Nonclinical Selection Criteria for Maximizing Yield of Nucleic Acid Amplification Tests in Tuberculosis Diagnosis

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In spite of the excellent performance of rapid tuberculosis (TB) nucleic acid amplification (NAA) tests and the clear benefits of immediate diagnosis of TB disease, NAA tests frequently are not used in the diagnosis of pulmonary TB cases, particularly TB cases with smear-negative sputa. Public health laboratories primarily perform TB NAA tests only on a targeted subset of specimens, usually including those that are smear positive and those for which a clinician has specifically requested NAA testing. As an alternative to targeted testing, some laboratories use TB NAA tests universally for all respiratory specimens, though this practice can be prohibitively costly and can be associated with an increased frequency of false-positive results due to testing of lower-risk patients. We propose a strategy for identifying individuals for NAA testing on the basis of nonclinical risk criteria that are routinely provided on the test requisition form, such as type of health care facility from which the specimen is received and patient age group. Use of this strategy at the Massachusetts Department of Public Health Laboratory would allow for NAA test identification of approximately 54 (74%) of 72 culture-positive pulmonary TB cases over a 1-year period while requiring NAA testing for only 933 (17%) of 5,469 individuals submitting respiratory specimens. We demonstrate that use of nonclinical NAA test selection criteria is an effective strategy for maximizing the number of TB cases that can be rapidly identified while minimizing the number of specimens that must be tested.

Early diagnosis and treatment of tuberculosis (TB) improve patient outcomes and reduce the risk of disease transmission. Nucleic acid amplification (NAA) tests can allow for identification of TB on the same day that the specimen is received, 2 weeks earlier than is usually achieved by conventional culture methods (5). In 2009, the U.S. Centers for Disease Control and Prevention issued updated guidelines for the use of TB NAA testing, stating that “NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management of TB control activities” (2). Although TB NAA testing has assumed an increasingly important role in clinical and public health settings, it can be costly and labor-intensive. As a result, most U.S. public health laboratories offer testing for only a subset of respiratory specimens, usually limiting testing to smear-positive specimens, specimens for which a clinician requests testing, or both. Among 45 U.S. public health laboratories for which data were available in 2009, 36% of culture-positive TB cases were identified within 48 h of specimen receipt, well below the Healthy People 2020 goal of diagnosing 75% of TB cases within 48 h (4).

The Massachusetts Department of Public Health Laboratory currently performs NAA testing on the first smear-positive respiratory specimen from each new patient and on any respiratory specimen as requested by the clinician. Although more than 50% of pulmonary TB cases confirmed by culture at the laboratory are smear negative, only a small percentage of these smear-negative specimens are flagged by clinicians for NAA testing. Consequently, targeting high-risk smear-negative specimens for NAA testing could result in substantial increases in the frequency of early detection of TB cases, in spite of the relatively low sensitivity of NAA tests among smear-negative specimens. In early 2010, we launched a campaign to inform clinicians of the availability of the TB NAA test and of the indications for ordering the test at our

laboratory. In spite of these efforts, the percentage of smear-negative TB culture-positive specimens for which NAA testing was requested decreased between 2009 and 2011, from 12.2% (7/57) to 7.0% (3/43).

Therefore, we considered whether it might be possible to identify specimens for NAA testing on the basis of nonclinical risk indicators that are routinely reported on the laboratory test requisition form, without reliance on clinician request. In this study, we examine 1 year of laboratory testing data with the objectives of (i) describing and defining the challenge associated with identifying high-risk smear-negative TB cases for targeted NAA testing and (ii) demonstrating the feasibility and potential utility of a strategy for identifying high-risk candidate specimens for NAA testing through the use of nonclinical risk factors that are available to laboratories on their test requisitions.

MATERIALS AND METHODS

All laboratory testing was performed at the Massachusetts Department of Public Health Laboratory, including a TB NAA test with the FDA-approved Gen-Probe Amplified *Mycobacterium tuberculosis* direct (MTD) test (Gen-Probe Inc., San Diego, CA). The laboratory receives primary specimens and mycobacterial reference isolates from a range of provider types, including acute-care hospitals, primary-care and TB clinics, reference laboratories, correctional facilities, and local boards of health. Data for specimens collected between 1 July 2010 and 30 June 2011 were extracted from the mycobacteriology laboratory information management

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TABLE 1 Numbers of individuals and culture-confirmed TB cases, by proposed test criterion

Test criterion	No. (%) of individuals (total, 5,469)	No. (%) of culture-positive TB cases (total, 72)	% of culture-confirmed TB cases/no. of individuals
1st smear positive	53 (1.0)	29 (40.3)	54.7
Smear negatives upon request	79 (1.4)	3 (4.2)	3.8
Submitted by correctional facilities	4 (0.1)	1 (1.4)	25.0
Submitted by public health hospital	95 (1.7)	13 (18.1)	13.7
Submitted by local board of health	46 (0.8)	4 (5.6)	8.7
Submitted by TB clinic	164 (3.0)	10 (13.9)	6.1
Age, 25–44 yr	660 (12.1)	38 (52.8)	5.8
All specimens	5,469 (100.0)	72 (100.0)	1.3

system and analyzed with the SAS statistical package, version 9.2 (SAS Institute Inc., Cary, NC). Only specimens identified as sputum, bronchial specimens, or tracheal aspirates were included. Only primary patient specimens from Massachusetts providers were included. Reference isolates were excluded.

