i.e., abandon the AFB smear for diagnosis, treat if positive, and reserve the AFB smear for other purposes, such as evaluating contagiousness, response to therapy, etc.).
5. Empiric treatment (i.e., ignore DAT, treat for clinical suspicion even if the AFB smear is negative).

Ms. Perry assigned costs for all of the above approaches based upon actual best estimates of costs at UCSD and data provided by the Lash Group Health Care Consultants (San Francisco, CA), and sensitivities and specificities for the five strategies based upon operating characteristics of the tests as studied at UCSD (14). She then modeled the answers to important questions for each of the five strategies. Among the questions were:

1. What is the average cost for each strategy under indemnity insurance plans, managed care, and Medicaid?
2. What is the return in cases detected for dollars spent for each strategy?
3. How do costs and effectiveness vary depending upon varying true tuberculosis prevalence?
4. How do costs and effectiveness vary with the specificity of the AFB smear (e.g., when false positive AFB smears are due to ***M. avium*** infection)?
5. How do costs and effectiveness vary with the sensitivity of the AFB smear (e.g., when false negatives are due to paucibacillary tuberculosis)?

Overall, she showed that, given a critical dissection of the components of such decision making, the issues are susceptible to thoughtful decision analysis, and that the "best strategy" will vary with the variables mentioned above, including epidemiologic characteristics, program goals, and other variables. In fact, the "all-or-none" adoption of one strategy or another may not be the best approach; it may be wisest to employ different strategies in different types of patients within a broad region.

Christopher J. L. Murray from the Harvard School of Public Health presented a global context for consideration of the DAT. He showed that the vast majority (> 97%) of new tuberculosis cases each year arise outside the U.S. in what are generally considered less developed countries. He presented data showing the annual risk of infection in the U.S. to be 0.019% per year, while the risks in both Pakistan and Korea, for example, are above 1% per year. He pointed to the recent success of China in substantially increasing previously disappointing therapy completion rates without the use of DAT. He presented a prioritized ranking (highest to lowest key priorities) of desired interventions to control tuberculosis worldwide:

1. Ultra-short-course chemotherapy for tuberculosis disease.
2. Depot, single-visit preventive therapy for tuberculosis infection.
3. Rapid diagnostic test for infection.
4. Rapid susceptibility testing.
5. Rapid diagnostic test for active disease.

In summary, while the DAT under discussion may have critical and valuable impact in the so-called more developed countries, their impact on the global tuberculosis problem is likely to be minimal.

Laboratory Focus Group Discussion

The Laboratory Focus Group addressed six topics related to diagnostic tests for tuberculosis that provide results in less time than currently is possible with the recommended culture methods (liquid and solid media combination) and identification techniques (probes or HPLC).

1. Desirable attributes of a rapid test.
2. FDA-approved and nonapproved uses of DAT.
3. Laboratory issues for performance of the current DAT.
4. Aspects of DAT that require additional research.
5. Standards for clinical trials for evaluation of new diagnostic products.

An ideal rapid diagnostic test. The group reached consensus concerning desirable attributes of a rapid test for diagnosis of tuberculosis. The top priority was high sensitivity and specificity; however, when evaluating the reliability of a new test for diagnosis of tuberculosis, the fact that the current reference method used in the laboratory (i.e., mycobacterial culture) is not perfect must be taken into consideration. Second on the

priority list was cost. It was the group's opinion that the ideal new rapid test must be inexpensive, and it must also be technically simple to perform. A test fulfilling these criteria would eliminate the need for further evaluation of negative specimens, which constitute the vast majority of the work load in mycobacteriology laboratories. The third most desirable attribute was a rapid test that would allow detection of organisms in the ***Mycobacterium*** genus in a "raw" specimen (i.e., digestion and concentration is not necessary). Fourth, a rapid test must efficiently fit into the workflow of a clinical laboratory. To meet this requirement, the test must not be labor-intensive, nor can it require any increase in laboratory personnel. Moreover, the test should be automated, preferably with random access and data management capabilities.

