MAJOR ARTICLE

Performance of Nucleic Acid Amplification Tests for Diagnosis of Tuberculosis in a Large Urban Setting

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(See the editorial commentary by Dorman on pages 55–7)

Background. A diagnosis of tuberculosis (TB) relies on acid-fast bacilli (AFB) smear and culture results. Two rapid tests that use nucleic acid amplification (NAA) have been approved by the US Food and Drug Administration for the diagnosis of TB based on detection of *Mycobacterium tuberculosis* from specimens obtained from the respiratory tract. We evaluated the performance of NAA testing under field conditions in a large urban setting with moderate TB prevalence.

Methods. The medical records of patients with suspected TB during 2000–2004 were reviewed. Analysis was restricted to the performance of NAA on specimens collected within 7 days after the initiation of treatment for TB. The assay's sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) were evaluated.

Results. The proportion of patients with confirmed or suspected TB whose respiratory tract specimens were tested by use of NAA increased from 429 (12.9%) of 3334 patients in 2000 to 527 (15.6%) of 3386 patients in 2004; NAA testing among patients whose respiratory tract specimens tested positive for AFB increased from 415 (43.6%) of 952 patients in 2000 to 487 (55.5%) of 877 patients in 2004 ($P < .001$ for both trends). Of the 16,511 patients being evaluated for pulmonary TB, 4642 (28.1%) had specimens that tested positive for AFB on smear. Of those 4642 patients, 2241 (48.3%) had NAA performed on their specimens. Of those 2241 patients, 1279 (57.1%) had positive test results. Of those 1279 patients, 1262 (98.7%) were confirmed to have TB. For 1861 (40.1%) of the 4642 patients whose specimens tested positive for AFB on smear, the NAA test had a sensitivity of 96.0%, a specificity of 95.3%, a PPV of 98.0%, and an NPV of 90.9%. For 158 patients whose specimens tested negative for AFB on smear, the NAA test had a sensitivity of 79.3%, a specificity of 80.3%, a PPV of 83.1%, and an NPV of 76.0%, respectively. For the 215 specimens that tested positive for AFB by smear, we found a sensitivity, specificity, PPV, and NPV of 97.5%, 93.6%, 95.1%, and 96.8%, respectively. A high-grade smear was associated with a better test performance.

Conclusion. NAA testing was helpful for determining whether patients whose specimens tested positive for AFB on smear had TB or not. This conclusion supports the use of this test for early diagnosis of pulmonary and extrapulmonary TB.

In the mid-1990s, the US Food and Drug Administration approved 2 rapid diagnostic tests—the Amplified *Mycobacterium tuberculosis* Direct (MTD) test (Gen-Probe) and the Amplicor *M. tuberculosis* test (Roche Diagnostics)—that use nucleic acid amplification (NAA) for the diagnosis of tuberculosis (TB) in patients whose sputum specimens tested positive for acid-fast bacilli (AFB) on smear [1]. In 1999, the US Food and Drug Administration approved the MTD test for use on specimens obtained from the respiratory tract that tested negative for AFB on smear [2]. The American Thoracic Society deems that NAA tests are a significant addition to the diagnostic tools available for the detection of TB, particularly in patients with possible pulmonary TB (i.e., patients whose sputum specimens

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tested positive for AFB on smear) [3]. The evaluation of rapid diagnostic tests for TB is particularly critical in places like New York City, because the city has a relatively high prevalence of TB and is home to large immigrant populations, and as a result, decisions need to be made promptly with regard to the treatment and/or respiratory isolation of patients with TB.

In small- to moderate-size studies [4–18], the use of NAA testing for the rapid diagnosis of the *M. tuberculosis* complex has been evaluated using specimens obtained from the respiratory tract and from other body sites. For respiratory tract specimens that tested positive for AFB on smear, some authors have reported test sensitivities of 73%–100%, specificities of 96%–100%, positive predictive values (PPVs) of 86%–100%, and negative predictive values (NPVs) of >99% [4–8, 10–13, 16, 17, 19, 20]. Although NAA testing had a lower sensitivity for respiratory tract specimens that tested negative for AFB on smear, compared with respiratory tract specimens that tested positive for AFB on smear, the sensitivity was higher than that reported using AFB microscopy and was close to that reported using mycobacterial culture [4, 5, 10, 16, 19, 20].

In addition, several studies have shown the benefits of NAA for testing specimens obtained from body sites other than the respiratory tract. Evaluations of the NAA test for use on tissue specimens (pleural, lung, lymph node, liver biopsies, and bone marrow) and body fluids (central nervous system, pleural, ascitic, synovial, pericardial, and peritoneal fluids) showed a sensitivity of 64%–86%, a specificity of 97–100%, a PPV of 81.5%–100%, and an NPV of 87.5–99.2% [5, 10–12, 14, 15, 18]. One study found a lower sensitivity (52.6%) among pleural biopsy specimens [14].

