Facilitating Test Verification and/or Modification of Algorithms for Detection of *Mycobacterium tuberculosis* Infections with Interferon Gamma Release Assays, 2018–2019

Executive Summary

SUMMARY

As we move toward tuberculosis (TB) elimination, implementation of interferon-gamma release assays (IGRAs) will play an important role in detection of latent tuberculosis infection (LTBI). In 2018, the Association of Public Health Laboratories (APHL), in partnership with the US Centers for Disease Control and Prevention (CDC), released a request for funding proposals to public health laboratories for the implementation or expansion of in-house IGRA testing to be completed between November 2018 and June 2019. Of 12 applicants, APHL awarded seven public health laboratories a total of $139,161 for this project. Major accomplishments of this project included:

- Seven public health laboratories verified and/or validated QuantiFERON®-TB Gold Plus.
- IGRA testing became available statewide in South Dakota, increasing access and affordability of testing.
- By expanding IGRA testing, Tennessee provided relief to their central laboratory, expedited shipping and turnaround time (TAT), and decreased indeterminate rates.
- El Paso established new working relationships with local hospital systems and home health providers to expand IGRA testing services.
- Milwaukee provided IGRA testing for the TB Prevention and Care Program, affording the program financial relief, and plans to expand services to outside clients in the future.

INTRODUCTION

In 2018, the United States reported 2.8 tuberculosis (TB) cases per 100,000 persons, the lowest rate on record.[1] Despite declining numbers of active TB cases in the US, the US Centers for Disease Control and Prevention (CDC) estimates up to 13 million US residents are living with latent TB infection (LTBI).[1] LTBI is not associated with any clinical symptoms and while those latently infected are not infectious, 5–10% of individuals with LTBI will go on to develop active TB disease in their lifetime. To achieve TB elimination, individuals with LTBI must be identified and treated. Interferon-gamma release assays (IGRA) are FDA-approved blood tests that aid in the diagnosis of infection with *Mycobacterium tuberculosis*. There are currently two IGRA tests available in the US: QuantiFERON®-TB Gold Plus (QFT-Plus) and T-SPOT® TB Test (T-Spot). According to CDC guidelines, IGRAs may be used in place of tuberculin skin tests in all situations, with preferential use among individuals with prior BCG vaccination and for those populations with lower rates of return for skin test readings. IGRAs require only one blood draw, results can be available in 24 hours, and prior BCG vaccination does not cause false-positive results.[2]

PARTICIPATING PUBLIC HEALTH LABORATORIES

- Colorado Department of Public Health and Environment
- El Paso Department of Public Health Laboratory
- Florida Department of Health
- Milwaukee Division of Disease Control and Environment
- New York City Public Health Laboratory
- South Dakota Department of Health
- Tennessee Department of Health (Knoxville Regional Laboratory)

1 CDC. (September 6, 2019). Tuberculosis (TB) Data and Statistics. Retrieved from https://www.cdc.gov/tb/statistics/default.htm

2 CDC. (May 4, 2016). Tuberculosis (TB) Interferon-Gamma Release Assays (IGRAs)—Blood Tests for TB Infection. Retrieved from https://www.cdc.gov/tb/publications/factsheets/testing/igra.htm

This project allowed for the implementation and expansion of IGRA testing services between November 2018 and June 2019. Participating public health laboratories (PHLs) selected one of three project options (**Table 1**):

Option 1: Public health laboratories in jurisdictions that do not currently perform IGRA will implement in-house testing capability with programmatic input.

Option 2: Public health laboratories currently performing IGRA will conduct verification studies for an in-house replacement test.

Option 3: Public health laboratories will modify current testing algorithms with input from TB control programs.

Table 1. Selected project implementation methods

Option	Public Health Laboratories
1	Colorado Milwaukee New York City
2	El Paso South Dakota
3	Florida Tennessee

PROPOSED APPROACHES

The request for funding proposals coincided with the discontinuation of the QuantiFERON®-TB Gold In-Tube (QFT-Gold) assay, so many public health laboratories needed to verify the performance of the new QFT-Plus assay. All seven awarded public health laboratories chose to implement and/or expand testing with QFT-Plus. One major change from the QFT-Gold assay to the QFT-Plus assay was the inclusion of the option of a four-tube or one-tube specimen collection method. This option allowed individual jurisdictions the flexibility to decide whether blood is collected directly into four specialized tubes at the time of specimen draw, or initially collected into a single Lithium Heparin (LiHe) tube and then aliquoted into four tubes when received at the laboratory. **Table 2** provides a summary of the specimen collection approaches that sites chose to evaluate. Of note, six out of seven sites met their goal of implementing their chosen collection method, however, New York City (NYC) encountered numerous obstacles that ultimately precluded the complete implementation of IGRA as proposed.

