Optimization of COVID-19 NGS to meet demand and provide optimum results in accordance with CLIA regulations

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Background

During the SARS-CoV-2 pandemic, IDOHL was approached by the epidemiology team about expanding capabilities to include testing that would determine lineages circulating throughout Indiana. The request was for this expanding capability to be reportable to the submitter, requiring CLIA validation of the method. Sequencing capabilities using Illumina technologies (Figure 1) were already in place at IDOHL, so once the reference genome and ARTIC protocols were available, validation plans started. This process was labor-intensive. When IDOHL was approached about purchasing a Clear Labs Instrument (Figure 2) to automate the process, we gathered information and made plans to acquire the necessary funding. IDOHL purchased two Clear Labs instruments to increase our NGS capacity. Figure 3 shows the comparison between the two methodologies.

Figure 1: Illumina MiSeq Instrument

Figure 2: Clear Dx Instrument

Materials and Methods

Validation of the NGS using Illumina technology and Qiagen primers and kits was completed with assistance from Wisconsin State Laboratory of Hygiene (WSLH) and the Centers for Disease Control and Prevention (CDC). Validation consisted of 15 specimens tested at IDOHL and WSLH as well as 14 files retrieved from National Center for Biotechnology Information (NCBI) used for analysis verification. This covered seven different lineages for analysis and four for the lab based on what was available at the time.

Validation of the Clear Lab instrument consisted of 13 specimens consisting of 12 lineages tested at IDOHL, two ATCC control strains, and 17 synthetic SARS-CoV-2 RNA controls representing 10 lineages. A dilution curve was used to determine the starting dilution for the synthetic controls.

Results

IDOHL re-evaluated the cycle threshold (Ct) cut-off for SARS-CoV-2 positive specimens to be sequenced due to an observed issue establishing lineages for specimens with Ct values above 30. A decision to make the Ct cut-off 28 for positives to be sequenced was made to reduce errors in variant calling.

Synthetic controls were produced by Twist Biosciences and included published lineages for each control. These published lineages were downloaded for comparison with our analysis process. IDOHL found a discrepancy with three of the synthetic controls which was confirmed with additional testing. IDOHL conducted further research and started discussion with Twist Biosciences about potential causes of the discrepancy.

The research and discussion revealed poor ARTIC primer binding due to the production method of one Twist control. Additional research on the other two discrepancies led to a discovery by Twist Bioscience of a labeling issue with two of the synthetic controls, which were later recalled. IDOHL worked closely with Twist Biosciences to identify the issue that led to the voluntary recall in June 2021.

Manual vs. Automated Sequencing

	Manual Sequencing	Automated sequencing
Procedure		
Method(s) utilized	Qiagen and Illumina	Clear Labs Clear DX™
Extraction Method	Automated (Qiagen QIAcube HT)	Automated (KingFisher Flex)
cDNA Preparation	Manual (Qiagen Primer)	Automated (Hamilton STAR)
Libraray Prepartion	Manual (Ilumina Library Prep)	Automated (Hamilton STAR)
Sequencer Type	Illumina MiSeq	Oxford Nanopore MinION (2)
Sequencer Technology	Short read	Long read
Time		
Hands-on Time	8 hours	< 1 hour
cDNA and Library Prep Time	2 days	< 1 day
Run time on Sequencer(s)	≈20 hours	≈9 hours
Total Sequencing Time	4 days	1 day
Other information		
Samples per run	48	32
Staff required	3 or 4	1 or 2
Total samples ran as of 4/1/2022	≈1300	≈4400
Validation Completion Date	3/10/2021	6/7/2021
Cost per sample	$172	$109

Figure 3: Comparison of Manual vs Automated processes for NGS

Conclusions

IDOHL validated two different methods under CLIA guidelines so that SARS-CoV-2 lineages could be reported to submitters as well as epidemiologist partners. The Illumina MiSeq instruments using Qiagen's QIASeq protocol were validated first, followed by the Clear Labs instrument to allow for a higher throughput without the strain on staff resources the Qiagen/Illumina protocol demanded. This allowed for a higher capacity in testing and the reporting out of variants of concern in a timely manner (Figure 4).

The validation of these methods also exposed an issue with the synthetic controls for SARS-CoV-2 that had not been previously identified. Overall, IDOHL was able to meet the demands of both submitters and epidemiology with methods that were validated, and we felt comfortable utilizing.

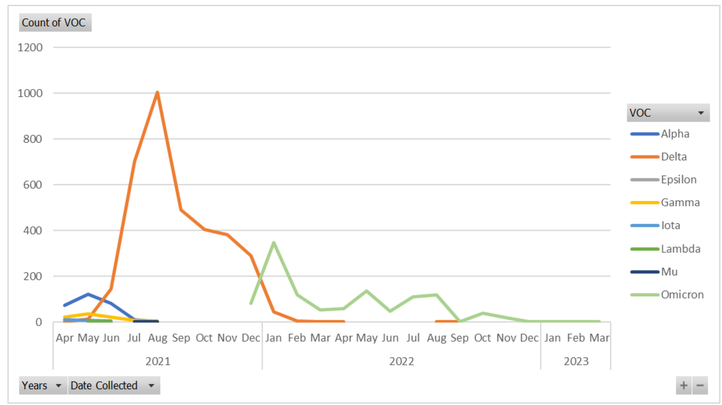

Figure 4: Timeline of Variants of concern sequenced at IDOHL

Acknowledgments

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