The present study retrospectively evaluates the performance of NAA tests among patients evaluated for TB in a large urban setting by several mycobacteriology laboratories in a real-life situation, using information entered in the New York City Department of Health and Mental Hygiene TB registry. The objectives of our study were (1) to describe the characteristics of patients whose specimens were tested by use of NAA and the factors associated with the decision to perform NAA testing; (2) to calculate the sensitivity, specificity, PPV, and NPV of NAA tests; and (3) to examine the relationship between the NAA test result, the AFB smear result and grade, and the culture result, per patient, for detection of the *M. tuberculosis* complex.

METHODS

Patients. The study population consisted of patients evaluated for TB and reported to the New York City Department of Health and Mental Hygiene's Bureau of Tuberculosis Control during the period from 2000 through 2004. A diagnosis of TB was confirmed using the Centers for Disease Control and Prevention's clinical or laboratory case definition [21].

Data. Our analysis relied on routine surveillance data from the Bureau of Tuberculosis Control's TB registry, which included sociodemographic, clinical, and laboratory data. The type of NAA test performed is not recorded in the TB registry. Most NAA testing in New York City is performed with the MTD test; the city and state public health laboratories use the MTD test (37% and 21% of the time, respectively) and performed the majority of the tests that occurred during the study period; an additional 10% of the testing was done at several private laboratories, all of which used the MTD test. The rest of the testing was performed either in out-of-city laboratories or in laboratories that were not recorded in the registry. All New York State laboratories follow the New York State Department of Health Mycobacteriology Standards for AFB smear and culture [22]. NAA testing was assumed to have been performed according to the manufacturer's recommendation [23]. The analysis was restricted to NAA tests performed on specimens collected within 7 days after the start of treatment for TB. New York City laboratories are required to report all positive test results identifying AFB or the *M. tuberculosis* complex and all negative test results associated with positive test results, such as a negative NAA test result on a specimen that tested positive for AFB on smear. In New York City, smears that are interpreted as inconclusive are considered to be positive for AFB, for the treatment of patients, and thus they were grouped with smears interpreted as positive for AFB in our analysis of smear results.

Statistical analysis. Data analysis was stratified on the basis of specimen source (i.e., sources from the respiratory tract vs. sources from other body sites). The sources of specimens from the respiratory tract included sputum, trachea, bronchus and/or bronchioles, bronchial fluid, and lung tissue. The sources of specimens from other body sites included cerebrospinal fluid, lymphatic tissue, gastric aspirate, and pleural and peritoneal fluid, of which at least 30 specimens were available for analysis of test performance.

For cases of pulmonary TB, we analyzed the performance of the NAA test by patient characteristics overall and then by stratifying patients into smear-positive and smear-negative patients. Categorical data were compared by use of the χ^2 test. To determine which factors predicted the use of NAA testing for patients with suspected pulmonary TB, multivariate logistic regression models were constructed. The analysis was performed by use of SAS, version 9 (SAS), and SPSS, version 14.0 (SPSS).

The sensitivities, specificities, PPVs, and NPVs were calculated for the NAA tests performed for patients who had specimens obtained from the respiratory tract, with the denominator being the number of patients, regardless of how many specimens were obtained for testing. The performance of NAA was evaluated by comparing NAA test results to 2 gold standards of TB diagnosis: (1) a culture positive for *M. tuberculosis,*

and (2) the combination of laboratory and clinical criteria to confirm TB, per the case definition for TB by the Centers for Disease Control and Prevention [21]. To determine whether there were any differences in test performance among patients who were evaluated for pulmonary TB on the basis of >1 specimen, we looked at the performance of NAA for the same specimen that tested positive by smear and/or culture and for different specimen that were not previously tested.

We also examined the correlation between culture positive for the *M. tuberculosis* complex—on the basis of smear grade (i.e., the number of bacilli per high-power field) by use of AFB microscopy—and NAA test result. A low grade was defined as either 1+ (i.e., bacilli were rare) or 2+ (i.e., bacilli were few in number), and a high grade was defined as either 3+ (i.e., there were a moderate number of bacilli) or 4+ (i.e., there were numerous bacilli). Smear results that were inconclusive were not included in the smear grade analysis, because they were inconsistently reported.

The performance of NAA was also evaluated for patients with suspected extrapulmonary TB, among whom we calculated the performance of NAA on the basis of the specimens tested, using, for comparison, AFB culture results as the gold standard and including only patients who had a culture result positive for *M. tuberculosis.* We examined and stratified the results on the basis of whether the patients had NAA and AFB culture performed on the same specimen or on a different specimen and found no difference in performance; therefore, we analyzed all test results, regardless of whether NAA and AFB culture were performed on the same specimen or different specimen.

The study was reviewed and approved by the New York City Department of Health and Mental Hygiene and the Centers for Disease Control and Prevention Institutional Review Boards.

RESULTS

During the 5-year study period, there were 5777 patient who received a confirmed diagnosis of TB and 13,003 patients who were suspected of having TB but for whom a diagnosis of TB was eventually excluded. Of these 18,780 patients, 16,511 were tested for TB by obtaining specimens from their respiratory tracts, and 4969 were tested for TB by obtaining specimens from other body sites.

