Use of Nucleic Acid Amplification Tests in Tuberculosis Patients in California, 2010–2013

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**Background.** Nucleic acid amplification tests (NAATs) have been used as a diagnostic tool for tuberculosis (TB) in the United States for many years. We sought to assess NAAT use in TB patients in California during a period of time when NAAT availability increased throughout the world.

**Methods.** We conducted a retrospective review of surveillance data from 6051 patients with culture-confirmed pulmonary TB who were reported to the California TB registry during 2010–2013.

**Results.** Only 2336 of 6051 (39%) TB patients had a NAAT for diagnosis before culture results. Although 90% (N = 2101) with NAAT had positive test results, 9% (N = 217) had falsely negative NAAT results, and 0.8% (N = 18) had indeterminate NAAT results. The median time from specimen collection to TB treatment initiation was shorter when NAAT was used (3 vs 14 days, P < .0001), and patients with a positive NAAT result initiated treatment earlier than patients with a falsely negative result (1 vs 11 days from NAAT report, P < .0001). We confirmed the increased sensitivity of NAAT compared with acid-fast bacilli (AFB) smear microscopy in our study population; 92 of 145 AFB smear-negative patients had positive NAATs. Median time from specimen collection to NAAT result report differed by health jurisdiction, from 1 to 11 working days.

**Conclusions.** Increased use of NAATs in diagnosis of pulmonary TB could decrease the time-to-treatment initiation and consequently decrease transmission. However, differential use and access to NAAT may prevent full realization of NAAT benefits in California.

**Keywords.** diagnosis; TB surveillance; treatment initiation; United States.
management decisions or TB control activities, and lack of demand from clinicians and public health authorities [2, 3]. Additional research is needed to explore the impact of NAAT use on outcomes among TB patients.

We sought to assess the use of NAATs in California using TB surveillance data. To determine the impact of NAAT on treatment decisions, we evaluated differences in demographic, clinical, and healthcare factors that could also affect clinical decision making. We asked the following specific questions. (1) How many TB patients had NAAT used in the diagnosis of TB? (2) How were patients with NAAT different from patients who did not have NAAT? (3) Did patients who had NAAT performed start treatment earlier than patients who did not have NAAT? (4) Did patients with a positive NAAT result start treatment earlier than patients with a falsely negative NAAT result? (5) How rapidly were the results of NAATs reported? (6) Did NAAT turnaround time (TAT) vary by the laboratory type reporting results or by jurisdiction?

MATERIALS AND METHODS
Study Design and Population
We retrospectively evaluated the use of NAATs for TB diagnosis of patients reported in the California TB registry during 2010–2013. Our study population was limited to patients with pulmonary, culture-confirmed TB and a known AFB smear result.

Definitions
For the purpose of this study, we categorized patients into 2 groups: those who had NAAT and those who did not have NAAT. Patients who had NAAT were defined as those whose NAAT result was reported before culture result, ie, a diagnostic NAAT. Patients who did not have NAAT were defined as those who either did not have a NAAT reported, or whose NAAT result was reported at/after culture. We reasoned that if the NAAT report date did not precede the culture report date, NAAT results were not used for rapid diagnostic purposes and would not influence the decision to start TB treatment. The NAAT report date was the date that the NAAT result was reported to the provider.

When comparing patients with and without NAAT, the time-to-treatment initiation was defined as the number of days between the earliest specimen collection and treatment initiation. We excluded patients who started treatment before or at the time of specimen collection for the analysis of treatment initiation time frames. Patients who started treatment before specimen collection were defined as starting treatment "presumptively.”

When comparing patients with NAAT-positive and NAAT-negative results, the time-to-treatment initiation was the number of days between the date of NAAT report and treatment initiation. We stratified treatment initiation in days by smear status, and we excluded patients who started treatment before NAAT.

The NAAT TAT was defined as the number of working days (ie, excluding weekends) between NAAT specimen collection and the NAAT report date. We compared TAT by reporting laboratory types that included public health, commercial, and other laboratories (eg, hospital laboratories and laboratories associated with federal public agencies). For analyses related to reporting jurisdiction in California, we limited our scope to jurisdictions that reported at least 20 TB cases during 2010–2013.

Statistics
SAS (version 9.3) software was used. Differences in proportions were assessed using χ² or Fisher’s exact tests (2-tailed). Differences in median time frames were compared using the Wilcoxon rank-sum test.

Ethical Review
This analysis was conducted as part of the California Department of Public Health’s mandate to routinely collect and analyze surveillance data for public health purposes.