Additional desirable attributes not prioritized include inclusion of controls and the ability to detect inhibitors, differentiation between live and dead mycobacteria, and semiquantitation of the number of organisms present. It also would be useful for a ***M. tuberculosis*** detection/identification test to simultaneously determine susceptibility of the isolate to antituberculous agents, especially isoniazid and rifampin. Finally, any rapid test for diagnosis of tuberculosis should allow replacement of, or a reduction in, the need to perform one or more currently performed tests.

FDA-approved and nonapproved uses. Currently, the FDA has approved the use of DAT for diagnosis of tuberculosis only for AFB smear-positive respiratory specimens from untreated patients. Agreement among group members concerning the value of these tests in this situation was unanimous. In addition, it was felt that the DAT will serve as valuable diagnostic tools in other circumstances as well. Some examples are: direct testing of AFB smear-negative respiratory specimens, specimens from extrapulmonary sites, and early identification of ***M. tuberculosis*** organisms in positive broth cultures. Although the optimal role ultimately will be determined based on data from further research (discussed in more detail in the following section), laboratories that have experience using the DAT may have data required for validation of the particular test evaluated, thus allowing use of the test in situations not approved by the FDA. However, before the test is put into practice in such a circumstance, education of clinicians, optimally by both verbal and written communication, of appropriate interpretation of results (based on personal experience plus a review of the published literature) is essential. One suggested mechanism for written communication is inclusion of an interpretation with each result for the AFB smear and DAT, as follows:

1. AFB smear-positive/DAT-positive: specimen contains *M. tu*berculosis-complex nucleic acid; culture required for susceptibility testing.
2. AFB smear-negative/DAT positive (non-FDA-approved use): nucleic acid amplification assay is more sensitive than smear for detection of ***M. tuberculosis,*** but nonspecific results have been known to occur; consider test tentatively positive unless confirmed by another test; culture required for final identification and susceptibility testing.
3. AFB smear-positive/DAT-negative: specimen most likely contains NTM, although ***M. tuberculosis*** complex may be present with DAT inhibited; culture required for final identification.
4. APB smear-negative/DAT-negative (non-FDA-approved use): ***M. tuberculosis*** complex not detected but may be present in very low numbers; culture required for final determination.

Laboratory issues for performance of DAT. Laboratory issues that must be addressed concerning use of DAT are level of proficiency and cost efficiency. The FDA has limited use of the current tests to levels II and III (College of American Pathologists Types 3 and 4) laboratories (i.e., those that perform mycobacterial culture and identify ***M. tuberculosis*** complex and/or other mycobacteria). The issue of cost efficiency has several facets that laboratory directors must examine and discuss with clinicians. First, the frequency of testing needed to have an impact on patient care must be determined. Testing once per week likely would have minimal effect; thus performing the test at least twice or three times per week will be necessary. Does a laboratory have sufficient personnel to add a new, relatively labor-intensive procedure to the current workload? This question must be answered by each laboratory director. Secondly, detailed cost analysis, accounting for equipment, testing frequency, technologist time, and kit wastage (discarding reagents not used prior to expiration), must be done. The cost of performing the test in-house must be weighed against the cost of referring the samples elsewhere for testing. Regardless of whether the test is done in-house or referred, the laboratory director must decide whether the test will be performed routinely on the first AFB smear-positive respiratory specimen from all patients or require a separate order, which potentially could delay testing. Those laboratories that refer samples elsewhere for testing must investigate the quality of the referral site, turnaround time, and shipping requirements.

Future research issues. An aspect not only of DAT, but of all rapid, direct detection/identification tests that would benefit from further investigation involves finding an answer to the question, "What constitutes a 'good' quality specimen?" Guidelines for differentiating acceptable from unacceptable sputum specimens submitted for aerobic bacterial culture exist but cannot be applied to mycobacterial culture (15). Identification of one or more parameters that would allow rejection of poor quality specimens submitted for the microbiological diagnosis of tuberculosis would increase efficiency in the laboratory and improve the reliability of the test results.