Testing of specimens obtained from respiratory tracts of patients. The proportion of patients with confirmed or suspected TB whose respiratory tract specimens were tested by use of NAA increased from 429 (12.9%) of 3334 patients in 2000 to 527 (15.6%) of 3386 patients in 2004; NAA testing among patients whose respiratory tract specimens tested positive for AFB increased from 415 (43.6%) of 952 patients in 2000 to 487 (55.5%) of 877 patients in 2004 ($P < .001$ for both trends). Of the 16,511 patients being evaluated for pulmonary TB, 4642 (28.1%) had specimens that tested positive for AFB on smear. Of those 4642 patients, 2241 (48.3%) had NAA performed on their specimens. Of those 2241 patients, 1279 (57.1%) had positive test results. Of those 1279 patients, 1262 (98.7%) were confirmed to have TB (figure 1). In addition, 2070 (44.6%) of the 4642 patients whose specimens were positive for AFB on smear were ultimately found not to have TB. Of 902 patients whose specimens were positive for AFB on smear but who did not receive a confirmed diagnosis of TB diagnosis, 885 (98.1%) had negative NAA test results.

Patients were mostly adult, male, nonwhite, and born in countries other than the United States. The proportion of patients whose specimens were tested by use of NAA varied according to demographic and clinical characteristics (table 1). Multivariate analysis of all patients evaluated for TB indicated that the following patients were statistically significantly more likely to have had NAA performed on their specimens: female patients ($P = .006$); Hispanic patients ($P = .001$); black, non-Hispanic patients ($P = .003$); patients whose specimens tested positive for AFB on smear ($P < .001$); patients with an abnormal and/or cavitary chest radiograph finding ($P < .001$) or an abnormal and/or noncavitary chest radiograph finding ($P < .001$); patients who were employed ($P < .001$), homeless ($P = .002$), and/or alcohol users ($P = .007$); and patients seen at private hospitals ($P = .045$). Patients who were seen by private physicians not affiliated with any hospital ($P = .026$) were less likely to have NAA performed on their specimens. Patients who were drug users were not more likely than patients who were not drug users to have had NAA performed on their specimens. In a separate multivariate model for patients whose specimens tested positive for AFB on smear, Hispanic and black non-Hispanic patients, patients with an abnormal (cavitary or noncavitary) chest radiograph finding, patients who had an unknown HIV status, and patients who were homeless, used alcohol, and/or were seen by a private physician were more likely to have had NAA performed on their specimens (data not shown). In the model with only patients whose specimens tested negative for AFB on smear, female patients, HIV-positive patients, patients with an unknown HIV status, and patients who were employed and/or were treated at a public hospital or by a private physician were more likely to have an NAA test performed; in contrast, patients with an abnormal chest radiograph were not more likely than those without an abnormal chest radiograph to have had NAA performed on their specimens (data not shown).

Test performance. For the 2418 patients who received a confirmed diagnosis of TB on the basis of either a positive culture result or clinical criteria, the NAA test had a sensitivity of 92.4%, a specificity of 97.3%, a PPV of 98.1%, and an NPV of 89.5%. For the 2241 patients whose specimens tested positive for AFB on smear, the NAA test had a sensitivity of 94.3%, a specificity of 98.1%, a PPV of 98.7%, and an NPV of 92.0%

