SARS-CoV-2 Sequencing Capacity in US Public Health Laboratories

2024 Survey Report

Background and Purpose

The COVID-19 pandemic saw the rapid implementation of large-scale genomic sequencing for SARS-CoV-2 in laboratories across the nation. As the public health response continues to wind down, the number of SARS-CoV-2 sequences being generated has decreased, but the need for sustained, representative, high quality genomic data remains.

The US Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) are working to develop recommendations that will inform the design of a SARS-CoV-2 surveillance network, which will assure sustained availability of necessary SARS-CoV-2 genomic data that will continue to guide public health action. The goal is to create a comprehensive approach to national SARS-CoV-2 genomic surveillance that produces timely, cost effective, quality data with adequate geographic coverage to support a robust surveillance system.

There is a wide variety of laboratory equipment and methods being utilized by public health laboratories (PHLs) to perform SARS-CoV-2 sequencing. There is also a diverse range of testing capacity across the laboratories. This survey aimed to assess the landscape of SARS-CoV-2 sequencing at PHLs in the US in order the help guide discussions regarding a national strategy for SARS-CoV-2 genomic surveillance. APHL utilized this data at a SARS-CoV-2 Sequencing Strategy Meeting that was held in April 2024 with CDC, APHL member laboratories and other partners.

Understanding the landscape of SARS-CoV-2 testing and resources in PHLs is important to help shape a guidance document for a National SARS-CoV-2 Surveillance Strategy. Laboratories have made significant investments in both equipment and staffing. Rather than mandating a single ideal method, the strategy will consider multiple platforms and workflows and implementing quality measures to ensure high-quality sequencing data.

Methodology

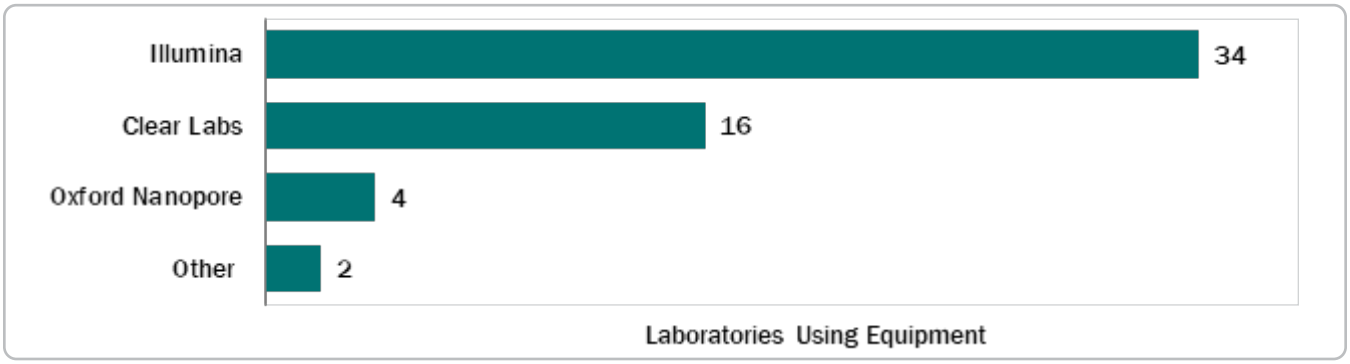
The 2024 SARS-CoV-2 Public Health Laboratory Sequencing Capacity Survey was launched in December 2023 via email to directors of APHL's member laboratories and closed in January 2024. A total of 112 laboratories—56 local and 56 state PHLs—were invited to participate, with a 38% (43/112) completion rate, with one local PHL responding that they did not perform SARS-CoV-2 sequencing. Of the PHLs that responded, 70% (30/43) were state PHLs and 30% (13/43) were local PHLs. The sample size for each topic varies based on the completeness of the answer to each question. If a response of "unknown" was given or the answer was incomplete, it was omitted from the results analysis. The survey was designed to get a snapshot of SARS-CoV-2 sequencing occurring in PHLs to help inform future strategies.

Results

SARS-CoV-2 Sequencing Equipment

All respondents were requested to report any sequencing equipment currently in use for SARS-CoV-2 testing, therefore we have a total of 56 responses from 42 PHLs (Figure 1). The majority of laboratories used Illumina (34/42, 81%), followed by Clear Labs (16/42, 38%) and Oxford Nanopore (4/42, 10%). Two PHLs (2/42, 5%) used other platforms (Aviti and Genexus). Laboratories were able to select as many platforms as they had in use. Of the 42 responding PHLs, 13 (31%) used two of these platforms, with nine of those 13 PHLs (21%) utilizing both Illumina and Clear Labs. One laboratory (1/42, 2%) reported having three platforms.