Other issues discussed related specifically to DA T. The first topic addressed the use of DAT for AFB smear-negative respiratory specimens. Before introducing the test for patient samples in this non-FDA-approved situation, all laboratories must perform an in-house validation. Unfortunately, the number of specimens required for such a validation has not been defined. Consultation with a statistician regarding an acceptable number of samples, referencing articles published in the peer-reviewed literature in the validation documentation, and following published guidelines as they become available were recommended. Another facet of this issue that requires additional research is the use of DAT for identification of tuberculosis in subpopulations of patients, for example those with HIV infection or children, who would receive optimal benefit from DAT, and in whom the test might perform differently than in the general population.

A second area in which clinical data are lacking concerns performance of DAT on nonrespiratory specimens, such as sterile body fluids (especially cerebrospinal and pleural fluids), gastric aspirates, and tissues, including samples fixed in formalin. Optimal design of a clinical study to evaluate test performance on extrapulmonary specimens would require the participation of multiple institutions. Protocols for standardizing specimen processing must be developed, and a sufficient number of specimens from each site, yielding an appropriate number of positive results, must be tested.

A third role of DAT that has great promise is direct identification of ***M. tuberculosis-complex*** organisms in positive my-

cobacterial broth cultures. Preliminary data indicate that, when used in this manner, the sensitivity and specificity of the DAT for detection of *M. tuberculosis* complex are 99% or greater, and results are available significantly sooner than is currently possible with available commercial culture and identification tests (16).

Standards for clinical trials. Clinical trials for evaluation of new diagnostic products should be designed to eliminate as many variables as possible. Good laboratory practice should be performed, and results compared against the physician's clinical assessments of the disease.

Clinical Issues Focus Group Discussion

The group considered separately issues concerning the use of DAT in AFB smear-positive and smear-negative patients, in patients who had not received prior chemotherapy or who were not receiving chemotherapy at the time the test is done, and in patients at high risk for having active tuberculosis as compared with those at low risk. Additionally, the group considered the impact of a rapid test on individual patient care, and briefly considered the impact on infection control in general. Finally, the group addressed questions for future research on the clinical role of rapid tests. It should be noted that a firm consensus was reached on relatively few points, reflecting deficiencies in many of the studies published to date. This point is amplified below.

An ideal rapid diagnostic test. All participants in the Clinical Issues Focus Group felt that there was a strong need for new diagnostic tests for tuberculosis, as the general approach to diagnosis of tuberculosis has changed little from the days of Robert Koch. Specifically, there was great interest and enthusiasm expressed for a test which could accurately diagnose tuberculosis. Excluding NTM as a cause of a positive sputum AFB smear, diagnosis of AFB smear-negative tuberculosis, assessment of response to therapy, detection of relapse, assessment of the infectiousness of a given patient, diagnosis of paucibacillary forms of tuberculosis (e.g., pediatric tuberculosis, extrapuhnonary tuberculosis), and the diagnosis of culture-negative tuberculosis were all identified as areas where an ideal test could have substantial impact clinically. It was in that framework that the Clinical Issues Focus Group discussed currently available DAT.

AFB smear-positive disease. Sputum samples which are positive by currently available staining techniques and also positive using DAT are overwhelmingly likely to contain *M. tuberculosis-complex in* the vast majority of laboratories. Having that information available within a day or so of the patient's presentation was felt by the group to be of significant value, aiding the clinician's interaction with the patient in several ways. Specifically, the public health authorities could be notified promptly to begin a contact investigation, the clinician's diagnostic uncertainty in some cases could be assuaged (particularly in regions where pulmonary disease due to NTM is common), and, in the event that the AFB smear-positive specimen is overgrown with non-acid-fast microorganisms, species confirmation will already have been provided. There were felt to be few risks of mistreatment associated with an AFB smear-positive, DAT-positive test result, other than the cost, owing to the very rare occurrence of false-positive tests in this setting.