RESULTS
Frequency of Nucleic Acid Amplification Test
Of 9008 TB cases reported during 2010–2013, 6051 (67%) patients met the required inclusion criteria (pulmonary, culture-confirmed TB, with a known AFB smear result). Among these patients, 3311 (55%) had a NAAT reported, and 2740 (45%) did not have NAAT reported. However, only 2336 (39% of 6051) patients had NAAT reported before the date of culture report, whereas 915 had NAAT reported at or after the date of culture report. We excluded 60 patients who had missing dates of NAAT or culture report (Figure 1). Although 90% (N = 2101) of patients with NAAT reported before culture had positive NAAT results, 9% (N = 217) had negative NAAT results, and 0.8% (N = 18) had indeterminate NAAT results.

Comparison of Sociodemographic and Clinical Characteristics
Patients who had NAAT were similar to patients without NAAT in most demographic and socioeconomic factors assessed (Table 1). However, there were differences in the clinical factors that likely correlated with the clinical suspicion of TB. Compared with patients who did not have NAAT, patients with NAAT were more likely to have a positive sputum smear (88% vs 53%, P = .001), an abnormal chest radiograph with cavities (29% vs 20%, P < .0001), a tuberculin skin test or interferon-gamma release assay performed (72% vs 68%, P = .001), and TB symptoms as the primary reason for TB evaluation (73% vs 62%, P = .001).

Timing of Diagnostic Tests and Treatment Initiation
For patients who started treatment after specimens were collected (N = 5061), the median time-to-treatment initiation from earliest specimen collection was 3 days for patients who had a diagnostic NAAT (N = 1960) and 14 days for patients who did
not have NAAT (N = 3101) (P < .0001). Among patients who were smear positive (N = 3308), the median treatment initiation time was 2 days for those who had NAAT (N = 1728) and 4 days for those who did not have NAAT (N = 1580) (P < .0001). For patients who were smear negative (N = 1753), the median treatment initiation time for those who had NAAT (N = 232) was 10 days, and 26 days for those who did not have NAAT (N = 1521) (P < .0001) (Table 2).

Among patients with positive or negative NAAT results who started treatment at or after NAAT was reported (N = 795), 90% (N = 714) also started treatment before culture report. Of 795 patients, 650 (82%) were smear positive and 145 (18%) were smear negative. Among those who were smear positive, 612 (94%) were NAAT positive and 38 (6%) were NAAT negative. Among patients who were smear negative, 92 (63%) were NAAT positive and 53 (37%) were NAAT negative (Figure 2). Compared with smear, NAAT identified 92 additional patients with TB before culture.

The median time-to-treatment initiation from NAAT report date among all patients who had a positive or negative NAAT result was 1 day for NAAT-positive patients (N = 704) and 11 days for NAAT-negative patients (N = 91) (P < .0001). Among 650 smear-positive patients, the median time was 1 day for those who had a positive NAAT result (N = 612) and 5.5 days for those who had a negative NAAT result (N = 38) (P < .0001). Among 145 smear-negative patients, the median time was 2 days for those who had a NAAT-positive result (N = 92) and 14 days for those who had a NAAT-negative result (N = 53) (P < .0001) (Table 3; Figure 2).

Of the 714 patients with NAAT who started treatment before culture, patients with a positive NAAT were different from patients with a negative NAAT. The NAAT-positive patients were older (median age = 54 vs 38, P = .003), more likely to have TB symptoms as the primary reason for TB evaluation (68% vs 38%, P < .0001), and to have cavities on chest radiograph (28% vs 8%, P = .001).

**Turnaround Time by Reporting Laboratory Type and Jurisdiction**

The NAAT median TAT was 2 days for 1254 patients served by public health laboratories (PHLs), 3 days for 869 patients served by commercial laboratories (P < .0001 compared with median TAT of PHLs), and 2 days for 196 patients served by other reporting laboratories (P = .110 compared with median TAT of PHLs). The median TAT for all patients with NAAT was 3 days (interquartile range, 2–4), and 22% of patients with NAAT had a TAT greater than 4 days (Figure 3). Among the 25 jurisdictions that reported at least 20 TB cases during 2010–2013, the NAAT median TAT ranged from 1 to 11 days, and the percentage of culture-confirmed patients with NAAT ranged from 2% to 61%.

