BACKGROUND

Whole genome sequencing (WGS) of *Mycobacterium tuberculosis* complex (MTBC) provides detailed genomic information that increases the accuracy of outbreak investigations and can identify drug-resistant strains. In combination with epidemiologic data, genotyping can help differentiate TB cases that may be part of an outbreak from unrelated sporadic TB cases. Thus, WGS data can be utilized to identify clusters of interest and focus intervention resources.

Beginning in 2012, the US Centers for Disease Control and Prevention (CDC) Division of TB Elimination (DTBE) provided WGS for clusters of interest for TB cases across the United States to determine if the clusters could be further refined. However, demand for WGS began to exceed DTBE’s internal capacity for performing the testing in a timely manner. In response to this need, the Association of Public Health Laboratories (APHL), in cooperation with the DTBE’s Laboratory Branch, conducted an initial pilot with the California Department of Public Health’s Microbial Diseases Laboratory. This small pilot demonstrated the potential feasibility of public health laboratories serving as centers for performing TB WGS. In response, APHL released a request for proposals (RFP) to identify up to six public health laboratories to act as WGS reference centers, providing WGS services and electronically delivering results to DTBE within two months. Reference centers had the option of sequencing samples provided by DTBE, jurisdictional samples or a combination of both. Contract years ran from July 1 through June 30. Nine public health laboratories responded to the RFP and, over the five contract years, six were selected to participate.

THE PROJECT

The initial TB WGS pilot was conducted in 2015 at the California Department of Public Health’s Microbial Diseases Laboratory. The laboratory sequenced 36 isolates provided by DTBE.

2015–2016: Following the RFP selection, the project expanded to include the University of Iowa’s State Hygienic Laboratory, the Michigan Bureau of Laboratories, New York State’s Wadsworth Center and the Ohio Department of Health Laboratory, in addition to the California Department of Public Health. Over this period, the five laboratories sequenced 954 isolates (569 DTBE samples, 385 jurisdictional samples).

REFERENCE CENTERS

- California Department of Public Health
- University of Iowa State Hygienic Laboratory
- Michigan Bureau of Laboratories
- New York State’s Wadsworth Center
- Ohio Department of Health Laboratory
- Virginia Division of Consolidated Laboratory Services

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<tr>
<th>Laboratory</th>
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<tr>
<td>California</td>
<td>DTBE and Jurisdictional</td>
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<td>Virginia</td>
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2016–2017: Virginia’s Division of Consolidated Laboratory Services was added to the project, following completion of internal validations. During this timeframe, the six laboratories sequenced a total of 2,514 isolates (1,483 DTBE samples, 1,031 jurisdictional samples).

2017–2018: All six laboratories continued to conduct TB WGS, with a total of 1,368 isolates (1,084 DTBE samples, 284 jurisdictional samples). During this period, New York implemented universal sequencing of all MTBC-positive cultures identified in-house. Thus, in addition to funding the sequencing of retrospective DTBE isolates, the TB WGS Project contributed to funding prospective sequencing in New York at a reduced rate of $100 per sample. This was done to eliminate redundant WGS with the agreement that New York would submit the WGS files for incorporation into the national surveillance data set.

2018–2019: California, Iowa, Michigan, New York and Ohio laboratories continued participation in the project. New York stopped retrospective DTBE sequencing but continued universal sequencing for jurisdictional samples. The other laboratories continued sequencing DTBE and jurisdictional samples, with a total of 1,840 isolates sequenced (1,224 DTBE samples, 616 jurisdictional samples).

From 2015 (pilot)–2019, the six participating laboratories sequenced a total of 6,712 MCTB isolates (Figure 1), which included 4,396 DTBE samples and 2,316 jurisdictional samples (including 811 WGS files from New York). The total project cost was $1,490,100 (Figure 2).

**Figure 1.** Total Number of MTBC Isolates Sequences per Year.
Note: Counts from New York beginning in 2017 included in-house prospective sequencing and the submission of raw WGS data to CDC for analysis.

**Figure 2.** Total Cost of the TB WGS Project per Year.

**REFERENCE CENTERS**

The California Department of Public Health sequenced 1,045 MTBC isolates (845 DTBE samples, 200 jurisdictional samples).

The public health laboratories in Iowa, Michigan and Ohio all participated in the project for four years, from 2015–2019. During that time, the University of Iowa’s State Hygienic Laboratory sequenced 935 isolates (923 DTBE samples, 12 jurisdictional samples); the Michigan Bureau of Laboratories 1,988 isolates (1,887 DTBE samples, 101 jurisdictional samples); and the Ohio Department of Health Laboratory 668 isolates (456 DTBE samples, 212 jurisdictional samples).

New York State’s Wadsworth Center also participated in the TB WGS project from 2015–2019. For the first two years, it sequenced jurisdictional isolates. In contract year 2017–2018, New York implemented universal, prospective WGS for TB isolates. It sequenced a total of 1,879 isolates (Contract for Reference Center TB WGS Work: 1,068 isolates; Contract for Prospective Universal TB Work: 811 isolates).

Virginia’s Division of Consolidated Laboratory Services participated in the project for only two years, from 2016–2018. During that time, the Virginia laboratory sequenced 197 isolates (195 DTBE samples, 2 jurisdictional samples).
CONCLUSIONS

Over the first five contract years of the TB WGS Project, the six reference centers sequenced a total of 6,712 MTBC isolates for which they were reimbursed $1,490,100. Of these isolates, 4,396 (65%) were retrospective DTBE isolates and 2,316 (35%) were jurisdictional isolates, including 811 New York universal sequencing isolates (Figure 3). Of the total cost, DTBE isolates accounted for 69%, jurisdictional isolates for 25% and New York’s Universal Sequencing for 6%.

SUCCESSES AND CHALLENGES

In 2015–2016, participating laboratories were asked to describe their major successes and challenges throughout the project.

Major TB WGS project successes included establishing routine and streamlined workflow and incorporating protocol improvements to increase efficiency and decrease testing turnaround time. New York noted turnaround time for WGS results fell from 28 days at the beginning of their participation in January 2016 to 13-15 days in June 2016. Compared to New York’s first line Drug Susceptibility Testing, WGS resulted in faster turnaround in 36 out of 46 cases by an average of 10 days. Many states reported that monthly conference calls, conducted between February and November 2016, fostered collaboration and idea exchange with other participating laboratories. One outcome of this exchange was protocol improvements; for example, states were able to reduce pipetting and extraction time, implement prioritization protocols and reduce the cost of DNA extraction.

Participating laboratories encountered multiple challenges over the course of the project. Protracted contractual processes (both on the legal and manufacturing fronts) and problems with supply orders and reagent pricing delayed project implementation. Instrument and reagent failures likewise slowed progress, as did issues with data storage and transfer. The latter issue was addressed at one laboratory with the addition of a new server; however, another reported problems uploading data to the CDC FTP site and one encountered a misunderstanding about whether 30X coverage was sufficient to meet testing requirements. Staffing and distribution of responsibilities was also an issue. One laboratory stated that it would need additional staffing and resources to support future sequencing, and all the laboratories requested more training and feedback on data quality and utilization.
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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