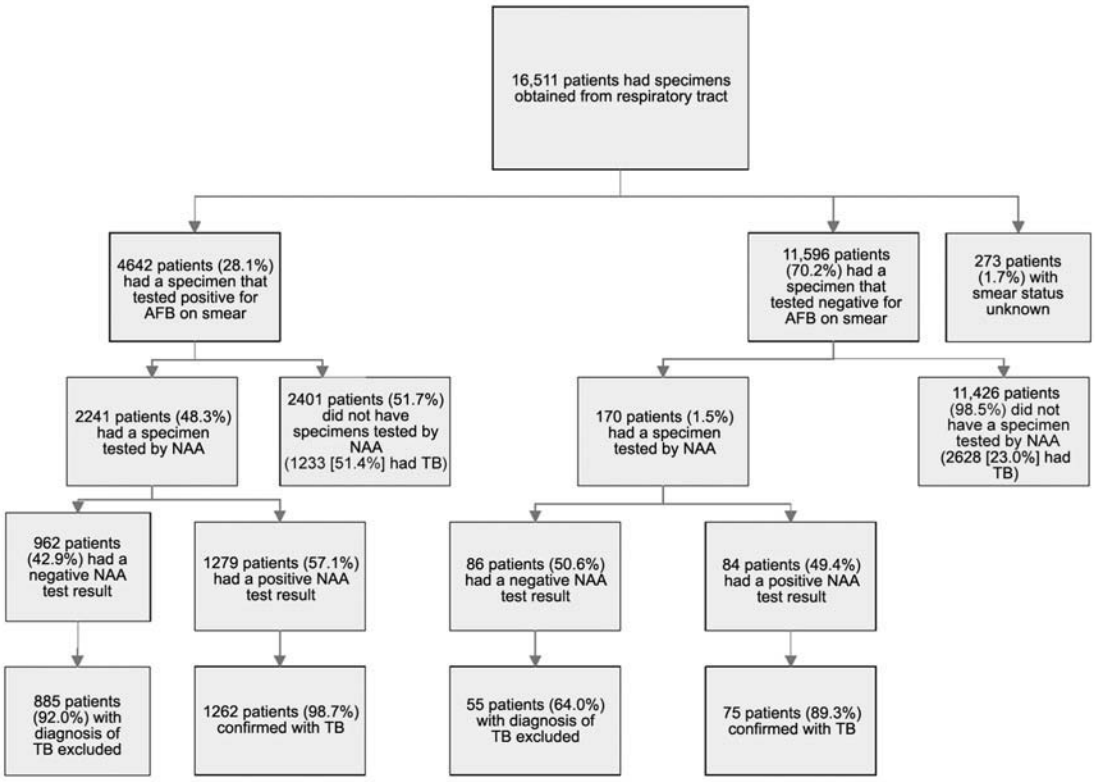

Figure 1. Distribution of nucleic acid amplification (NAA) test results and acid-fast bacilli (AFB) smear results for specimens obtained from the respiratory tract of patients suspected of having tuberculosis (TB) in New York City during 2000–2004.

(table 2). For the 170 patients whose specimens tested negative for AFB on smear, the NAA test had a sensitivity of 70.8%, a specificity of 85.9%, a PPV of 89.3%, and an NPV of 64.0%.

For the 2021 patients who received a confirmed diagnosis of TB based on a positive culture result, the NAA test had a sensitivity of 95.0%. For the 1861 patients whose specimens tested positive for AFB on smear, the NAA test had a sensitivity of 96.0%. For the 150 patients whose specimens tested negative for AFB on smear, the NAA test had a sensitivity of 79.3% (table 2). In addition, for patients whose specimens tested positive for AFB on smear and who were eventually confirmed not to have TB, the NAA test had a specificity of 92%. Data on the performance of the NAA test (i.e., the performance measures) at the 2 laboratories where most of the testing was performed were similar (data not shown).

Test performance based on AFB smear grade. The sensitivity of NAA testing was higher for the 900 patients whose specimens had a high-grade smear than it was for 848 patients whose specimens had a low-grade smear (97.6% vs. 92.2%) (table 3). For the 800 patients whose specimens had a high-grade smear and a culture positive for *M. tuberculosis* (i.e., the gold standard of TB diagnosis), the NAA test had a sensitivity of 98.2%, a specificity of 94.0%, and a PPV of 99.4%. For the 668 patients whose specimens had a low-grade smear and a culture positive for *M. tuberculosis*, the NAA test had a sensitivity of 94.4%, a specificity of 92.8%, and a PPV of 96.0% (table 3).

Compared with the use of AFB smear microscopy, the use of NAA testing greatly improved the predictability of the AFB culture result for the diagnosis of TB. Regardless of the results of NAA testing performed on their specimens, 780 (62.9%) of 1240 patients who had a specimen with a low-grade smear and 1067 (88.5%) of 1205 patients who had a specimen with a high-grade smear also had a specimen that tested positive for *M. tuberculosis* on culture. In comparison, 407 (96.0%) of 424 patients who had a specimen that tested positive for TB by use of NAA and who had a specimen with a low-grade smear also had a specimen that tested positive for *M. tuberculosis* on culture, and 720 (99.4%) of 724 patients who had a specimen that tested positive for TB by use of NAA and who had a specimen with a high-grade smear also had a specimen that tested positive for *M. tuberculosis* on culture (figure 2).

False-positive and false-negative results. NAA testing resulted in some false-positive and false-negative results. With the use of overall TB diagnosis as the comparison, 26 of 1366 patients had false-positive NAA test results, resulting in a false-

Table 1. Demographic and clinical characteristics of patients with suspected and confirmed pulmonary respiratory specimens tested in New York City during 2000–2004.

