

Public Health Laboratory Expansion of Nucleic Acid Amplification Testing for *Mycobacterium tuberculosis* Complex

Summary of Final Reports from TB NAAT Expansion Sub-Grant

BACKGROUND

In October 2010 the Association of Public Health Laboratories (APHL), in cooperation with the Centers for Disease Control and Prevention's (CDC) Division of Tuberculosis Elimination (DTBE), released a Request for Proposals (RFP) that sought to award one-time funding to state and selected local and territorial Public Health Laboratories (PHLs). The RFP aimed to expand the use of nucleic acid amplification testing (NAAT) for the identification of *Mycobacterium tuberculosis* complex (MTBC) directly from clinical specimens and/or the molecular detection of drug resistance (MDDR) in *M. tuberculosis* in their jurisdiction. Funds could be used to implement, expand or improve access to NAAT in PHLs or to contract with an outside facility to provide these services.

APHL and DTBE received, reviewed and approved 54 applications, including two joint applications, which would expand or improve testing in a total of 56 jurisdictions. Funds were distributed to 52 of those laboratories through a subgrant mechanism. Laboratories that received funds were expected to complete mid-term and final progress reports.

EXPANSION OF NAAT FOR MTBC

Table 1 summarizes the frequency of reported NAAT expansion activities conducted by the 52 funded laboratories. Laboratories reported all NAAT expansion activities they conducted. Responses were categorized into seven NAAT expansion activities; the number of laboratories that implemented each activity is also provided.

The responses showed that of the 52 funded laboratories, only four implemented NAAT in-house where it had previously not been available (either through in-house test or referral method). Five of the funded laboratories brought NAAT in-house instead of referring the testing to an outside laboratory. For two low-volume laboratories, establishing mechanisms for referral of NAA testing was deemed most appropriate. Thirty-eight of the 52 laboratories with previous NAAT capability used subgrant funds to transition to a new NAAT method that would decrease cost and/or TATs.

Furthermore, 24 laboratories began increasing the frequency of NAAT to shorten TAT, from specimen receipt to results reporting. As the data signify, the two most

Table 1

Summary of Reported NAAT Expansion Activities in 52 Jurisdictions	
NAAT Expansion Activity	Frequency of Laboratories (n=52) ¹
Change NAAT platform to shorten turnaround time (TAT) or reduce cost.	38
Increase frequency of testing to shorten TAT. ²	24
Assess staff proficiency/ provider education.	11
Expand patient population eligible for NAAT.	10
Make improvements in specimen storage and transport (e.g., institute courier services).	6
Implement NAAT in-house where it had previously not been available.	4
Implement NAAT in-house where testing was previously referred	5
Institute referral of TB NAAT.	2

¹ Some laboratories listed multiple approaches to NAAT expansion.

² To improve efficiency, laboratories may delay testing until enough specimens are received, a practice known as batching. For lower-volume laboratories, batching may lead to delays in TAT.

commonly reported activities were targeted on improving TAT, indicating that having NAAT capability does not, on its own, ensure an effective testing program.

Additionally, six laboratories focused on pre-analytical factors, such as updating equipment and implementing courier services for better specimen storage and transport. These changes were designed to improve the quality of the specimens received for NAAT. Ten laboratories expanded eligibility criteria for NAAT, including testing specimens from suspected TB patients regardless of smear status and reaching out to new submitters.

Laboratories have also aimed to expand knowledge of NAAT services through a public health systems approach. Such activities include enrolling in staff proficiency testing programs and educating providers on the benefits and proper utilization of NAAT services. Reported educational ac-

