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LETTER TO THE EDITOR

Implementation of a Streamlined SARS-CoV-2 Whole-Genome Sequencing Assay for Expeditious Surveillance during the Emergence of the Omicron Variant

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Next-generation sequencing technology (NGS) has rapidly advanced and emerged from research applications to find growing adoption by clinical laboratories for use in patient care. With the emergence of variants, there is growing interest for SARS-CoV-2 genotyping by whole-genome sequencing (WGS) in clinical care as it enables detection of mutations that confer resistance to antiviral and monoclonal antibody therapies (1). Furthermore, we and others have demonstrated its use for infection control investigations (2–4). Despite the clinical utility of NGS assays, adoption by clinical laboratories has been limited, largely due to the molecular skills required and sufficient bioinformatics expertise needed for sequencing data analysis (5).

During our transition from a SARS-CoV-2 genotyping research endeavor to a clinical assay with reporting in the electronic medical record (EMR), we evaluated a WGS methodology using the AmpliSeq SARS-CoV-2 Insight Research Assay on Ion Torrent Genexus system (Thermo Fisher Scientific; Waltham, MA) that allowed for on-board library preparation and sequencing. This platform significantly reduces the amount of hands-on time and training needed. We compared the performance of the Ampliseq assay to generate high quality sequence data and SARS-CoV-2 variant calls against CleanPlex SARS-CoV-2 Panel (Paragon Genomics; Hayward, CA) on Illumina MiSeq. The CleanPlex and Ampliseq amplicon based WGS assays utilize 343 and 237 overlapping amplicons that span the entire SARS-CoV-2 genome, respectively (6, 7). Samples yielding sequence data that met the APHL recommended minimum criteria for submission to GISAID of >90% target base coverage and average coverage depth ≥10× were included in this study (8). Further, to ensure high confidence in lineage calls for a clinical assay we set the following thresholds for sequencing quality: average coverage depth >1,000×, base reads on-target >90%, and target base coverage at 100× >98%. A total of 75 samples met the APHL recommended criteria; of these, 91% (68/75) and 75% (56/75) of samples met our more stringent QC thresholds for the Ampliseq and CleanPlex assays, respectively (Table 1). This demonstrates that the Ampliseq assay was not inferior to the CleanPlex assay in its ability to generate high quality data. Since variants were included from different phases of the pandemic, the original CleanPlex samples had lineage calls performed at different time points using different versions of the Pangolin COVID-19 Lineage Assigner. To avoid this source of analysis variability, consensus sequences generated by both methods were analyzed using the same version of Pangolin (version 3.1.19, lineages version 2022-01-20). Lineage calls generated by the two assays were considered concordant if the same variant classification scheme defined by the SARS-CoV-2 Interagency Group (SIG) were identified (9). The lineage calls were also considered concordant when a nonvariant was identified by both methods. The two methodologies were highly concordant at 94.7% (71/75) and 98.2%

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TABLE 1 Concordance between Ion AmpliSeq SARS-CoV-2 and reference method