Table 2: Proposed collection method approaches for implementation/expansion of QFT-Plus

Collection Method	Public Health Laboratories
Four-Tube Collection	Colorado New York City Florida (already validated four-tube collection)
One-Tube (LiHe) Collection	Milwaukee Tennessee
Both	El Paso (primarily four-tube, but conducted small sensitivity study comparing both methods) South Dakota

APHL asked each jurisdiction to describe the advantages and disadvantages of their chosen method of specimen collection. Three laboratories provided descriptions on one-tube collection and one laboratory provided descriptions on the four-tube method (**Table 3**).

In addition, APHL requested that jurisdictions describe direct comparisons, if any, utilized during the validation and verification process as well as indicate whether an automated platform was used during the project. Tennessee, South Dakota, El Paso and Milwaukee directly compared results from their newly implemented IGRA tests against other methods for identification of infection with *M. tuberculosis*, including QFT-Gold tests and tuberculin skin tests. One site also included information about clinical use of x-rays to rule out active disease. Additionally, all sites except El Paso utilized an automated platform (DSX instrument) to perform ELISA assays.

TRAINING AND PURCHASES

Project funds covered crucial reagents and kits as well as several substantial purchases, including a microplate washer and absorbance reader in El Paso, a microplate washer and incubator in Milwaukee, service agreements for refurbished instruments in New York City, and nine incubators to be distributed to five state clinics and four Indian Health Service (IHS) clinics in South Dakota.

Furthermore, approximately two to four public health laboratory staff at each site received training from a manufacturer (Qiagen, Dynex or BioTek), with approximately 20 public health laboratory staff trained across the seven sites. Beyond the laboratory staff, Florida, Milwaukee and South Dakota provided training for nine TB control staff to ensure effective

Table 3. Advantages and disadvantages of the four- and one-tube collection methods as described by participating laboratories

Collection Method	Advantages	Disadvantages
Four-Tube Collection	• Similar to previous method (QFT-Gold), thus many clinical and laboratory staff are already trained in protocols • Less hands-on laboratory time as the specimen is already aliquoted into four tubes at the draw site • Laboratories already have the resources and supplies for performing this method	• Short time frame between blood draw and drop off of the specimen at a laboratory (16 hours) • More challenging for staff to obtain four tubes of blood, especially in remote locations or with certain patient populations
One-Tube (LiHe) Collection	• Allows laboratory staff to aliquot the exact volume of blood needed into the four tubes, potentially allowing for more precise measurements • Easier use in remote locations as only one vial of blood is collected, reducing hands-on time and collection tube inventory • More flexible hold time (up to 53 hours), allowing for the potential to ship samples on cold packs overnight	• Increased laboratory processing time due to aliquoting the specimen from one to four tubes • Risk of sample exceeding recommended temperature during shipment due to potentially longer time in transit • Increased risk of cross-contamination and pathogen exposure of laboratory staff due to additional sample manipulation • Additional costs of procuring additional supplies for outside providers submitting specimens and new equipment and laboratory supplies to accommodate new methods

sample collection and processing. South Dakota also arranged phlebotomy training at five locations for multiple disease intervention specialists (DIS) and highlighted QFT-Plus at their annual DIS workshop.

POPULATIONS REACHED

In their proposals, public health laboratories included target populations to whom the jurisdiction wanted to offer IGRA testing. The identified target populations included:

- Incarcerated persons
- Persons in contact investigations
- Individuals of Native American descent
- Persons experiencing homelessness
- Refugee populations
- Individuals in congregate settings
- Individuals exposed to active TB
- Low-income residents
- Individuals with diabetes, HIV or who are immunocompromised
- Persons with travel history to TB-endemic regions

Five out of seven public health laboratories reached their target populations with several states also able to expand beyond their original target populations. Due to a variety of obstacles, El Paso and New York City were unable to reach their target populations; however, El Paso did reach unplanned populations by partnering with the local hospital system to provide IGRA testing. South Dakota reached new populations through expanded testing services with testing now available state-wide. Florida also expanded beyond their original target populations of persons identified through contact investigations and individuals in correctional facilities to provide testing to individuals experiencing homelessness and residing at shelters.

PROJECT OUTCOMES

APHL asked the jurisdictions to provide several project outcomes including efficiencies gained, new or strengthened partnerships, how the project addressed laboratory needs, turnaround time (TAT), cost per test, workflow adjustments and challenges encountered.