For AFB smear-positive, DAT-negative specimens, most in the group felt comfortable in delaying antituberculosis therapy and waiting for culture confirmation of what is likely a case of disease due to NTM. This feeling was based primarily on data from the AMPLICOR® *M. tuberculosis* test trials showing that, of 30 patients with AFB smear-positive, AMPLICOR® *M. tuberculosis* test-negative specimens, only three (10%) patients ultimately had tuberculosis (17). This is reflected in the overall sensitivity of 95–96% for both the Roche and Gen-Probe assays (17-19). Some clinicians from areas where disease due to NTM is not common felt that they would not be comfortable withholding therapy for AFB smear-positive, DAT-negative specimens, and would treat this category of cases until culture results were available. Others felt that they would, in fact, withhold antituberculosis therapy in this situation, and await culture results. The overall opinion was that the greatest impact of the DAT would be in AFB smear-positive patients who were nonetheless believed to be at low risk for having tuberculosis on clinical grounds. In that setting, a negative DAT might allow therapy to be stopped, based on available data.

AFB smear-negative disease. The Clinical Issues Focus Group next considered the use of the DAT in AFB smear-negative tuberculosis suspects, noting at the outset that any such use would be an off-label procedure as far the FDA was concerned and would require additional information as described above in the Laboratory Focus Group discussion.

The group did not feel that the test should routinely be done in all circumstances. However, they noted that the published data for both the Gen-Probe@ MTD test and the Roche AMPLICOR® *M. tuberculosis* test indicate that these assays will identify about one-half to three-quarters of all AFB smear-negative, culture-positive cases of tuberculosis (3, 20). It was noted that this is a substantial limitation of these tests compared with the ideal test. The members felt strongly that a diagnosis of tuberculosis could not be excluded in a AFB smear-negative, DAT-negative patient if there was a reasonably high clinical suspicion (based on demographic, clinical, and/or radiographic features) of tuberculosis. There was significant debate and little consensus, concerning the definition of a high-risk cohort of patients; however, cohorts with prevalence of tuberculosis ranging from 10 to 50% were put forth as examples of high-risk categories. Some in the group felt that, in a very low-risk (the definition of the term "low risk" also remained a matter of debate) patient, a negative DAT might justify a delay in empiric therapy and an active search for other diagnoses.

Others felt that a positive DAT with a negative AFB smear could justify initiation of therapy, and that DAT might be a reasonable intermediary step before resorting to bronchoscopy for a diagnosis of tuberculosis. However, it was pointed out that data presented to the FDA indicated that only 19 out of 33 AFB smear-negative, AMPLICOR® *M. tuberculosis* test-positive patients were ultimately determined to have culture-proven tuberculosis. The same principle applies to the use of the Gen-Probe@ MTD product, so that AFB smear-negative, DAT-positive patients have an intermediate probability of having active tuberculosis, using the culture result as the gold standard. It was noted that patients with culture-negative tuberculosis are not reported in the currently available clinical trials, and the group strongly urged that rigorous clinical reporting and diagnosis be a part of any future clinical trials of these or related assays. There was no consensus concerning the appropriate use of DAT in AFB smear-negative patients.

Patients previously treated or currently receiving treatment. With regard to the evaluation of patients previously treated, or currently receiving treatment for tuberculosis, the group noted that there have been numerous published and unpublished reports indicating that DAT may remain positive for many months after the institution or completion of therapy, even in the face of apparent good clinical response and out-

come (21-23). Based on these data, the group felt that, precluding systematic studies that provide detailed clinical information and outcome data concerning tuberculosis patients and suspects, currently available DAT should not be used for patients on antituberculosis therapy.

The evaluation of patients with pediatric tuberculosis and other paucibacillary form of tuberculosis. The group felt that an ideal test should aid greatly in the diagnosis of pediatric tuberculosis and the various presentations of extrapulmonary tuberculosis, such as tuberculosis meningitis and tuberculosis pleurisy. The novel use of specimens such as peripheral blood was also mentioned as a possible potential use of these tests. However, because of the lack of significant data to date about the use of DAT in these situations, the group could reach no consensus concerning their routine use in such situations. In particular, it was noted that data presented at the plenary session indicated that in one series, 39% of children with tuberculosis infection without evidence of active disease had ***M. tuber***culosis-complex DNA detected from gastric aspirates (24). This underscored to the group the importance of rigorous clinical follow-up of patients in the field trials in order to identify culture-negative active tuberculosis.