Characteristic	Patients whose specimens were tested by NAA (n = 2418)	Patients whose specimens were not tested by NAA (n = 14,093)	Adjusted OR	P
Sex				
Female	1038 (42.9)	5458 (38.7)	1.18	.006
Male	1380 (57.1)	8634 (61.3)	Ref	
Race/ethnicity				
White, non-Hispanic	319 (13.2)	1324 (9.4)	Ref	
Hispanic	687 (28.4)	3688 (26.2)	1.37	.001
Black, non-Hispanic	773 (32.0)	3699 (26.2)	1.34	.003
Asian	426 (17.6)	2564 (18.2)	1.15	.19
Other or unknown	213 (8.8)	2818 (20.0)	1.14	.28
Birthplace				
United States	1010 (41.8)	3722 (26.4)	Ref	
Country other than the United States	1134 (46.9)	5816 (41.3)	Not obtained[a]	
Unknown	274 (11.4)	4555 (32.3)	Not obtained[a]	
AFB smear status				
Positive	2241 (92.7)	2401 (17.0)	48.5	<.001
Negative	170 (7.0)	11,426 (81.1)	Ref	
Unknown	7 (0.3)	266 (1.9)	2.0	.07
Type of radiograph				
Cavitary	548 (22.7)	937 (6.6)	2.69	<.001
Abnormal and/or noncavitary	1526 (63.1)	10,101 (71.7)	1.9	<.001
Normal	202 (8.4)	1980 (14.0)	Ref	
Unknown	142 (5.9)	1075 (7.6)	1.93	<.001
HIV status				
Positive	500 (20.7)	2513 (17.8)	0.86	.08
Negative	996 (41.2)	3390 (24.1)	Ref	
Unknown	922 (38.1)	8190 (58.1)	0.77	<.001
Type of provider				
Public hospital	570 (23.6)	5747 (40.8)	0.97	.81
Private hospital	1387 (57.4)	3999 (28.4)	1.25	.045
Private practice	83 (3.4)	487 (3.5)	0.75	.023
Public health clinic	182 (7.5)	3084 (21.9)	Ref	
Employment status				
Employed	2010 (83.1)	5956 (42.3)	1.42	<.001
Unemployed	408 (16.9)	8137 (57.7)	Ref	
Living status				
Homeless[b]	187 (7.7)	641 (4.5)	1.44	.002
Not homeless	2231 (92.3)	13,452 (95.5)	Ref	
Alcohol use				
History of alcohol abuse[c]	370 (15.3)	764 (5.4)	1.28	.007
No history of alcohol abuse	2048 (84.7)	13,329 (94.6)	Ref	

NOTE. Data are no. (%) of patients. ABF, acid-fast bacilli; HIV, human immunodeficiency virus; NAA, nucleic acid amplification; OR, odds ratio; Ref, reference.

[a] This variable was not kept in the model, and a significance level was not obtained.
[b] Homeless at or within 12 months of TB diagnosis.
[b] Alcohol abuse within 12 months before TB diagnosis.

Table 2. Data on the performance of nucleic acid amplification testing of respiratory tract specimens for the *Mycobacterium tuberculosis* complex in New York City during 2000–2004.

Type of patient, performance measure	Patients who received a TB diagnosis on the basis of either a positive culture result or clinical criteria (n = 2418)	Patients who received a TB diagnosis solely on the basis of a positive culture result (n = 2021)
All patients		
Sensitivity, %	92.4	95.0
Specificity, %	97.3	97.3
PPV, %	98.1	97.1
NPV, %	89.5	89.4
Patients whose specimens tested positive for AFB on smear[a]		
Sensitivity, %	94.3	96.0
Specificity, %	98.1	98.1
PPV, %	98.7	98.7
NPV, %	92.0	94.5
Patients whose specimens tested negative for AFB on smear[b]		
Sensitivity, %	70.8	79.3
Specificity, %	85.9	85.9
PPV, %	89.3	88.3
NPV, %	64.0	75.3

NOTE. AFB, acid-fast bacilli; NPV, negative predictive value; PPV, positive predictive value.

[a] There were 2241 patients who received a diagnosis on the basis of either a positive culture result or clinical criteria and 1861 patients who received a diagnosis of TB solely on the basis of a positive culture result.

[b] There were 170 patients who received a diagnosis on the basis of either a positive culture result or clinical criteria and 158 patients who received a diagnosis of TB solely on the basis of a positive culture result.

positive rate of 1.9%. Among 800 patients who had a specimen with a high-grade smear, there were only 3 who had false-positive results, resulting in a false-positive rate of 0.4%. In contrast, there were 110 of 1450 patients with false-negative results overall (for a false-negative rate of 8%) and 70 of 1390 patients with false-negative results when culture was used as a gold standard (for a false-negative rate of 5%); the false-negative rate decreased further, to 2.4% overall among the 18 patients who had a specimen with a high-grade smear and to 1.8% among the 13 patients who had a high-grade smear and whose culture result was positive for AFB culture (i.e., the gold standard).

Patients with extrapulmonary TB. During the study period, 4969 patients were evaluated for extrapulmonary TB by the testing of 12,171 specimens obtained from body sites other than the respiratory tract. There were 682 specimens tested by use of both NAA and AFB culture. Specimens tested included 159 lymph node tissue specimens (88 tested by culture and NAA), 94 gastric aspirate specimens (65 tested by culture and NAA), 83 pleural fluid specimens (56 tested by culture and NAA), 56 peritoneal fluid specimens (31 tested by culture and NAA), and 257 cerebrospinal fluid specimens (188 tested by culture and NAA). For the 682 specimens tested by use of NAA, we found an overall sensitivity, specificity, PPV, and NPV of 89.3%, 74.5%, 79.3%, and 86.5%, respectively (table 4). For the 215 specimens that tested positive for AFB by smear, we found a sensitivity, specificity, PPV, and NPV of 97.5%, 93.6%, 95.1%, and 96.8%, respectively. For the 383 specimens that tested negative for AFB by smear, we found a sensitivity, specificity, PPV, and NPV of 83.2%, 65.6%, 70.7%, and 79.7%, respectively. As shown in table 4, the performance of NAA testing varied by type of specimen tested.