Efficiencies and Partnerships

Multiple laboratories reported programmatic efficiencies as well as increased communication and strengthened relationships with local clinics, health departments, community-based organizations and TB control programs.

- El Paso noted that cost savings helped with the purchase of new equipment, allowed for validation of a newly purchased BioTek instrument and allowed for expansion of services. Notably, El Paso also reported the creation of an unexpected partnership with the local hospital system. Hospitals transitioned to the four-tube collection method and assisted the PHL with a one-tube LiHe evaluation. El Paso also reported that local home health providers showed interest in partnering with the laboratory.
- Florida was able to show proof of concept for the one-tube collection method for outbreak investigations and demonstrated efficiency of the one-tube method in remote areas. Although the four-tube method is still more broadly utilized in Florida, their evaluation provided compelling support for expansion of services in the future.
- By bringing QFT-Plus testing in-house, Milwaukee had the opportunity to implement a laboratory information system that enabled direct reporting of test results to the TB Prevention and Control Clinic resulting in more efficient and timely reporting compared to reference laboratory reporting. Milwaukee also set up a direct courier between the collection site and the department of health laboratory, improving the time frame between blood draw and specimen receipt.
- By adding QFT-Plus to the Knoxville Regional Laboratory testing menu, specimens from Eastern Tennessee no longer had to be sent to Nashville. As a result, the rate of indeterminate results and specimen transport time decreased, which is important for improving contact investigations and patient treatment and decreasing the need for second blood collection from patients.
- Although not directly purchased with grant funds, Colorado obtained a DSX automated platform instrument which was used as part of the QFT-Plus assay validation. The automated platform will save hands-on time, increase capacity and accuracy and allow for additional assays to be performed at the laboratory.

Laboratory Needs

This funding proposal allowed multiple sites to purchase crucial equipment and reagents, to validate IGRA methods and to increase testing availability and capacity.

- In Milwaukee, the grant provided financial relief for the Milwaukee TB Prevention and Control Clinic and provided opportunities to expand clientele and generate funding to recuperate previous costs.
- South Dakota reported that the funding supported the state laboratory's migration from QFT-Gold to QFT-Plus and expanded the IGRA testing offered.
- Colorado now offers IGRA testing, previously unfeasible due to time restrictions, which increases accessibility to TB testing and ultimately aids Colorado in the goal of becoming TB-free.
- By adding the QFT-Plus method to the Knoxville Regional Laboratory testing menu, Tennessee expedited TB testing in the eastern region of Tennessee filling an essential gap and relieving the Nashville Central Laboratory which received all IGRA samples in Tennessee until this project.
- At the NYC PHL, the grant provided funds to refurbish two Dynex Agility instruments, acquire new service agreements, commission training for the instruments, and integrate the instruments into the laboratory information system. The implementation of IGRA at the NYC PHL was intended to provide relief to the Bureau of TB Control. Due to numerous challenges, IGRA implementation did not occur, but the integration of the instruments and training of staff during the project period have ensured that the laboratory is prepared for implementing testing in the future.

Turnaround Time

Jurisdictions were asked to compare TAT (specimen receipt to reporting test results) at the start of the project and after implementation of changes indicated in their project proposal. Four sites (Florida, Milwaukee, South Dakota and Tennessee) reported that they were able to reduce TAT; Florida and Milwaukee reduced TAT from two days to one day. Due to delays in funding and challenges encountered after completion of the project, Colorado was not able to provide TAT results. The NYC PHL has not yet implemented IGRA testing and thus has not monitored TAT; however, they anticipate that TAT may increase by 24-48 hours when implementation does occur. This is a result of numerous factors associated with the shift from collection and processing samples at the Bureau for TB Control to the NYC PHL, including different laboratory working hours at the NYC PHL and the shift to one-tube collection. El Paso's TAT remained unchanged but stated that automation of the system would positively impact TAT when/if additional funding became available.

Cost

Jurisdictions compared the cost of IGRA testing to their previous in-house method or, for laboratories not previously performing IGRA testing, the cost of testing at local reference laboratories to whom they previously submitted samples. Jurisdictions were not provided a cost template. Thus, differences in reported cost per test could reflect variability in reporting methods.

The cost of IGRA testing remained the same in Florida, Colorado, and South Dakota. Several jurisdictions noted an increase in test costs: El Paso (+$0.70), Milwaukee (+$15.00) and Tennessee (+$9.44). There were a number of contributing factors reported for the increased costs. Tennessee reported dramatic changes in supply pricing. El Paso reported increased costs due to the unexpected need for additional supplies (longer pipette tips) and providing hospital submitters with LiHe collection tubes. Plans to begin batch testing are in place to reduce costs in Colorado.