We defined an individual as being smear positive if any specimen collected within 1 week of the first specimen was smear positive. An individual was considered to be smear negative if all specimens collected within 1 week of the first specimen were smear negative. Individuals who produced a smear-positive specimen after more than 1 week of producing only smear-negative specimens were considered to be smear negative. We chose these definitions with the assumption that initial smear results are most relevant in the timely identification of candidates for NAA testing. Of note, only 34% of individuals tested during the study period submitted more than one specimen.

We considered several nonclinical selection criteria for identifying high-risk smear-negative specimens as candidates for NAA testing, focusing on categories that included high numbers of culture-confirmed TB cases relative to the total number of specimens tested. We then considered sets of selection criteria, adding each new criterion one at a time while examining the incremental and cumulative effects, in terms of number of culture-confirmed TB cases identified and number of specimens to be tested. Individuals meeting more than one criterion were associated only with the first criterion met. Because smear-positive specimens are already being targeted for NAA testing, most of the TB cases identified by additional selection criteria would be smear negative. Therefore, the number of additional TB cases identified by NAA test with each additional selection criterion was calculated by multiplying the number of culture-positive TB cases in each category by 72%, the sensitivity of the MTD test among smear-negative specimens as stated by the test manufacturer.

All analyses were based on results of routine laboratory testing protocols. All primary respiratory specimens were decontaminated and concentrated, smeared for acid-fast bacillus microscopy, and inoculated onto solid and liquid culture media, as previously described (6). Mycobacterial isolates were identified by DNA probes (AccuProbe; Gen-Probe Inc.) or by biochemical testing. The first smear-positive respiratory specimen collected from each patient was subjected to MTD testing, as were smear-negative respiratory specimens for which NAA testing was requested by the ordering physician. For each specimen tested, MTD was performed on an undiluted sediment and on a 1:10 dilution of the sediment in order to minimize the effects of potential MTD reaction inhibitors, as previously described (6, 7). A specimen that was negative on both undiluted and diluted sediments was considered negative. A specimen with a positive result on the undiluted and diluted sediment was considered positive. A specimen with a negative result for the undiluted sediment and a positive result for the diluted sediment was considered positive. A specimen with a positive result on the undiluted sediment and a negative result on the diluted sediment was considered positive if both relative light unit values were borderline; otherwise, the specimen was considered equivocal and subjected to repeat testing. On the basis of data collected over a 30-month period (1 January 2009 to 30 June 2011), the sensitivity of this modified

MTD test was 97.8% (MTD was positive in 88 of 90 TB culture-positive specimens) among smear-positive specimens and 93% (MTD was positive in 14 of 15 TB culture-positive specimens) among smear-negative specimens. The specificity was 98.3% (MTD was negative in 57 of 58 TB culture-negative specimens) among smear-positive specimens and 98.7% (MTD was negative in 153 of 155 TB culture-negative specimens) among smear-negative specimens.

RESULTS

Among specimens collected between 1 July 2010 and 30 June 2011, 9,382 were respiratory specimens from 5,475 individuals, including 78 culture-positive TB cases (1.4%). Among the 5,475 individuals tested, 72 (1.3%) were smear positive and 5,403 (98.7%) were smear negative, including 11 who produced at least one smear-positive specimen after producing only smear-negative specimens during the first week. Thirty-five (48.6%) of 72 smear-positive individuals were ultimately confirmed as culture-positive TB cases, compared to 43 (0.8%) of 5,403 smear-negative individuals. Among 78 TB cases with positive cultures during the study period, 6 were not tested by NAA because they had previously been identified as TB cases. These were excluded from subsequent analyses. Among the remaining 72 culture-positive TB cases, 31 were smear positive and 41 were smear negative. Of the 31 smear-positive TB cases, 29 (93.5%) were correctly identified by NAA testing and 2 were not NAA tested for unknown reasons. Of the 41 smear-negative TB cases, only 4 (9.8%) were tested by NAA, including 3 that were NAA positive and 1 that was NAA equivocal.