Specimen number	Ct value	Paragon Genomics CleanPlex[a]	Ion AmpliSeq SARS-CoV-2	Avg base coverage depth	Base reads on target	Target base coverage at 100×	Concordance
1	29.38	B.1.2	B.1.2	3,165	99.58%	99.83%	Yes
2	27.38	B.1.427	B.1.427	3,418	99.76%	99.87%	Yes
3	26.57	B.1.240	B.1.240	4,114	99.81%	99.91%	Yes
4	25.6	B.1.1	B.1.1	4,060	99.94%	99.84%	Yes
5	27.87	B.1.429	B.1.429	3,619	99.75%	99.68%	Yes
6	24.27	B.1.1	B.1.1	4,809	99.45%	99.65%	Yes
7	23.88	B.1.1.7	B.1.1.7	4,189	99.89%	99.71%	Yes
8	22.83	P.1.17	P.1.17	4,374	99.91%	99.65%	Yes
9	27.33	AY.20	AY.20	3,834	99.55%	99.32%	Yes
10	29.88	AY.103	AY.103	1,875	99.81%	99.57%	Yes
11	28.49	AY.100	AY.100	2,431	99.43%	98.70%	Yes
12	29.11	AY.26	AY.26	2,248	98.54%	99.41%	Yes
13	31.82	B.1.2	B.1.2	2,631	98.52%	99.82%	Yes
14	31.27	B.1.2	B.1.2	994	96.61%	98.36%	Yes
15	30.34	B.1.429	B.1.429	9,205	98.23%	99.78%	Yes
16	30.17[c]	B.1.429	B.1.429	623	94.90%	95.47%	Yes
17	31.45	B.1.429	B.1.361	4,654	99.69%	99.87%	No
18	30.8	None	B.1.429	2,090	97.93%	99.73%	No
19	30.34	B.1.427	B.1.429	454	78.76%	88.26%	Yes[b]
20	18.87	B.1.427	B.1.427	7,644	99.93%	99.87%	Yes
21	25.55	B.1.427	B.1.427	2,168	99.08%	99.70%	Yes
22	31.36[c]	B	B.1.429	3,340	91.18%	99.57%	No
23	28.5[c]	B.1.427	B.1.429	1,690	93.30%	99.05%	Yes[b]
24	15	B.1.526	B.1.526	1,949	99.95%	99.68%	Yes
25	20.64	P.1	P.1	3,692	99.96%	99.67%	Yes
26	27.04	AY.44	AY.44	2,725	99.93%	99.76%	Yes
27	10.82	BB.2	BB.2	8,006	99.95%	99.57%	Yes
28	18.57	P.1	P.1	10,070	99.94%	99.87%	Yes
29	28.11	B.1.241	B.1.241	1,949	99.87%	99.68%	Yes
30	26.81[c]	B.1.1.239	B.1.1.239	498	94.46%	96.82%	Yes
31	15.1	B.1.1.291	B.1.1.291	9,327	99.93%	99.82%	Yes
32	16.62	B.1.526	B.1.526	8,781	99.95%	99.83%	Yes
33	21.33	B.1.1.7	B.1.1.7	2,633	99.04%	99.23%	Yes
34	29.75[c]	B	B.1.429	327	79.92%	90.43%	No
35	14.45	B.1.1.7	B.1.1.7	2,998	99.80%	99.68%	Yes
36	19.37	P.1	P.1	5,640	99.77%	99.62%	Yes
37	20.98	P.1.17	P.1.17	6,679	99.79%	99.83%	Yes
38	24.9	AY.103	AY.103	2,730	98.69%	99.28%	Yes
39	19.81	AY.119	AY.119	3,383	99.56%	98.81%	Yes
40	16.67	AY.116.1	AY.116.1	6,547	99.76%	99.82%	Yes
41	17.77	B.1.637	B.1.637	4,703	99.70%	99.77%	Yes
42	18.34	B.1.526	B.1.526	3,220	99.78%	99.59%	Yes
43	20.18	B.1.1.7	B.1.1.7	3,749	99.80%	99.97%	Yes
44	19.59	B.1.1.7	B.1.1.7	4,018	99.79%	99.83%	Yes
45	27.37	B.1.421	B.1.421	2,039	98.76%	99.66%	Yes
46	24.48	B.1.421	B.1.421	3,585	99.73%	99.40%	Yes
47	19.75	B.1.421	B.1.421	5,402	99.79%	99.80%	Yes
48	14.42	B.1.421	B.1.421	10,677	99.75%	99.91%	Yes
49	28.81	B.1.404	B.1.404	1,315	96.18%	99.62%	Yes
50	27.86	B.1.404	B.1.404	4,447	99.80%	99.54%	Yes
51	28.03	B.1	B.1.438.4	1,136	95.45%	98.43%	Yes[b]
52	27.03	B.1.399	B.1.399	4,699	99.76%	98.96%	Yes
53	17.42	B.1.399	B.1.399	20,056	99.96%	99.09%	Yes
54	14.77	B.1.399	B.1.399	5,681	99.96%	99.74%	Yes
55	25.64	B	B.1.324	1,016	98.23%	99.37%	Yes[b]
56	25.53	B	B.1.609	2,834	97.45%	99.37%	Yes[b]
57	18.71	B.1.324	B.1.324	8,486	99.96%	99.84%	Yes
58	14.06	B.1.298	B.1.298	4,901	99.97%	100%	Yes
59	19.54	B.1	B.1.265	6,085	99.94%	99.44%	Yes[b]
60	25.76	B.1.232	B.1.232	3,925	99.77%	99.13%	Yes
61	24.19	B.1.232	B.1.232	3,213	97.54%	99.39%	Yes