DISCUSSION

Our analysis showed that the performance of NAA for the diagnosis of TB based on detection of *M. tuberculosis* in respiratory tract specimens in clinical settings was consistent with published reports. The MTD manufacturer's package insert reports a sensitivity of 96.9%, a specificity of 100%, and a PPV of 100% for patients' specimens that also tested positive for AFB on smear [23], whereas for patients' specimens that tested negative for AFB on smear, there was a sensitivity of 72%. Compared with published studies [4–13, 16] that reported sensitivities of 73%–93% and specificities of 96%–100%, our study, which is based on a large number of patients' respiratory tract specimens obtained over several years, showed a sensitivity as high as 96% among patients whose specimens tested positive for AFB on smear and a sensitivity as high as 79% among patients whose specimens tested negative for AFB on smear;

Table 3. Data on the performance of nucleic acid amplification testing of specimens obtained from patients who also had specimens that tested positive for acid-fast bacilli (AFB) on smear in New York City during 2000–2004, by smear grade.

Type of patient, performance measure	Patients whose specimens had a high-grade smear (n = 900)	Patients whose specimens had a low-grade smear (n = 848)
All Patients		
Sensitivity, %	97.6	92.2
Specificity, %	98.1	97.2
PPV, %	99.6	97.4
NPV, %	90.0	91.7
Patients whose specimens tested positive for *M. tuberculosis* on culture[a]		
Sensitivity, %	98.2	94.4
Specificity, %	94.0	92.8
PPV, %	99.4	96.0
NPV, %	82.9	90.1

NOTE. NPV, negative predictive value; PPV, positive predictive value.

[a] There were 800 patients whose specimens tested positive for AFB and had high-grade smear and 668 patients whose specimens tested positive for AFB and had low-grade smear (for details about smear grades, see Methods).

these sensitivities indicate that the performance of NAA testing in the real-life setting of New York City was as good as in the manufacturer's controlled study. The value of using both NAA and AFB microscopy on smear, even for patients with a high-grade smear, was demonstrated by the higher rate of *M. tuberculosis* culture positivity among patients with high-grade smear and positive NAA test results (i.e., 99.4%), compared with the rate of *M. tuberculosis* culture positivity among patients with high-grade smear alone (i.e., 88.5%). In New York City, a significant number of patients had NAA performed on specimens obtained from body sites other than the respiratory tract, of which the sensitivity of the NAA test was high (~90% overall). Our results were higher than the results reported in some published studies [14, 18] and validated the results reported in other published studies [5, 10, 12].

These results are encouraging, although our study has a few limitations. NAA testing was performed at several laboratories, with the resulting inability to control for laboratory processing. Although most of the testing was performed at laboratories known to use the MTD test, for approximately one-third of the specimens, the type of NAA test was not known. Although this lack of knowledge is a limitation, it does not diminish the value of our study of the performance of NAA in a real-life situation, given the large sample size. The rate of false-negative results was high overall at 8% (compared with the rate of false-negative results for all patients with TB) and was comparable those reported in previous studies [6, 7, 10, 11], but rates of false-negative results reported in the literature vary widely. The rates of false-negative results were perhaps the result of laboratory processing variability or errors and/or the result of the presence of inhibitors. Therefore, a negative NAA test result, by itself, should not be used to exclude a diagnosis of TB in patients in whom there is a high suspicion of TB. Our retrospective study could not evaluate the presence of inhibitors or specimen suitability. It is notable that, among patients who had a specimen with a high-grade smear, the rate of false-negative results decreased to 2.4%. Therefore, we believe that a negative NAA test result is consistent with a low likelihood of TB. In contrast, the rate of false-positive results was low overall (i.e., >2%). The reasons for false-positive NAA test results are unclear and include possible specimen contamination in the laboratory. The association of a high-grade smear and a positive NAA test result is highly associated with active TB, because there were only 3 instances of false-positive results in this group. Another limitation is that negative NAA test results may not have always been reported to the New York City Department of Health and Mental Hygiene, thus potentially inflating the study results. However, we believe that the failure to report negative NAA test results is infrequent, particularly for patients whose specimens tested positive for AFB on smear. Additional limitations include the retrospective nature of the study, which precluded studying the correlation with presenting symptoms, and the variability in the reading of AFB smear results, which may lead to the misclassification of the smear status of some patients. Despite these limitations, our study is a unique natural experiment in which a large number of patients were studied in a TB program setting; only a few laboratories performed NAA, and results were consistent with those reported in other studies.