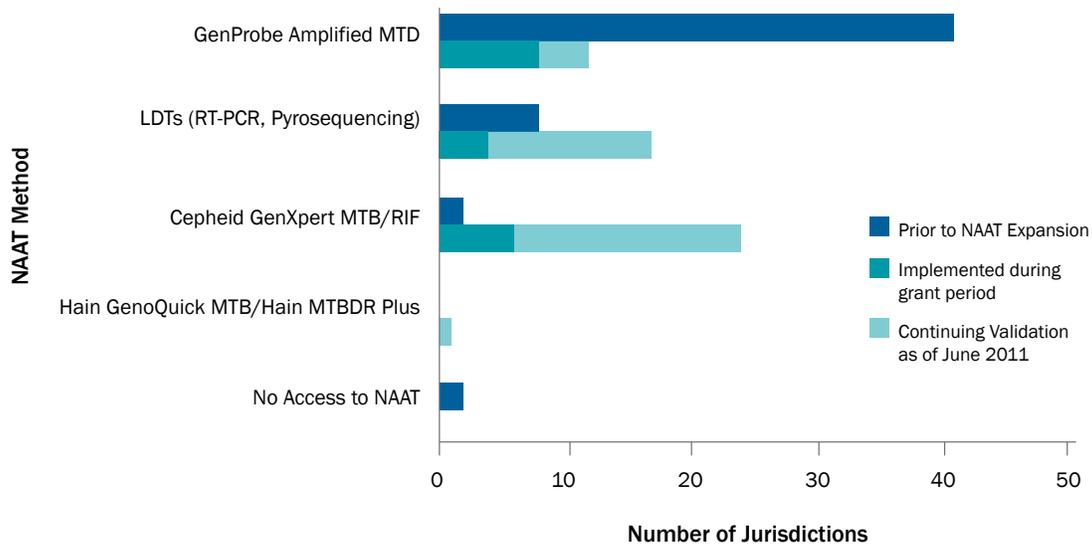
tivities include trainings, webinars, phone calls and other electronic or hard-copy communications.

TRENDS IN NAAT METHODS

Laboratories had the option of using funds to evaluate and implement new methods and platforms for direct detection of the MTBC and the molecular detection of drug resistance. As shown in Figure 1, prior to the start of the NAAT expansion grant, the most commonly utilized platform among applicants was the GenProbe Amplified MTD test (41 jurisdictions). By the end of the grant period in June 2011, a majority of laboratories had implemented or were in the process of validating different NAAT methods, including laboratory-developed tests (LDTs), such as real-time polymerase chain reaction (RT-PCR) and pyrosequencing, and the Cepheid GeneXpert MTB/RIF™ assay (research use only). One jurisdiction

Figure 1 summarizes the effect of the NAAT expansion awards on implementation of new NAAT methods at the conclusion of the grant period in June 2011.

Figure 1: Effect of Subgrants on Distribution of NAAT Methods



reporting no previous access to NAAT was able to use award funds to bring in-house the Cepheid GeneXpert MTB/RIF™ assay. Only one jurisdiction evaluated the Hain GenoQuick MTB for TB direct detection and the Hain GenoType MTBDRplus for drug resistance testing.

Popularity of Cepheid GeneXpert MTB/RIF™ Assay

Laboratories had the option of using funds to evaluate and implement new methods and platforms for direct detection of the MTBC and the molecular detection of drug resistance. As shown in Figure 1, prior to the start of the NAAT expansion grant, the most commonly utilized platform among applicants was the GenProbe Amplified MTD™ test (41 jurisdictions). By the end of the grant period in June 2011, a majority of laboratories had implemented or were in the process of validating different NAAT methods, including laboratory-developed tests (LDTs), such as real-time polymerase chain reaction (RT-PCR) and pyrosequenc-

ing, and the Cepheid GeneXpert MTB/RIF™ assay (research use only). One jurisdiction reporting no previous access to NAAT was able to use award funds to bring in-house the Cepheid GeneXpert MTB/RIF™ assay. Only one jurisdiction evaluated the Hain GenoQuick MTB for TB direct detection and the Hain GenoType MTBDRplus for drug resistance testing.

Use of LDTs

Eight jurisdictions use a laboratory-developed RT-PCR as their current NAAT method and 13 jurisdictions are continuing to validate a laboratory-developed method.

Agreement between Methods

Of the 659 patients that jurisdictions reported testing positive by NAAT, 500 were positive by an acid fast bacilli (AFB) smear test (76%), but 84 were negative by that test (13%). Some jurisdictions did not report AFB smear test results for all patients testing positive by NAAT.

Of the 12 jurisdictions that reported sensitivities and specificities based on a comparison of their previous and new NAAT methods, six attained 100% sensitivity and 100% specificity for TB detection during validation. In general, AFB smear positive samples performed better than AFB smear negative samples. The lowest sensitivity and specificity reported for AFB smear positive samples was 98.2% and 100%, respectively. For AFB smear negative samples, the lowest sensitivity and specificity values reported were 72.5% and 62.5%, respectively.

EXPANSION OF NAAT CAPACITY

Jurisdictions reported the number of specimens previously received for NAAT from January to June 2010 and again following the end of the NAAT expansion grant period, from January to June 2011. As a result of the funding awards, jurisdictions experienced an average of an 85% increase in the number of specimens received for NAAT during the six-month grant period in 2011.