The following analyses were based on the assumptions that 5,469 individuals were candidates for NAA testing (the 5,475 individuals described above, less the 6 previously identified TB cases) and that 72 culture-positive TB cases could have been identified by NAA testing. Our current practice of performing NAA testing on smear-positive patients allowed us to identify 29 TB culture-positive cases after testing only 53 individuals, a yield of 54.7%. Alternatively stated, this practice allowed us to identify 40.3% of a total of 72 culture-positive TB cases while only requiring us to test 1.0% of a total of 5,469 respiratory specimens (Table 1). The practice of NAA testing smear-negative specimens upon clinician request has a yield of 3.8%, allowing us to identify 3 culture-positive TB cases (4.2% of all culture-positive TB cases) after testing 79 smear-negative individuals (1.4% of all smear-negative individuals).

Proposed selection criteria are listed in Table 1, along with the numbers of individuals and the number of culture-confirmed TB cases that each criterion encompasses. The categories include individuals submitting specimens via any correctional facility, a particular public health hospital, any local board of health, and any

TABLE 2 Incremental and cumulative number of individuals tested, number of culture-positive TB cases identified, and yield with addition of each new test criterion

Test criterion	Incremental no. (%) of			Cumulative no. (%) of		
	Incremental no. (%) of specimens tested with each additional criterion (<i>n</i> = 5,469)	Incremental no. (%) of culture-positive TB cases identified with each additional criterion (<i>n</i> = 72)	Yield (%)	Cumulative no. (%) of specimens tested with each additional criterion (<i>n</i> = 5,469)	Cumulative no. (%) of culture-positive TB cases identified with each additional criterion (<i>n</i> = 72)	Cumulative yield (%)
1st smear positive	53 (1.0)	29 (40.3)	54.7	53 (1.0)	29 (40.3)	54.7
Smear negatives upon request	79 (1.4)	3 (4.2)	3.8	132 (2.4)	32 (44.4)	24.2
Submitted by correctional facility ^a	4 (0.1)	1 (1.0)	18.0	136 (2.5)	33 (45.4)	24.1
Submitted by public health hospital ^a	90 (1.6)	6 (9.0)	7.2	226 (4.1)	39 (54.4)	17.3
Submitted by local board of health ^a	42 (0.8)	2 (3.0)	5.1	268 (4.9)	41 (57.4)	15.4
Submitted by TB clinic ^a	157 (2.9)	6 (8.0)	3.7	425 (7.8)	47 (65.4)	11.1
Age, 25–44 yr ^a	508 (9.3)	6 (9.0)	1.3	933 (17.1)	54 (74.4)	5.7
Universal testing ^a	4,536 (82.9)	7 (10.0)	0.2	5,469 (100.0)	61 (84.4)	1.1

^aAssuming that all additional specimens in this category are smear negative and that MTD test sensitivity is 72% for smear negatives.

TB clinic. We also considered NAA testing all individuals aged 25 to 44 years, because 42% of patients with newly identified TB cases in Massachusetts are aged 25 to 44 years, while only 12% of individuals submitting respiratory specimens for mycobacterial testing are aged 25 to 44. The ratio of the number of culture-confirmed TB cases to the number of individuals that would need to be tested was greater for each of these five proposed criteria than for the strategy of NAA testing smear-negative individuals on the basis of clinician request.

Addition of multiple selection criteria to our two existing criteria (NAA test for smear-positive specimens and any specimen by clinician request) produces incremental and cumulative effects in terms of number of culture-confirmed TB cases identified and number of specimens to be tested, as presented in Table 2. By NAA testing smear-positive specimens, and smear-negative specimens as requested by the clinician, we would identify 32 (44.4%) of 72 culture-positive TB cases after testing 132 (2.4%) of all 5,469 individuals. If we add the four provider criteria, we would identify an additional 15 (21.0%) of 72 culture-positive TB cases after testing an additional 293 (5.4%) of all 5,469 individuals, assuming a 72% MTD test sensitivity among smear-negative specimens. Using six criteria, we would identify 47 (65.4%) of 72 culture-positive TB cases after testing 425 (7.8%) of all 5,469 individuals. If we used all seven criteria, we would identify 54 (74.4%) of 72 culture-positive TB cases after testing 933 (17.1%) of all 5,469 individuals. Figure 1 illustrates how the ratio of the percentage of TB cases identified to the percentage of individuals tested decreases as less-selective criteria are added, until the strategy of universal testing maximizes both the percentage of specimens tested at 100% (5,469/5,469) and the percentage of culture-confirmed TB cases identified by NAA testing at 84.4% (61/72).

DISCUSSION

In this analysis, we demonstrate that application of nonclinical selection criteria for TB NAA testing can substantially increase the number of TB cases identified without the prohibitive expense associated with a strategy of universal testing. We suggest a strategy by which NAA testing criteria are implemented in descending order of yield, with the highest-yield categories being added first to the list of selection criteria and the lower-yield categories being

added only as allowed within the constraints imposed by limited testing resources.