**DISCUSSION**

Our retrospective descriptive analysis of the use of NAAT demonstrated a potential benefit of NAAT to TB patients: earlier treatment initiation. However, only 39% of pulmonary culture-confirmed TB patients in California had a diagnostic NAAT. Patients who had NAAT were different than patients who did not have NAAT; they had an increased frequency of clinical factors that are hallmark signs of TB disease (eg, cavities on chest radiograph), suggesting that providers ordered NAAT to confirm the TB diagnosis that they had already considered to be probable. Patients who had NAAT started treatment...
Table 1. Sociodemographic and Clinical Characteristics of Pulmonary Culture-Confirmed TB Patients Who Had NAAT vs Patients Who Did Not Have NAAT, California 2010–2013

| Patient Characteristic | All Patients N (%) (n = 5991)* | Patients Who Had NAAT N (%) (n = 2336) | Patients Who Did Not Have NAAT N (%) (n = 3655) | P Value |
|------------------------|-------------------------------|----------------------------------------|-----------------------------------------------|---------|
| **Demographic characteristic** |                               |                                        |                                               |         |
| Age, median (IQR)      | 53 (35–69)                    | 52 (34–68)                             | 53 (36–70)                                   | .02     |
| Sex                    |                               |                                        |                                               | .13     |
| Male                   | 3815 (64)                     | 1515 (65)                              | 2300 (63)                                    |         |
| Female                 | 2176 (36)                     | 821 (35)                               | 1355 (37)                                    |         |
| **Race**               |                               |                                        |                                               |         |
| Asian/Pacific Islander | 2987 (50)                     | 1124 (48)                              | 1863 (51)                                    | .03     |
| Black                  | 339 [6]                       | 135 [6]                                | 204 [6]                                      | .75     |
| Hispanic               | 2180 (36)                     | 889 (38)                               | 1291 (35)                                    | .03     |
| White                  | 472 [8]                       | 183 [8]                                | 289 [8]                                      | .92     |
| US born                |                               |                                        |                                               | .79     |
| Yes                    | 1163 [19]                     | 458 [20]                               | 705 [19]                                     |         |
| No                     | 4816 [81]                     | 1876 [80]                              | 2940 [81]                                    |         |
| **Socioeconomic factors** |                               |                                        |                                               |         |
| Homeless within past year (n = 5987) | 364 [6]                       | 151 [6]                               | 213 [6]                                      | .14     |
| Correctional facility resident (n = 5986) | 198 [3]                       | 74 [3]                                | 124 [3]                                      | .79     |
| Long-term care facility (n = 5988) | 149 [2]                       | 51 [2]                                | 98 [3]                                       | .44     |
| Injecting drug use (n = 5989) | 85 [1]                        | 30 [1]                                | 55 [2]                                       | .06     |
| Noninjecting drug use (n = 5988) | 391 [7]                       | 137 [6]                               | 254 [7]                                      | .05     |
| Excess alcohol use (n = 5988) | 603 [10]                      | 233 [10]                              | 370 [10]                                     | .16     |
| **Clinical characteristic** |                               |                                        |                                               |         |
| Sputum smear           |                               |                                        |                                               | .001    |
| Positive               | 3985 (67)                     | 2006 (88)                              | 1919 (53)                                    |         |
| Negative               | 2006 (33)                     | 270 (12)                               | 1736 (48)                                    |         |
| Initial chest radiograph (N = 5864)b |                           |                                        |                                               |         |
| Normal                 | 181 [3]                       | 47 [2]                                 | 134 [4]                                      | <.0001  |
| Abnormal with no cavities | 4314 (74)                    | 1579 (69)                              | 2735 (77)                                    | .92     |
| Abnormal with cavities | 1369 [23]                     | 674 (29)                               | 695 [20]                                     | <.0001  |
| Tuberculin (Mantoux) skin test or IGRAc |                           |                                        |                                               | .001    |
| Done                   | 4161 (69)                     | 1693 (72)                              | 2468 (68)                                    |         |
| Not done               | 1830 (31)                     | 643 (28)                               | 1187 (32)                                    |         |
| Primary reason evaluated for TB |                           |                                        |                                               |         |
| TB symptoms            | 3994 (67)                     | 1712 (73)                              | 2282 (62)                                    | .001    |
| Abnormal chest radiograph | 1193 (20)                    | 411 (18)                               | 782 (21)                                     | <.0001  |
| Contact investigation   | 149 [2]                       | 59 [3]                                 | 90 [2]                                       | .88     |
| Targeted testing       | 107 [2]                       | 46 [2]                                 | 61 [2]                                       | .39     |
| Immigration medical exam | 192 [3]                       | 30 [1]                                 | 162 [4]                                      | .001    |
| Incidental laboratory result | 278 [5]                       | 61 [3]                                 | 217 [6]                                      | .001    |
| HIV status             |                               |                                        |                                               | .24     |
| Positive               | 264 [4]                       | 112 [5]                                | 152 [4]                                      |         |
| Negative               | 5727 (96)                     | 2224 (95)                              | 3503 (96)                                    |         |
| Additional TB risk factors |                               |                                        |                                               |         |
| TNF-α antagonist therapy | 38 [1]                       | 18 [1]                                 | 20 [1]                                       | .29     |
| Postorgan transplantation | 34 [1]                       | 10 (0)                                 | 24 [1]                                       | .25     |
| Diabetes mellitus      | 1596 (27)                     | 686 (29)                               | 910 (25)                                     | <.0001  |
| End-stage renal disease | 172 [3]                       | 63 [3]                                 | 109 [3]                                      | .52     |
| Immunosuppression (not HIV/AIDS) | 368 [6]                     | 144 [6]                               | 224 [6]                                      | .96     |
| Other                  | 1621 (27)                     | 653 (28)                               | 968 (26)                                     | .21     |