The impact of NAAT expansion on meeting CDC-recommended TATs, from specimen receipt to NAAT results reporting, was also assessed. According to the CDC's *Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis*, "NAA test results should be available within 48 hours of specimen collection. Laboratorians should treat an initial positive NAA test result as a critical test value, immediately report the result to the clinician and public health authorities..."

To determine whether this requirement was being met, each jurisdiction was required to report on its TAT. On average, 20% of results that were positive for MTBC were reported within 48 hours of specimen receipt. Additionally, on average, 18% of specimens tested or referred for NAAT were also tested or referred for molecular detection drug resistance.

BARRIERS TO IMPLEMENTATION

Despite high interest in expanding NAAT capacity, many awardees were unable to meet the grant objectives and encountered common barriers to implementing the proposed goals and changes. Most commonly cited barriers were: limited number of samples available for validation, delays due to the approval process for contract or payment, limited staff or staff turnover and limited budget or funds (Table 2).

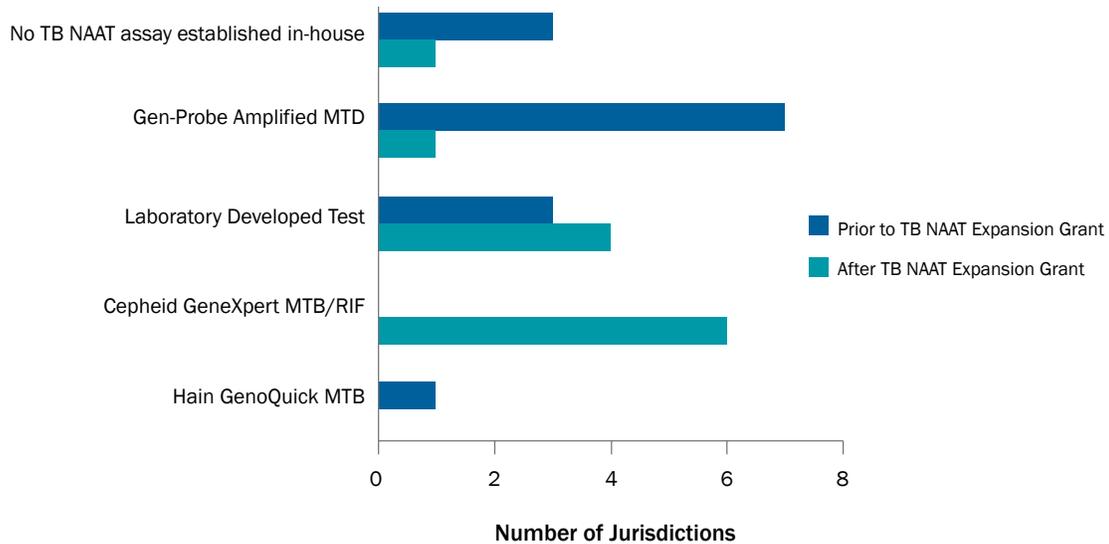
Table 2: Most Commonly Reported Barriers to Implementation

Barriers	# of Jurisdictions
Limited samples for validation	12
Payment, contract or approval mechanisms	11
Limited or loss of staff	9
Limited budget or funds	9

Overall, TB NAAT expansion awards were largely successful in addressing the need for faster and improved TB testing practices. Capacity for NAAT increased, laboratories were empowered to evaluate and establish testing methods that worked most efficiently within their current system, many laboratories established new in-house NAAT capability and the public health system was strengthened through improved education and communication. Because a majority of the reported barriers were a result of the short funding period, laboratories expressed confidence that many could be overcome over time. For many laboratories bringing in new NAAT methods, access to an additional—and significant—source of funding enabled them to offset the often substantial initial investment associated with startup costs.

Figure 2 shows TB NAAT methods used by 13 selected PHLs before and after the grant period. This information came from follow-up interviews.

Figure 2: Implementation of TB NAAT in Select Public Health Laboratories (n=13)



After establishing preliminary capability and capacity of the NAAT program, however, jurisdictions needed to ensure its continued strength. To evaluate the long-term sustainability and impact of the awards, APHL contacted a subset of award recipients in January 2013 for follow-up.

EVALUATING THE SUSTAINABILITY OF TB NAAT: STATUS IN 2013?

APHL invited 13 selected subgrant recipients to participate in a 30-45 minute "pulse check" interview to provide an update on the continued impact of the award on general TB NAAT practices, as well as progress related to ongoing objectives described in final reports submitted to APHL. APHL staff conducted the interviews over a three-week period between February and March 2013. Participating laboratories responded to a series of nine standard questions, along with an additional one to three questions tailored to the specific objectives proposed by the grantee.