Infectiousness, response to therapy and other uses. The group noted that, based on current data, the amplification tests are superior to sputum AFB smear, in terms of accuracy, in diagnosing active pulmonary tuberculosis in previously untreated individuals. However, because clinical trials to date provide no information about the interpretation of the test regarding infectiousness of individual patients, response to therapy, prediction of relapse, and drug susceptibility, the DAT cannot replace either sputum AFB smears or cultures for follow-up of cases on treatment.

Future issues. Overall, the group welcomed the possibility of a new era in tuberculosis diagnostics. It also identified several areas where future clinical trials of such tests should aim to answer specific questions, including, but not limited to, the following:

1. Improvement of the performance of the test in AFB smear-negative, culture-positive disease.
2. Patient-based, rather than laboratory-based clinical trials, to specifically address the issue of culture-negative tuberculosis.
3. Studies to address use of the tests in large numbers of previously treated individuals.
4. Studies to address the use of the tests in pediatric populations.
5. Studies to address the use of the tests in paucibacillary forms of tuberculosis, such as tuberculosis meningitis, pericarditis, and other extrapulmonary manifestations.
6. Studies relating to the interpretation of the test ***vis-á-vis*** infectiousness of individual patients.
7. Studies relating to the interpretation of the test ***vis-á-vis*** response to therapy.

The Clinical Issues Focus Group felt strongly that making clinical recommendations for the use of DAT based on studies that have primarily been focused on the laboratory is a difficult task. However, the recommendations of the group have been made in view of the best evidence available to it. The focus group urges the performance of additional clinical trials framed around the above questions.

Public Health Focus Group Discussion

Introduction. The Public Health Focus Group opened its discussion by having Kenneth Castro, Director of the Division of Tuberculosis Elimination at the Centers for Disease Control and Prevention (CDC), remind us that the public health goals with respect to tuberculosis are to identify cases, initiate and complete adequate treatment, reduce disease transmission and identify and evaluate contacts and others at high risk for developing tuberculosis. From the public health perspective, the value of the DAT is the measure to which they help meet these goals. (Since this workshop, the CDC has published its interim guidelines for the uses of the DAT, which reflect some of the discussions that took place at this meeting [25].)

Five general themes emerged from the discussion:

1. The need to educate clinicians about issues surrounding these new tests.
2. The "added value" of DAT compared to tests currently available.
3. The usefulness of DAT in deciding when to commence treatment, isolation and contact investigation when clinical suspicion of tuberculosis is high or low and when the AFB smear is positive or negative.
4. The public health research questions raised by the use of these tests.
5. The use of DAT in international settings.

It was agreed that cost ***per se*** would not be considered the most important factor in the group's discussion, since. the price per unit would probably be reduced with time and with increased utilization.

Need to educate clinicians. Based on the group's understanding of the concerns of ***laboratorians,*** it was agreed that increased emphasis should be placed on collecting adequate (in both quantity and quality) clinical specimens. The group also discussed the need to delineate clear guidelines for the clinician regarding the value and limitations of the DAT, the meaning of test results, and the importance of considering the level of sensitivity, specificity and predictive value (which will differ according to the prevalence of disease in the populations tested).

Added value of DAT. There were many more questions than answers in this part of the discussion. What is the goal of using DAT? Is it sufficient that the new tests are marginally better than those currently available, or must they meet higher standards? The group felt that currently available DAT would not reduce the incidence or mortality of tuberculosis, but could be expected to improve patient care. Since the number of undiagnosed cases of tuberculosis is small, it was also recognized that, despite the potential increased cost of DAT, they could serve to conserve and focus resources through decreased need to treat, isolate, and undertake contact investigations.

