Tackling the COVID-19 Pandemic by Utilizing Next Generation Sequencing Technologies

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ABSTRACT

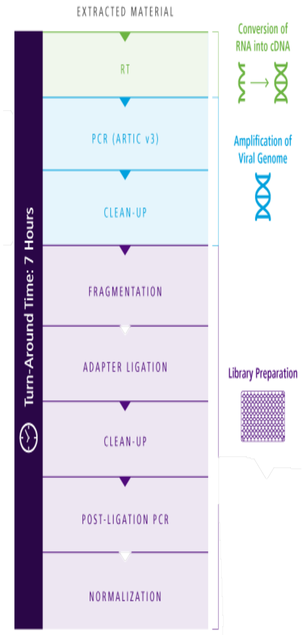
The COVID-19 outbreak caused by SARS-CoV-2 has led to major socio-economic disruption worldwide and the pandemic has yet to recede after more than 18 months. As of November 2021, SARS-CoV-2 has infected over 251 million people and caused over 5 million deaths worldwide1. The discovery of aggressive variants of the virus has shown that monitoring for mutations associated with changes to infection outcome and transmission of SARS-CoV-2 is critical to ensure the success of vaccination programs and establish robust public health responses. A sample identified positive for COVID-19 using an RT-PCR assay can then be sequenced to determine if this sample has a SARS-CoV-2 Variant of Concern (VOC). Next Generation Sequencing (NGS) is ideal for this as the high resolution enables scientists to identify all mutations, known and unknown, providing insights to SARS-CoV-2 viral evolution.

Our laboratory is one of the largest COVID-19 testing laboratories in the US, which was set up at the apex of the largest outbreak of SARS-CoV-2 infections in the state of California. The laboratory was set up in a total of 8 weeks which included obtaining reagent supply, staffing, and operating a high-throughput, large scale SARS-CoV-2 testing facility capable of processing 150,000 samples per day. To date, our laboratory has tested over 8 million patient samples for COVID-19 since its inception in November 2020 with a total positivity rate of 8.07% at this reporting. Due to the ability of the laboratory to process SARS-CoV-2 patient samples at such a high-throughput with a turnaround time averaging 12.2 hours per sample, we quickly utilized our ability to rapidly mobilize the lab's skilled scientists to efficiently implement NGS at the lab. We exercised the collaborative efforts of the lab team along with experts from multiple other divisions including R&D, Genomics, and Business to build and test a research use only NGS sequencing kit that utilizes robust NGS reagents in conjunction with a community designed Artic Primer set2. Discussion in the lab about implementing NGS took place in February 2021 and by March 2021, we were fully operational and had begun sequencing these positive samples for epidemiological purposes. A streamlined process was established within four weeks using robotic automation as well as training an entry-level staff running 4 shifts for a 24/7 lab operation. The laboratory utilizes two Illumina platforms, the NovaSeq and MiSeq systems, to process high-throughput and high priority samples.

At this reporting, more than 115,000 samples have been sequenced using the NGS kit to determine the populations of VOCs present in California. This is broken up into two workflows: routine high-throughput, automated processing on the NovaSeq with a turnaround time of 7 days, and high-priority samples using a manual processing method on the MiSeq with a turnaround time of 48 hours. The lab can process up to 24 high priority samples daily and an average of 5000 routine patient samples per week. SARS-CoV-2 patient samples measured by qPCR have a cycle threshold (Ct) cutoff value that determines the presence and amount of virus detected in a sample. Of these samples approximately 35% (~39,200) have been weakly detected with a Ct value greater than 33. Samples with high Ct values indicative of lower viral titer traditionally do not meet the requirements for sequencing set forth in other laboratories. Lower viral titer samples have lower nucleic acid concentration which causes under clustering, leading to flowcell failure and, more importantly, inconclusive patient results. Due to our high-throughput automated NGS workflow plus the robust NGS reagents in conjunction with a community designed Artic Primer set, our laboratory was able to successfully sequence 40% of these high Ct value samples which would have been rejected elsewhere.

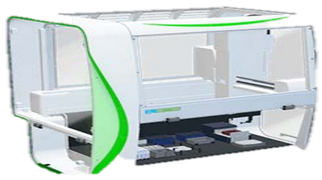
SARS-CoV-2 PCR Kit, NGS Kit and Automation

Patient samples are tested using the Revvity New Coronavirus Nucleic Acid Detection Kit. This kit utilizes Real Time PCR for the detection of nucleic acid from the SARS-CoV-2 virus. Once a sample is reported as positive, the laboratory can begin NGS library preparation for epidemiological tracking.



The NEXTFLEX® Variant-Seq™ SARS-CoV-2 kits offer 1,536 unique dual index (UDI) barcodes, ideally suited to ultra high-throughput multiplexing on the Illumina NovaSeq sequencer or other Illumina platform. By utilizing all 1536 UDI barcodes, the laboratory was able to fully utilize an S4 flowcell to its capacity.

The proprietary chemistry used in the NEXTFLEX® Variant-Seq™ SARS-CoV-2 kits allows for 96 well manual library preparation to be completed in as little as 7 hours.



Our laboratory adopted the Revvity JANUS G3 MDT™ Automated Workstation to fully automate the library preparation workflow. By utilizing the JANUS G3 MDT the laboratory was able to reduce library preparation to just under 5 hours.