For our laboratory specifically, we plan to continue to test smear positives and smear negatives upon request. However, we also plan to test an additional 293 (5.4% of 5469) specimens received from correctional facilities, the public health hospital, TB clinics, and local boards of health, thereby increasing by 15 (22.5%) the number of TB cases identified by NAA testing. In fact, we anticipate being able to identify up to 19 (26.4%) additional TB cases, reflecting our actual smear-negative sensitivity of 93%

FIG 1 Total number (percent) of culture-positive TB cases identified annually by NAA testing versus the total number (percent) of respiratory specimens that would be tested for each additional NAA test selection criterion added. Selection criteria: a, none; b, smear positives only; c, smear negatives upon clinician request; d, specimens from correctional facilities; e, Department of Public Health hospital; f, local boards of health; g, TB clinics; h, patients aged 25 to 44 years old; i, universal testing.

rather than the 72% sensitivity used in this analysis. During the study period, the median time to identification of a TB case by NAA testing in our laboratory was 2 days, versus 21 days for identification by culture. Therefore, the estimated potential increase in the number of TB cases identified by NAA testing would likely have a substantial clinical and public health impact by limiting patient morbidity and mortality, reducing the opportunity for disease transmission, and expediting contact investigation. If laboratory funding improves in future years, we could consider adding to our list of testing criteria the category of patients aged 25 to 44 years. It is unlikely, though, that we would ever consider a strategy of universal testing, even if we had the financial resources to do so. After selecting out the high-risk individuals with the first 6 or 7 criteria, the prevalence of TB in the remaining smear-negative individuals would be so low (0.2%) that the false-positive rate would become unacceptably high (83%) (calculations not shown).

Another general approach to improving the yield of TB NAA testing, especially among smear-negative specimens, could be to perform NAA testing on two or more specimens per patient. One recent study found that overall TB NAA sensitivity for smear-negative specimens increased from 72.5% to 85.1% when two specimens per patient were tested instead of only one (1). In our study, only four smear-negative individuals with culture-confirmed TB were tested by NAA, of which only one did not have a positive NAA test result. If, during the study period, we had performed NAA testing on a second specimen from each patient whose initial specimen was NAA test negative (assuming that we received a second specimen from each of these individuals, which we did not), then we would have had to have performed an additional 100 NAA tests in order to have had the possibility of correctly identifying the one TB case. The yield on this strategy is too low to consider at present. However, we will reevaluate our data when the number of smear-negative TB cases tested by NAA increases, as we expect to occur after we begin targeted NAA testing of specimens submitted by high-risk providers.

Although our results are specific to our own laboratory and its associated patient population, other public health laboratories performing similar analyses of nonclinical risk factors in their own settings would likely be able to identify comparable NAA test selection criteria of similarly high yield. This strategy could also be applied in the clinical laboratory setting, where nonclinical risk criteria might include hospital wards (e.g., medical versus surgical versus intensive care), subspecialty groups (e.g., pulmonary versus infectious disease versus oncology), or satellite clinics identified as being high risk. In addition, it is likely that selection criteria that we considered and dismissed could be useful in a different setting. For example, we speculate that race and country of origin could be useful as test selection criteria, since these factors have strong associations with TB incidence in Massachusetts and nationally (2). However, these data are not required on our test requisition and are too inconsistently available to be of value as selection criteria in our laboratory. This may not be the case in other laboratories.

We acknowledge several limitations of this analysis. First, this

study is based on retrospective review of data that were collected along with routine diagnostic specimens, without the benefit of active real-time data quality management and control. Second, we may have underestimated the number of previously known TB cases that were excluded from analysis. Our only means of identifying a previously known TB case was by finding the individual's name and positive culture result in an earlier portion of our laboratory database. We would not have identified previously known TB cases that were diagnosed without laboratory confirmation or that were culture confirmed at a different laboratory. However, we expect that the number of these cases should be low, since 75% of TB cases nationally were laboratory confirmed (3), and most TB cases in Massachusetts are confirmed by our laboratory. Finally, our analyses are based on only 1 year of testing data, with most of our proposed selection categories being associated with fairly small numbers of specimens and TB cases. Similar analyses of the yield of various NAA test selection criteria would need to be conducted on an ongoing and routine basis to ensure consistency in results and to identify changes in the risk categories associated with the highest yields.

In conclusion, we propose a strategy by which public health laboratories can optimize the use of TB NAA testing without resorting to universal testing and without the challenges associated with clinician education campaigns. This approach provides laboratories with a simple, well-defined, and feasible strategy for effectively increasing the number of TB cases that are identified by rapid diagnostic methods.

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