Decision algorithms. DAT are not stand-alone tests. An algorithm was developed by the focus group, reflecting the opinions of the people in the room at that time. It should be noted that this algorithm is not intended to represent an official recommendation, from either the CDC or the ATS. The algorithm suggests one clinical (the decision to treat) and two public health (the decisions to isolate and quickly begin a contact investigation) actions in situations integrating the following variables: high or low clinical suspicion of tuberculosis; AFB smear-positive or -negative results; and DAT-positive or -negative results. The algorithm compares actions with or without knowledge of DAT results. (Refer to Table 2 for the algorithm.) In some circumstances, the group could not agree upon the action to take, as indicated on the table by a question mark.

The greatest potential impact of the DAT on the decisions to initiate antituberculosis treatment, isolation or contact investigation would be in just those areas where there is still uncertainty about DAT's sensitivity, specificity, and predictive

TABLE 2

POTENTIAL ACTION WHILE WAITING FOR CULTURE RESULT

		High Clinical Suspicion of Tuberculosis				Low Clinical Suspicion of Tuberculosis			
		AFB Smear-(+)		AFB Smear-(-)		AFB Smear-(+)		AFB Smear-(-)	
Potential Action	DAT Results	Action without DAT Results	Action with DAT Results	Action without DAT Results	Action with DAT Results	Action without DAT Results	Action with DAT Results	Action without DAT Results	Action with DAT Results
Treat	(+)	Yes	Yes	Yes	Yes	Yes	Yes	No	?
Isolate	(+)	Yes	Yes	Yes	Yes	Yes	Yes	No	?
Begin contact investigation	(+)	Yes	Yes	No	**Yes**	Yes	Yes	No	No
Treat	(-)	**Yes**	?	Yes	?	Yes	No	No	No
Isolate	(-)	Yes	?	**Yes**	No	**Yes**	No	No	No
Begin contact investigation	(-)	**Yes**	No	No	No	Yes	No	No	No

Definition of abbreviations: DAT = direct amplification test; AFB = acid-fast bacilli; ? = divided opinion among attendees; bold typeface = different actions with and without knowlege of DAT results.

value. The most likely application of DAT would be earlier discontinuation of nonindicated treatment and isolation, eliminating the need to perform a contact investigation, and possibly earlier identification of tuberculosis cases. Possible harm if the tests were wrong would be to delay public health activities or, in the rare case of a false positive DAT, erroneous initiation of treatment.

Public health research agenda. The area of greatest discordance regarding the decision to initiate treatment, isolation, and contact investigation was the situation where the clinical suspicion of tuberculosis is high, but the AFB smear is negative. Based on the algorithms developed by the group, in this clinical situation, if the DAT is positive, contact investigations may be started prematurely. On the other hand, when both the AFB smear and DAT are negative, treatment or isolation may not be started when they might be needed. Thus, for the DAT to be useful to tuberculosis control programs, sensitivity and specificity must be improved when the AFB smear is negative. Another issue that must be addressed is whether the AFB smear or DAT constitutes the gold standard for initial decisions to treat, isolate, and begin contact investigations. Are DATs too sensitive as a measure of infectiousness? Is it appropriate to use them at all to measure infectiousness, given that we are amplifying and identifying the presence of mycobacterial DNA or RNA, rather than the bacilli themselves?

International settings. Given that most cases of tuberculosis occur outside of the United States, there was some debate on the role of DAT in international settings. Some participants felt that these tests had no role for tuberculosis control programs of developing countries, where limited financial and program resources preclude the use of even the most basic tool, the AFB smear. Others felt that since these countries are at different stages of financial and technical development, and their populations are very heterogeneous, it was not the role of this group to make any statement on the use of these tests worldwide.

Conclusions of the public health group. In general, the public health group felt that while DAT are potentially useful to the control of tuberculosis, they are currently in their first generation and are not yet sensitive or specific enough in AFB smear-negative patients to have a great impact on public health decision-making. Further research is needed in those areas of discordance delineated by the group.