Workflow

Workflow adjustments were noted in five laboratories that processed samples.

Systematic Adjustments

- Changes were made to Tennessee shipping labels to route specimens from eastern Tennessee health departments to Knoxville as opposed to the Nashville Laboratory which previously handled all IGRA testing.
- Scheduling changes were made to allow for an influx in demand and incorporation of a new assay including:
    - Additional testing
    - Addition of a weekend courier
    - Rearrangement of current testing schedules.
- Additional laboratory staff was allocated to support IGRA pre- and post-analytical processing.
- A laboratory information system was implemented to allowed test results to be electronically submitted to TB control.

Laboratory Adjustments

- Processing schedule was adjusted to maximize testing time by performing centrifugation the day prior to testing.
- An automated pipette was purchased for aliquoting the specimen from a single tube to four tubes to reduce hands-on time and provided ergonomic relief to PHL staff.
- Standardized timing of incubation was implemented on testing days (i.e., 4:00 pm to 11:00 am for the minimum 16-hour incubation) to improve workflow.

Challenges

Colorado encountered a delay in funding, which resulted in delayed implementation of IGRA services. As a result, Colorado was able to validate, but not implement, testing. The Colorado laboratory began receiving samples in October 2019; however, in the months since the project's conclusion, their courier company dissolved, resulting in difficulties

transporting samples from collection sites to the laboratory. Colorado is searching for a new courier and will continue to monitor improvements in TAT and cost.

The NYC PHL encountered numerous obstacles over the course of the project, including the Brooklyn measles outbreak and funding decreases resulting in reductions of TB staff. The serology laboratory, where the assay was to be implemented, was allocated to the measles outbreak, thus making implementation of the assay impractical. The TB unit at the NYC PHL requires additional staff and funding to move forward with IGRA implementation. Validation of the assay was performed on an automated platform, however, due to the aforementioned obstacles, the NYC PHL was unable to complete implementation of IGRA testing and therefore, did not provide results regarding TAT, cost, workflow, efficiencies or partnerships.

El Paso encountered a shift in service as a result of the collaboration of the State TB Control Program and the Texas Department of State Health Services. The Texas State TB Control Program no longer utilized the El Paso laboratory for TB testing, resulting in a large decrease in samples sent to the laboratory. El Paso also noted an unexpected spike in indeterminate results, from 1% to 5%, but hypothesized this could be due to a change in patient populations, from testing Texas TB Control patients to testing immunocompromised hospital patients.

In South Dakota, due to the federal government shutdown in early 2019 and staff reorganization within IHS, incubators had not been placed in the four IHS clinics by the conclusion of this project. However, the South Dakota Public Health Laboratory is currently working to place these incubators and is hoping to complete placement by May 2020.

NOTABLE ACHIEVEMENTS

The El Paso Laboratory is currently trying to secure a fee-for-service contract with a local home health provider that would increase the annual number of IGRA tests over 200 and allow the laboratory to be self-sustaining in regard to IGRA testing. Florida demonstrated that the Bureau of Public Health Laboratories can make one-tube collection available to any county in the state for contact investigations or shelter programs; however, this service is currently not available statewide. The South Dakota PHL expanded testing services statewide through procurement of incubators for nine local and IHS clinics and provided appropriate training to clinical and laboratory staff.

POST-PROJECT PLANS

Moving forward, all laboratories except the NYC PHL will continue to provide IGRA testing. The NYC PHL hopes to begin and sustain IGRA testing once funding is available.

IGRA testing in El Paso is currently supported by their annual budget and will be continued for local hospital partners. The Texas TB Control Program may resume sending samples to the EL Paso laboratory in the future, which could result in an increase of testing volume by approximately 1,600 patient samples per year.

Florida will continue to provide both one- and four-tube collection testing to two counties. Continuation of services will be supported by the TB Control Program.

South Dakota's IGRA testing is self-sustained through a fee-for service model. This model is available for clinical and public health partners, with no-cost testing offered to the Office of Disease Prevention services and IHS.

Colorado currently provides IGRA testing for TB control samples but plans to expand services and adopt a fee-for-service model after completion of the grant-funded pilot.

Milwaukee is currently discussing offering fee-for-service testing to local health departments and continues to collaborate with and test for the TB Prevention and Control Clinic.

Tennessee is looking to expand IGRA to accommodate testing for correctional facilities and other state-run facilities. Knoxville will perform approximately 3,000 tests per year and service 35 counties. The continuation of the program will be supported by the TB Elimination and Laboratory Cooperative Agreement.

Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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