Abbreviations: AIDS, acquired immune deficiency syndrome; HIV, human immunodeficiency virus; IGRA, interferon-gamma release assay; IQR, interquartile range; NAAT, nucleic acid amplification test; TB, tuberculosis; TNF, tumor necrosis factor; TST, tuberculin skin test.

NOTE: NAAT: NAAT was reported before culture was reported. No NAAT: NAAT was not reported or was reported at time of or after culture was reported.

*Excluding 60 patients who had NAAT reported with NAAT or culture dates missing.

bInitial chest radiograph excludes 187 patients with missing results.

cTB skin test excludes 1 missing value (TST result was missing).
earlier than patients who did not have NAAT. However, differences in patients with NAAT vs those without NAAT indicate that NAAT may not have been the sole trigger for the clinical decision to start TB treatment. Compared with patients who had positive NAAT results, patients who had negative NAAT results had delayed treatment, suggesting that NAATs did influence TB treatment initiation decisions. Patients with negative NAATs were also different than patients with positive NAATs, with a smaller proportion of patients with cavities on chest radiograph, and with TB symptoms as the primary reason for TB evaluation. We documented varying TATs, with 22% of TB patients having NAAT results reported more than 4 days from specimen collection. These reporting delays may have been due to referral of specimens to out-of-jurisdiction laboratories. We also found that different jurisdictions had different proportions of patients with NAAT. Taken together, these findings suggest that variable access to NAAT in California local health jurisdictions may have contributed to its low utilization during the study time frame.

The longest median times-to-treatment initiation for NAAT-positive vs NAAT-negative patients were for smear-negative patients, who were less likely to be infectious and to have extensive disease. Thus, the potential negative consequences of delayed treatment in this group were likely fewer than those for delayed treatment in the smear-positive group. On the other hand, compared with smear-positive patients, smear-negative patients had the greatest decline in time-to-treatment initiation, and therefore the greatest benefit, when they had a positive NAAT.

In response to the relatively low frequency of NAAT usage, the California Department of Public Health TB Control Branch has implemented steps to increase the use of NAAT. We are assessing NAAT availability in California laboratories, and we are implementing a TB program improvement indicator to monitor and encourage use of NAAT. Additional efforts focused outside public TB clinics, where presumptive treatment of patients with TB symptoms is less common, could have a greater impact.

We confirmed that NAAT was more sensitive than AFB smear microscopy in our study population: NAAT detected and treatment was initiated in an additional 92 patients that the AFB smear missed. Our findings are in contrast to the reported sensitivity of Xpert for smear-negative induced sputum samples from patients in a hospital in Montreal (sensitivity of 28% with 95% confidence interval 10%–56%) [8]. These differences in sensitivity may be explained by population differences. Whereas the majority of our study’s TB patient population reported TB symptoms (67% among all patients and 73% among patients with NAAT), only 18% of the Montreal study population reported symptoms. The authors of the Montreal study suggest

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**Table 2. Median Time-to-Treatment Initiation From Earliest Specimen Collection for Patients Who Had NAAT vs Patients Who Did Not Have NAAT**

| Group             | Patients Who Had NAAT Median Daysb (N) | Patients Who Did Not Have NAAT Median Daysb (N) | P Value for Wilcoxon Rank-Sum Test |
|-------------------|----------------------------------------|-----------------------------------------------|----------------------------------|
| All               | 3 (1960)                               | 14 (3101)                                    | <.0001                           |
| Smear positive    | 2 (1728)                               | 4 (1580)                                     | <.0001                           |
| Smear negative    | 10 (232)                               | 26 (1521)                                    | <.0001                           |

Abbreviations: NAAT, nucleic acid amplification test; TB, tuberculosis.
*Pulmonary culture-confirmed TB patients initiating treatment after specimen collection (N = 5061).
bTime (days) between date of earliest specimen collection and treatment initiation.