CONCLUSIONS

Conference participants were in agreement that DAT or possibly other classes of rapid diagnostic tests for ***M. tuberculosis*** were highly desirable. Important attributes of such a test would include cost-effectiveness and ease of use in the laboratory without conventional sputum digestion. Such a test would be useful in distinguishing positive APB smears due to ***M. tuberculosis*** from those due to NTM. The tests should also be useful in identifying AFB smear-negative tuberculosis. Other desirable uses would include evaluation of nonrespiratory specimens, determining infectiousness, following the response to therapy, and identifying early relapse or its risk. It was also recognized that such a test could be potentially quite useful in prioritizing individuals for isolation and contact tracing.

Data were presented on two DAT that had undergone substantial clinical trials; it was recognized by all that the current performance of these tests was such that not all of the above expectations could be realized. This conclusion was based upon actual test performance and upon the clinical trials used to evaluate them. The majority of published studies were laboratory based, utilizing culture result as a major endpoint and neglected to integrate the available clinical information into the decision process. In future trials comparing DAT with current laboratory methods, good laboratory practice should be strictly exercised, and clinical data and assessments must be included in the design.

It is possible to draw some conclusions about the current roles for DAT. There was fairly broad agreement that a positive DAT result in AFB smear-positive patients is of benefit in distinguishing tuberculosis from NTM. There was not complete agreement on how to manage patients who were AFB smear-positive/DAT-negative. Some individuals would treat these patients for tuberculosis and others would regard them as AFB smear-positive due to NTM. The local prevalence of NTM infections and the overall clinical suspicion of tuberculosis would be important in this setting. There was not agreement about the need for isolation in this situation, but the public health group did feel that contact tracing could be deferred until culture results were available.

In AFB smear-negative situations, there was not complete agreement with regard to when a DAT should be performed or how the result should be interpreted. There was recognition that, given an overall sensitivity for both the Gen-Probe and

the Roche tests of about 50% in this setting, and despite their fairly high specificity, both false-positive and false-negative results can be expected. It was recognized that underlying clinical suspicion would be very important in using these results.

There was considerable agreement about directions for future research. Important issues need to be addressed, such as (I) the use of DAT in large numbers of previously-treated individuals; (2) the use of DAT in subpopulations such as HIV-positive patients and children; (3) the use of DAT in paucibacillary forms of tuberculosis (culture-negative pulmonary tuberculosis, meningitis, pericarditis, and other extrapulmonary manifestations); (4) the interpretation of the test *vis-á-vis* the infectiousness of individual patients; (5) the interpretation of DAT *vis-a-vis* the response to therapy; and (6) the interpretation of DAT *vis-á-vis* the decision to isolate a patient and to begin contact investigations. These and other questions need to be answered in well designed, multidisciplinary studies.

One overriding theme throughout the meeting was the recognition that a better understanding of how to use these tests in conjunction with available clinical information is essential. The completed clinical trials were not designed to do this, and, in fact, although many participants indicated that the clinical suspicion of tuberculosis would play a major role in their response to a DAT result, particularly when it was discrepant with the AFB smear, it was acknowledged that the definition of low versus high risk was inexact at best. Complicating this was the recognition that the actual diagnosis of tuberculosis was itself not as clear cut as one might like. The participants expressed the need for research to develop a clinical standard for the diagnosis of tuberculosis. In addition, culture-negative tuberculosis was accepted by all as a real entity and a situation in which these tests might be of value. There was agreement between clinicians, investigators, manufacturer representatives, and FDA staff that increased cooperation was essential in order to resolve this latter issue.

These DAT represent a significant scientific advance. Their judicious use can expedite TB control activities in certain circumstances. They can be expected to evolve and become more useful in the future. At present, they add to, but do not replace, the current armamentarium of diagnostic measures that assist TB treatment and control. As the technologies improve and evolve and more, better designed clinical trials are conducted, the appropriate use for the rapid diagnostic tests will be re-examined, resulting in more definitive recommendations.

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