Figure 1. Sequencing Equipment Used to Sequence SARS-CoV-2 (n=42)

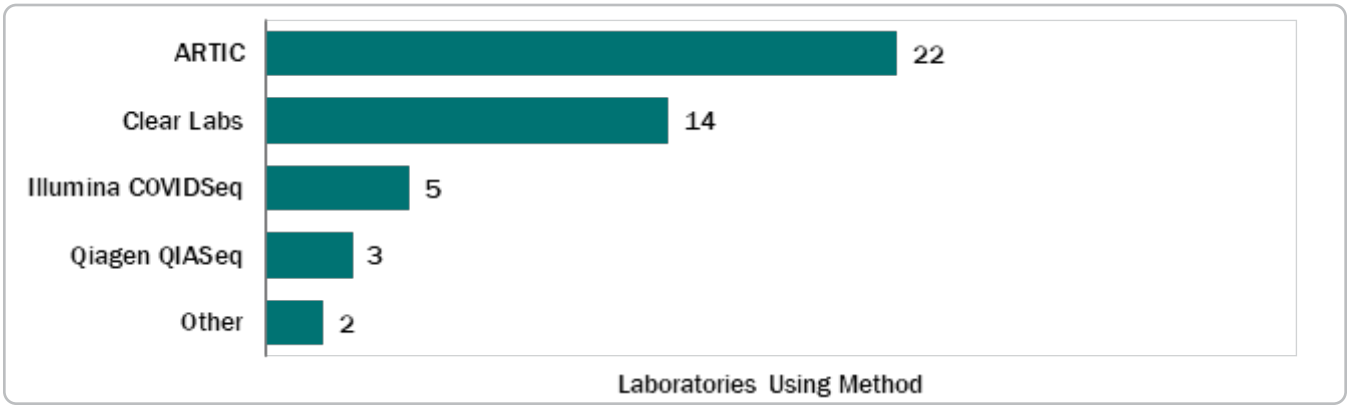


SARS-CoV-2 Sequencing Testing Methods and Protocols

To capture the diversity of practices being used to sequence SARS-CoV-2, respondents were given space to detail their specific methodologies—which could include more than one method—for a total of 46 responses from 39 PHLs. The answers were then categorized and summarized (Figure 2). All versions of ARTIC primers were categorized as ARTIC; however, if a laboratory used ARTIC on a Clear Labs instrument those responses were categorized as Clear Labs.

The majority of laboratories were using ARTIC primers and protocols (22/39, 56%), followed by Clear Labs (14/39, 36%), Illumina COVIDSeq (5/39, 13%) and Qiagen QIAseq (3/39, 7%). The “other” category (2/39, 5%) includes one laboratory using a laboratory developed test and one laboratory using Ampliseq Insite for Genexus. Seven PHLs were using two different methods, with four of the seven laboratories using ARTIC and Clear Labs.

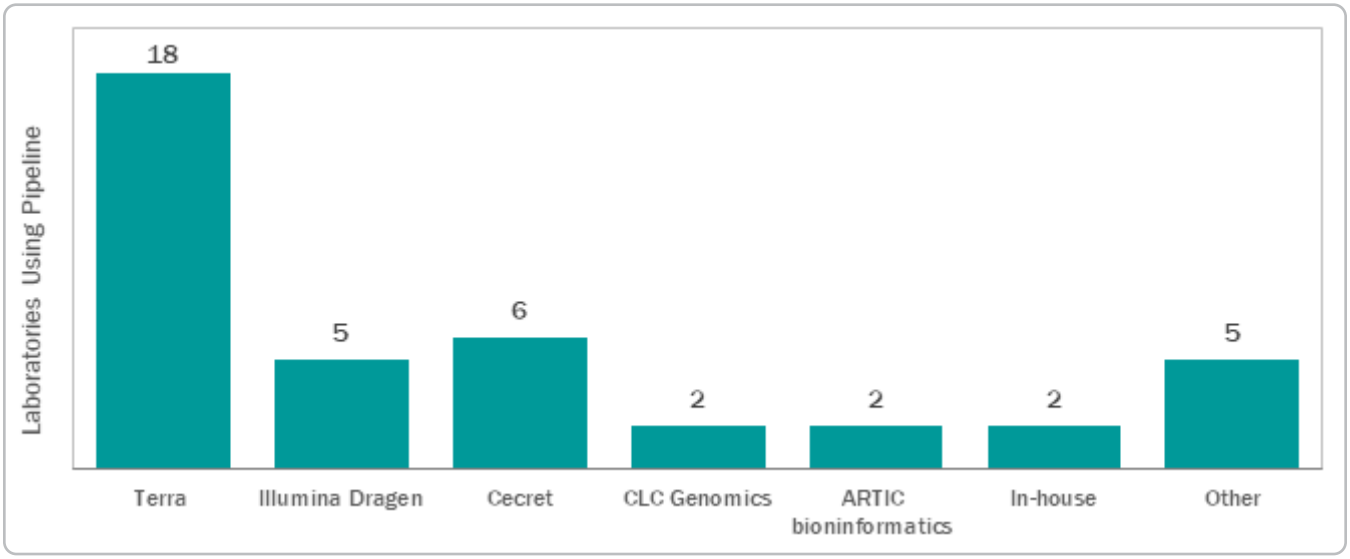
Figure 2. SARS-CoV-2 Methods (n=39)



SARS-CoV-2 Sequencing Analysis Pipelines

With several respondents using multiple analysis pipelines, 40 responses were captured from the 38 PHLs that completed this question (**Figure 3**). The majority of respondents were using Terra (18/38, 47%) followed by Cecret (6/38, 16%), Illumina Dragen (5/38, 13%), CLC Genomics (2/38, 5%), ARTIC Bioinformatics Pipeline 2/38, 5%), an in-house pipeline (2/38, 5%) or "Other" (5/38, 16%). The "Other" category consisted of one PHL using each of the following pipelines: Genexus, HiperGator, Freyja, Monroe and Viralrecon.