Although the value of NAA testing for rapid TB diagnosis

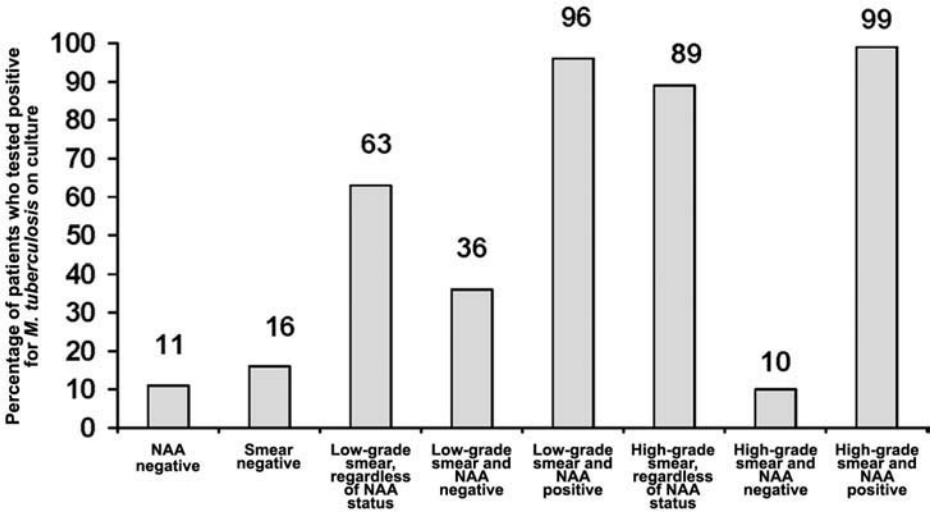

Figure 2. Bar graph comparing patients who tested positive for *Mycobacterium tuberculosis* in New York City during 2000–2004, by acid-fast bacilli smear status and nucleic acid amplification (NAA) status. For details about smear grades, see Methods.

is clear, the test remains underutilized [18, 24]. New York City has a large number of patients (>40%) who have had respiratory tract specimens that tested positive for AFB on smear but in whom a diagnosis of active TB is eventually rejected, which makes NAA testing very valuable. Our results are in line with the new Centers for Disease Control and Prevention guidelines [25] and support the following use of NAA test results for patient management:

1. A positive NAA test result in conjunction with an AFB-positive smear, with the patient showing clinical symptoms consistent with TB, is highly predictive of TB disease. Such patients should receive anti-TB medications, airborne isolation precautions should be implemented, and an investigation of exposed contacts for possible TB transmission should be performed. A mycobacterial culture is still needed for species identification and for drug-susceptibility testing.

2. Similarly, ordering NAA tests for patients whose specimens tested negative for AFB on smear but who are highly suspected of having TB disease is recommended, because a positive NAA test result for such patients warrants starting treatment for TB.

3. A negative NAA test result for a patient whose specimen tested positive for AFB on smear indicates that the AFB are probably nontuberculous mycobacteria. In general, respiratory isolation of the patient and a contact investigation may be discontinued; treatment for TB may be withheld or continued, depending on the patient's clinical signs and symptoms. However, in instances for which clinical suspicion for TB remains high and the patient will be discharged into a high-risk setting, such as a

Table 4. Performance of nucleic acid amplification (NAA) testing of patients' specimens obtained from body sites other than the respiratory tract, compared with culture positivity, in New York City during 2000–2004.

Type of specimen tested	No. of specimens[a]	Sensitivity, %	Specificity, %	PPV, %	NPV, %
All	682	89.3	74.5	79.3	86.5
Positive for AFB on smear	215	97.5	93.6	95.1	96.8
Negative for AFB on smear	383	83.2	65.6	70.7	79.7
Cerebrospinal fluid	188	84.9	62.1	68.7	80.8
Lymph node tissue	88	97.0	66.7	90.3	87.5
Gastric aspirate	65	100.0	90.0	95.7	100.0
Pleural fluid	56	100.0	87.5	76.2	100.0
Peritoneal fluid	31	92.3	77.8	75.0	93.3

NOTE. AFB, acid-fast bacilli; NPV, negative predictive value; PPV, positive predictive value.

[a] Number of specimens that were tested for *Mycobacterium tuberculosis* on culture and by NAA.

congregate housing facility with a high prevalence of the human immunodeficiency virus, caution should be exercised.

4. A negative NAA test result for a patient whose specimen tested negative for AFB on smear and for whom the clinical suspicion for TB is low indicates a low likelihood of active TB and suggests that treatment for TB can be delayed.

5. A negative NAA test result for a patient whose sputum specimen tested positive for AFB on smear can be used to discontinue an investigation of exposed contacts for possible TB transmission and to discontinue airborne isolation.

In summary, the findings from our study on the performance of NAA tests for diagnosis of TB in this large urban setting corroborate the value of using NAA testing to reliably and rapidly identify patients with pulmonary tuberculosis. The sensitivity, specificity, and predictive values of the test in a real-life situation were high for patients who had a specimen that tested positive for AFB on smear, and they were at acceptable levels for patients who had a specimen that tested negative for AFB on smear. To improve the use of rapid testing for TB, in May 2006, the New York State Department of Health updated its mycobacteriology guidelines to mandate NAA testing on all specimens that initially tested positive for AFB on smear [22]. The Centers for Disease Control and Prevention updated its guidelines to recommend NAA testing on specimens obtained from the respiratory tract of patients suspected of pulmonary TB [25].