SARS-CoV-2 and Sequencing Data

Figure 1. Number of SARS-CoV-2 Samples Tested to Date

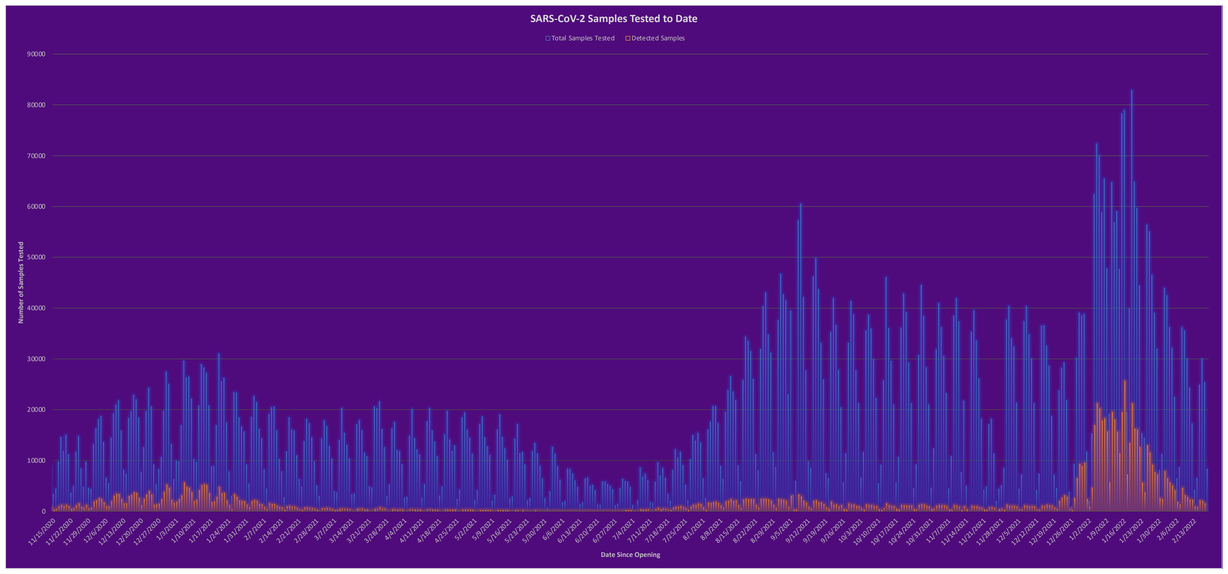


Figure 1. illustrates the number of samples tested at our lab by date. The figures in blue represent the total number of samples tested and the figures in orange represent the total number of detected samples.

Figure 2. Number of Detected Samples Sequenced by Date

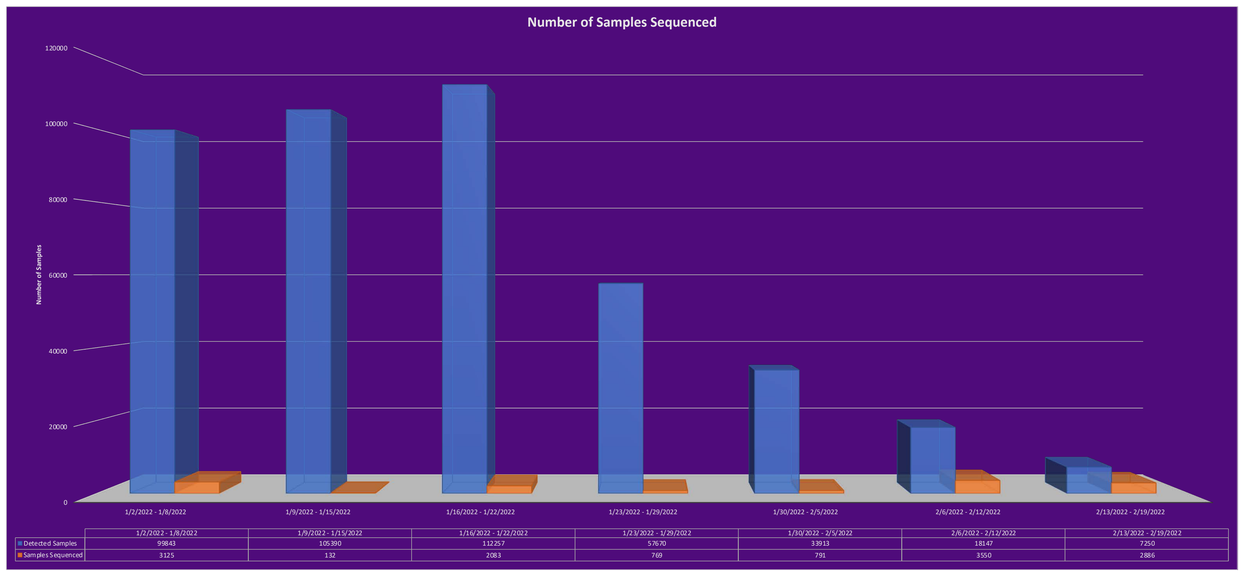


Figure 2. represents the number of detected samples sequenced by date. The figures in blue represent the number of detected samples reported and the figures in orange represents the number of samples sequenced by date.

CONCLUSION

The Revvity New Coronavirus Nucleic Acid Detection Kit has one of the highest sensitivity on the market. Due to the sensitivity of the kit and the laboratory's unique sequencing method enables the state of California to closely monitor and track emerging VOCs within the state. We have traced the increase of pathogenic variants such as Delta as it first appeared in California which, was circulating in the population at 85% of all VOCs detected at its peak. The laboratory is currently tracking the Omicron and all subsequential VOCs in real time. This epidemiological information is a crucial tool in fighting the COVID-19 pandemic, providing relevant data to authorities and researchers to understand SARS-CoV-2 infection and transmission. The success and accomplishments made by the laboratory are due in large part to the excellent design of the kit along with dedicated staff, scientists and support from state of California.

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