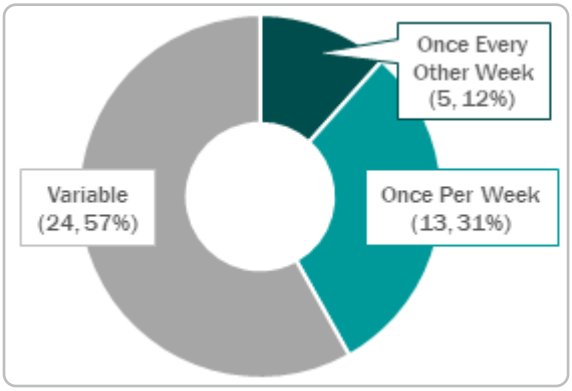
Figure 3. SARS-CoV-2 Pipelines (n=38)



Frequency of SARS-CoV-2 Testing

Of the 42 laboratories that responded to this question, the majority of laboratories (n=24, 57%) responded that SARS-CoV-2 testing frequency is variable—ranging from not testing at all to daily. Thirteen laboratories (31%) routinely perform testing once per week and five laboratories (12%) perform testing every other week (**Figure 4**). Respondents reported that the testing variability depended on availability of reagents, volume of positive specimens and preference to batch SARS-CoV-2 sequencing, as it is more efficient and less costly.

Figure 4. SARS-CoV-2 Sequencing Frequency (n=42)



Public Databases

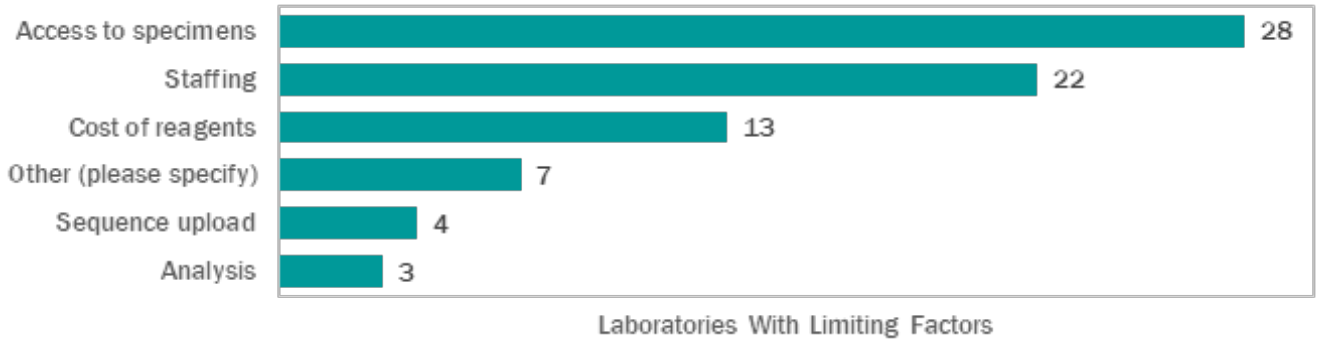
Nearly all respondents (40/42, 95%) routinely uploaded their sequences to public databases. Most laboratories were uploading to GISAID and NCBI GenBank, with a few also uploading to SRA. Sequences are typically uploaded after each run or within 48 hours, but some upload weekly (or monthly).

SARS-CoV-2 Sequence Generation and Capacity

The average number of SARS-CoV-2 sequences generated per week was 90 (minimum 0, maximum 600). However, there is a much larger capacity available in the PHL system to generate SARS-CoV-2 sequence data. The average weekly capacity for the responding PHLs was 468 sequences per week (minimum 0, maximum 3,000).

The biggest rate limiting factors to generating more SARS-CoV-2 sequences is access to specimens (28/42, 67%), followed by staffing (22/42, 52%), cost of reagents (13/42, 31%), sequence upload (4/42, 1%) and analysis (3/42, 1%) (**Figure 5**). Barriers specified in the "other" category (7/42, 17%) included instrument availability, laboratory space constraints, specimen quality issues, access to training and declining demand or interest in the data. Laboratories were able to select multiple factors.

Figure 5. **Rate Limiting Factors to Generating More SARS-CoV-2 Sequence Data (n=42)**



Discussion

The survey results reveal a diverse landscape of SARS-CoV-2 sequencing practices across the respondent PHLs. This diversity is reflected in the various combinations of equipment, methods, protocols and pipelines used, which has allowed laboratories to tailor their processes to their specific strengths and needs. However, it creates challenges for standardizing and optimizing national surveillance efforts.

The capacity for SARS-CoV-2 sequencing within PHLs exceeds the current utilization primarily due to limited number of specimen submissions. Efforts to address these limits by enhancing the respiratory surveillance network challenges is key to a successful national strategy.

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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