Standard questions focused on current NAAT capabilities for the identification of *M. tuberculosis*, expansion of testing services, sustainability of project objectives beyond the grant period, molecular detection of mutations associated with drug resistance, as well as challenges faced and lessons learned. Over a three-week period, APHL interviewed 10 PHLs, nine state PHLs and one local PHL. Three PHLs chose to submit written responses.

Current Implementation of TB NAAT Methods

Since the end of the grant period in June 2011, 12 out of 13 jurisdictions participating in the follow-up had sustained the capability to perform NAAT for the detection of MTBC (Figure 2). One jurisdiction continued to maintain pre-grant NAAT methods, while seven had transitioned to a new NAAT method. Two jurisdictions had no prior TB NAAT capabilities, and two were still in the process of validating a new method.

Of the nine jurisdictions that had transitioned or were in the process of validating or establishing TB NAAT, six chose to implement the Cepheid GeneXpert MTB/RIF™ assay, including the two jurisdictions with no previous TB NAAT capability. Two jurisdictions switched to a laboratory-developed test (LDT), and one switched to the Hain Genotype MTBDRplus. Six of the seven jurisdictions that implemented new assays were previously using the Gen-Probe Amplified MTD test.

Update on Impact of TB NAAT Expansion Activities

Figure 3 shows the activities conducted during the TB NAAT expansion grant award period. These activities were designed to improve utilization of TB NAAT and increase detection of positive specimens by, for example, expanding eligibility criteria (e.g., testing of AFB smear negatives), expanding access

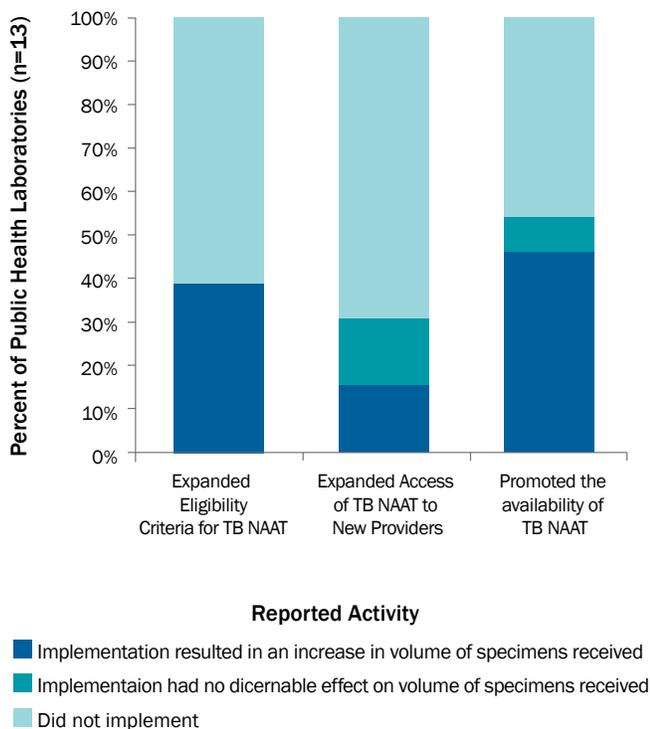
to new providers and promoting the availability of testing through communications.

During the grant period, nine out of 13 participating jurisdictions conducted activities to expand the utilization of TB NAAT services and increase detection of positive specimens. Seven jurisdictions (54%) promoted their NAAT testing capabilities to clients. Examples of promotional activities included mailing marketing materials to clients or sending them electronically, developing information packets and documents on the use and benefit of NAAT, conducting training via teleconference and waiving associated testing costs. As a result of these activities, six jurisdictions (86%) reported an increase in volume of specimens received for testing.

Four jurisdictions (31%) expanded access to new providers, which included hospitals, university clinics, correctional facilities and immigration facilities. Two of these jurisdictions (50%) reported an increase in volume of specimens received for testing.

Five jurisdictions (38%) expanded eligibility criteria for NAAT (e.g., allowing all smear negative or a subset of smear negative specimens to receive NAAT; incorporating testing of extrapulmonary specimens). All five of these jurisdictions (100%) reported that the new criteria increased the volume of specimens received for testing (Figure 3). In addition, four out of five jurisdictions (80%) reported an increase in the number of MTBC positive specimens identified as a result of the expanded eligibility criteria (data not shown).