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References

1. Centers for Disease Control and Prevention (CDC). Nucleic acid amplification tests for tuberculosis. MMWR Morb Mortal Wkly Rep **1996**; 45:950–2.
2. Centers for Disease Control and Prevention (CDC). Update: nucleic acid amplification tests for tuberculosis. MMWR Morb Mortal Wkly Rep **2000**; 49:593–4.
3. American Thoracic Society. Rapid diagnostic tests for tuberculosis—what is the appropriate use? Am J Respir Crit Care Med **1997**; 155: 1804–14.
4. Bradley SP, Reed SL, Catanzaro A. Clinical efficacy of the amplified *Mycobacterium tuberculosis* direct test for the diagnosis of pulmonary tuberculosis. Am J Respir Crit Care Med **1996**; 153:1606–10.
5. Coll P, Garrigó M, Moreno C, Martí N. Routine use of Gen-Probe amplified *Mycobacterium tuberculosis* (MTD) direct test for detection of *Mycobacterium tuberculosis* with smear-positive and smear-negative specimens. Int J Tuberc Lung Dis **2003**; 7:886–91.
6. Conaty SJ, Claxton AP, Enoch DA, Hayward AC, Lipman MC, Gillespie SH. The interpretation of nucleic acid amplification tests for tuberculosis: do rapid tests change treatment decisions? J Infect **2005**; 50: 187–92.
7. Dalovisio JR, Montenegro-James S, Kemmerly SA, et al. Comparison of the amplified *Mycobacterium tuberculosis* (MTD) direct test, Amplicor MTB PCR, and IS6110-PCR for detection of MTB in respiratory specimens. Clin Infect Dis **1996**; 23:1099–106.
8. Gallina M, Troupioti P, Rocco G, Sensalari G, Libanori E. Predicting culture results for *Mycobacterium tuberculosis* complex: amplified *Mycobacterium tuberculosis* direct test and acid-fast bacilli microscopy. Chest **2000**; 118:28–32.
9. Lim TK, Gough A, Chin N, Kumarasinghe G. Relation between estimated pretest probability and accuracy of automated *Mycobacterium tuberculosis* assay in smear-negative pulmonary tuberculosis. Chest **2000**; 118:641–7.
10. Maugein J, Fourche J, Vacher S, Grimond C, Bebear C. Evaluation of the BDProbeTec ET DTB assay for direct detection of *Mycobacterium tuberculosis* complex from clinical specimens. Diagn Microbiol Infect Dis **2002**; 44:151–5.
11. Moore DF, Curry JI. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by Amplicor PCR. J Clin Microbiol **1995**; 33:2686–91.
12. Pfyffer GE, Kissling, P, Jahn EM, Welscher HM, Salfinger M, Weber R. Diagnostic performance of amplified *Mycobacterium tuberculosis* direct test with cerebrospinal fluid, other non-respiratory, and respiratory specimens. J Clin Microbiol **1996**; 34:834–41.
13. Pfyffer GE, Kissling P, Wirth R, Weber R. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. J Clin Microbiol **1994**; 32:918–23.
14. Ruiz-Manzano J, Manterola JM, Gamboa F, et al. Detection of *Mycobacterium tuberculosis* in paraffin-embedded pleural biopsy specimens by commercial ribosomal RNA and DNA amplification kits. Chest **2000**; 118:648–55.
15. Shah S, Miller A, Mastellone A, et al. Rapid diagnosis of tuberculosis in various biopsy and body fluid specimens by the Amplicor *Mycobacterium tuberculosis* polymerase chain reaction test. Chest **1998**; 113: 1190–4.
16. Smith MB, Bergmann JS, Harris SL, Woods, GL. Evaluation of the Roche Amplicor MTB assay for the detection of *Mycobacterium tuberculosis* in sputum specimens from prison inmates. Diagn Microbiol Infect Dis **1997**; 27:113–6.
17. Tan MF, Ng WC, Chan SH, Tan WC. Comparative usefulness of PCR in the detection of *Mycobacterium tuberculosis* in different clinical specimens. J Med Microbiol **1997**; 46:164–9.
18. Wiener RS, Della-Latta P, Schluger NW. Effect of nucleic acid amplification for *Mycobacterium tuberculosis* on clinical decision making in suspected extrapulmonary tuberculosis. Chest **2005**; 128:102–21.
19. 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.
20. Campos M, Quartin A, Mendes E, et al. Feasibility of shortening respiratory isolation with a single sputum nucleic acid amplification test. Am J Respir Crit Care Med **2008**; 178:300–5.
21. Centers for Disease Control and Prevention (CDC). Case definitions for infectious conditions under public health surveillance. MMWR Morb Mortal Wkly Rep **1997**; 46(RR10):1–55.
22. New York State Department of Health. Clinical laboratory standards of practice. January **2008**. Available at: http://www.wadsworth.org/labcert/clep/files/Microbiology.pdf. Accessed 27 April 2009.
23. Amplied MTD Test (amplified *Mycobacterium tuberculosis* direct test for in vitro diagnostic use) [package insert]. San Diego, CA: Gen-Probe, **2001**.
24. Lim TK, Zhu D, Gough A, Lee KH, Kumarasinghe G. What is the optimal approach for using a direct amplification test in the routine diagnosis of pulmonary tuberculosis? A preliminary assessment. Respirology **2002**; 7:351–7.
25. Centers for Disease Control and Prevention (CDC). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb Mortal Wkly Rep **2009**; 58:7–10.