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**Figure 2.** Among patients with nucleic acid amplification test (NAAT), NAAT results for patients who started treatment after NAAT was reported, by smear status.
that less extensive disease may explain the lower sensitivity of Xpert in their study population setting. On the other hand, a large study of Xpert in multiple countries demonstrated a sensitivity of 72.5% for smear-negative TB [9]. Our comparatively lower sensitivity of NAAT in smear-negative patients may be explained by lower bacillary burden or the NAAT platforms used, which were likely not Xpert (see Limitations below).

The smear-positive patients with negative NAAT results demonstrate a potential pitfall with airborne infection isolation (AII) procedures outlined in the revised device labeling for the Xpert assay [10]. Although studies have suggested that 1 or 2 NAATs could replace serial sputum monitoring and result in fewer hours of unnecessary AII in low-TB-burden settings [11–13], our study demonstrated the frequency of negative NAATs was not negligible (with 6% negative NAATs from smear-positive patients and 9% negative NAATs from all pulmonary culture-confirmed patients who were tested). If a negative NAAT result on 1 sputum specimen were the only criterion for the decision to release a patient from AII, then findings from our study population suggest that 6 of every 100 smear-positive and eventually culture-confirmed and infectious patients would be inappropriately released. Our study was not able to assess whether 2 NAATs would reduce the percentage of negatives.

A recently proposed infection prevention strategy that combines clinical prediction rules to detect patients at high risk for culture-positive pulmonary TB and NAATs for TB detection would be a more conservative approach that may prevent inappropriate release from AII [14].

Our findings are also consistent with those of Marks et al [6] that demonstrated significantly decreased time to diagnosis in patients, among other benefits. However, if NAAT TAT is more than 1 day or 2, the benefits of rapid NAAT are reduced. We determined that a subset of California’s TB patients, from specific local health jurisdictions, were associated with excessively long NAAT TATs, with the highest at 11 working days. A long TAT suggests that patient specimens were likely referred to laboratories outside of California [15], a process that delays testing and reporting results, and diminishes the benefits of a rapid test. We expect that increased availability of Xpert will result in increased use of NAAT in TB patients.

**LIMITATIONS**

Although we did not directly assess all factors that contributed to the provider decision to treat the TB patient, which would require patient chart review and provider interview, we described evidence that NAAT contributed to earlier treatment initiation.

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**Table 3. Median Time-to-Treatment Initiation From the Date That NAAT Was Reported Among Patients Who Had NAAT (N = 795)**

| Group       | NAAT Positive Results | NAAT Negative Results | P Value for Wilcoxon Rank-Sum Test |
|-------------|-----------------------|-----------------------|-----------------------------------|
|             | Median Daysb (N)      | Median Daysb (N)      |                                   |
| All         | 1 (704)               | 11 (91)               | <.0001                            |
| Smear positive | 1 (612)               | 5.5 (38)              | <.0001                            |
| Smear negative | 2 (92)                | 14 (53)               | <.0001                            |

Abbreviations: NAAT, nucleic acid amplification test; TB, tuberculosis.

*Pulmonary culture-confirmed TB patients initiating treatment at/after NAAT report (N = 795).

*bTime (days) between date NAAT reported and treatment initiation.

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**Figure 3.** Number of days between specimen collection and report of nucleic acid amplification test (NAAT) results among all patients with NAAT.
The specific type of NAAT was not collected, so we could not assess the use of different NAATs. We hypothesize that most of the NAATs were of the pre-Xpert variety (eg, the enhanced Amplified Mycobacterium Tuberculosis Direct Test [E-MTD; Hologic Gen-Probe, San Diego, CA] and laboratory-developed tests) because the US Food and Drug Administration approval of Xpert occurred later in our cohort. We do not know whether NAAT was performed on the same specimen as smear microscopy, so it is possible that we either underestimated or overestimated the frequency of discordant results of NAAT and smear.

**CONCLUSIONS**

In California, NAAT was not used in the majority of pulmonary TB patients with positive culture. We confirmed the increased sensitivity of NAAT compared with smear microscopy, and we determined that differential use and access to NAAT may serve as a barrier to harnessing the benefit offered by NAAT. Increased use of NAATs in the diagnosis of pulmonary TB could decrease time-to-treatment initiation for the patient and transmission in the community.

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