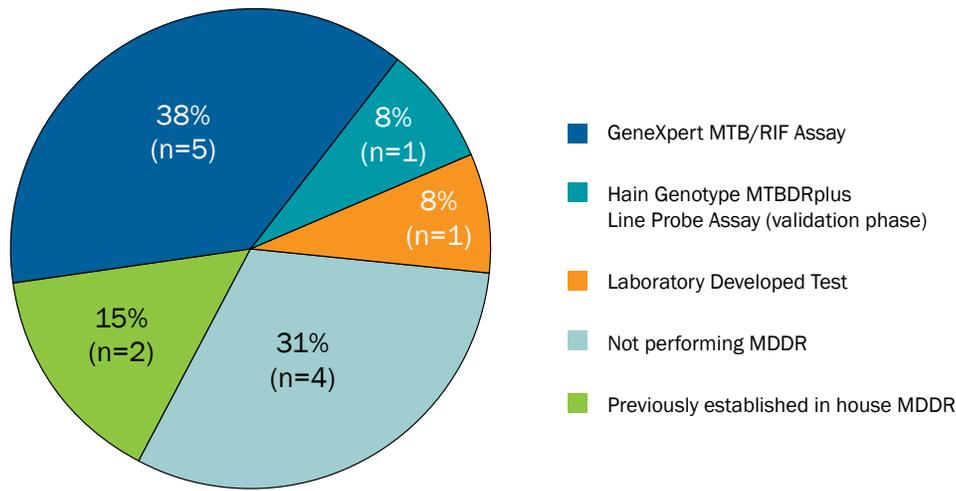
Figure 3: Activities Conducted to Increase Utilization of TB NAAT (n=13)



Molecular Detection of Drug Resistance

Figure 4 shows which of the 13 selected jurisdictions were able to conduct in-house molecular detection of drug resistance (MDDR) tests on *M. tuberculosis* positive isolates or clinical specimens, the assay platform used and if this capability was the result of the TB NAAT expansion grant funds.

Figure 4: Implementation of MDDR for TB



As of March 2013, seven of the participating jurisdictions (54%) had in-house capacity for MDDR. Two jurisdictions had previously established MDDR capacity (pyrosequencing; Hain Genotype MTBDRplus Line Probe Assay). Five jurisdictions (71%) have access to MDDR testing as a result of the award, and all five have access as a result of implementing the GeneXpert MTB/RIF™ assay. Two (2) jurisdictions continue to refer specimens for testing. One jurisdiction was in the process of validating the Hain Genotype MTBDRplus Line Probe Assay, and another was validating a LDT (Figure 4).

difficulties implementing non-FDA approved and research use only methods due to extensive and time-consuming validations, as well as lack of clear guidance on results interpretation and reporting. One laboratory explained that reaching out to its CDC laboratory consultants and other PHLs for advice was important when implementing a new test method. In general, while the participating laboratories often reiterated the challenges to making progress during the short six-month grant period, the award turned out to be very valuable, as evidenced by the continued sustainability of their current TB NAAT activities.

LESSONS LEARNED AND CONSIDERATIONS FOR IMPLEMENTATION OF NAAT FOR MTBC

The 13 participating laboratories were given the opportunity to offer retrospective insight in the form of free responses indicating any challenges or lessons learned as a direct result of the TB NAAT expansion grant opportunity. Many cited administrative difficulties, including staff turnover and mechanisms for procuring instruments and reagents. Some laboratories also reported

Lessons learned from TB NAAT promotional activities can provide insight into developing standards for future test implementation. Expansion and promotion activities should be carefully considered and accompany the introduction of any new TB diagnostics, and laboratories should occasionally remind providers of the available testing services and their benefits.

Association of Public Health Laboratories

This publication was supported by Cooperative Agreement Number U60HM000803 from the Centers for Disease Control and Prevention (“CDC”). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC or the Department of Health and Human Services.

National Center for Environmental Health (NCEH)
National Center for Zoonotic, Vector-borne, and Enteric Diseases (CK)
National Center for Immunization and Respiratory Diseases (IP)
National Center for HIV, Viral Hepatitis, STDS and TB Prevention (PS)
National Center for Infectious Diseases (NCID) (CID)
Office of the Director, Centers for Disease Control & Prevention (ODCDC)
National Center for Health Marketing (HM)

The TB NAAT Expansion Grants were financed 100% by federal funds. The total cost of the project was \$1,200,000.