(Continued on next page)

TABLE 1 (Continued)

Specimen number	Ct value	Paragon Genomics CleanPlex[a]	Ion AmpliSeq SARS-CoV-2	Avg base coverage depth	Base reads on target	Target base coverage at 100×	Concordance
62	25.9	B.1.126	B.1.126	3,699	99.78%	98.30%	Yes
63	16.35	B.1.429	B.1.429	9,198	99.97%	99.82%	Yes
64	23.84	B.1.1.7	B.1.1.7	6,214	99.95%	99.98%	Yes
65	27.82	B.1.1.228	B.1.1.228	2,319	99.87%	99.02%	Yes
66	29.39	AY.54	AY.54	2,607	99.77%	99.44%	Yes
67	21.89	BB.2	BB.2	10,089	99.89%	99.78%	Yes
68	21.34	B.1.526	B.1.526	10,482	99.97%	99.73%	Yes
69	26.24	B.1.126	B.1.126	6,545	99.71%	98.51%	Yes
70	13.94	AY.103	AY.103	5,381	99.96%	99.78%	Yes
71	14.06	P.1	P.1	8,572	99.95%	99.93%	Yes
72	17.4	B.1.1.7	B.1.1.7	6,248	99.95%	99.99%	Yes
73	14.02	B.1.1.7	B.1.1.7	17,717	99.94%	100%	Yes
74	16.35	B.1.429	B.1.429	4,951	99.95%	99.79%	Yes
75	16.35	B.1.429	B.1.429	19,243	99.94%	99.96%	Yes

[a]Underlined CleanPlex lineage calls did not pass more stringent QC thresholds.
[b]Lineage calling was interpreted as concordant when Pango nomenclatures (e.g., B.1.617.2, AY.X) classified as the same variant based on SIG classification (i.e., Delta) or both Pango nomenclatures are considered a nonvariant that will only be reported out as nonvariant.
[c]Samples did not meet QC cutoffs on first attempt. These samples were repeated and pooled with other low titer samples. Low titer was considered Ct >25.5 or approximately 1,000 copies/mL. QC metrics were improved and lineage calls were unchanged. Results are from low titer pooled repeat testing run.

(55/56) when comparing lineage calls of samples that met APHL recommended criteria and our more stringent QC criteria, respectively (Table 1). We were able to accurately detect multiple variants including alpha, epsilon, and delta. The discordant result from samples that met the more stringent QC threshold had 2-fold greater average coverage depth using the Ampliseq assay at 4,654×, compared to 1,800× for the CleanPlex.

Following implementation, all clinical specimens positive for SARS-CoV-2 by RT-PCR with sufficient viral titer (cycle threshold (Ct) <30) are automatically reflexed to WGS. Samples were batched into full runs of 14 patient samples plus 2 controls, twice weekly, to allow for turnaround time (TAT) range of 24–96 h. While the Ampliseq assay has about half the throughput of the CleanPlex assay on a weekly basis, it requires less than a tenth of the hands-on time (Table 2). Weekly testing throughput quickly expanded as we transitioned to Monday–Friday testing once a case of S-gene dropout detected by the TaqPath COVID-19 RT-PCR assay suspicious for the omicron variant (lineage B.1.1.529) was identified in December 2021. The automated features of the Ion Torrent Genexus system allowed us to quickly reflex to SARS-CoV-2 WGS. Within 30 h of initial SARS-CoV-2 detection by RT-PCR, the omicron variant was reported in the EMR. The analysis was significantly simplified through software plugins, which automatically list mutations and produce SARS-CoV-2 variant calls (Table 2). The time to genotyping result was approximately 44 h from the time specimen was received by the laboratory. Such rapid TATs can facilitate clinically actionable results, enabling critical outbreak investigations and therapeutic management within days rather than weeks. Since implementation, the genomic epidemiology has rapidly transitioned to omicron predominance (Fig. 1).

The system currently has some limitations. The throughput is only 16 samples per run for this assay, including any controls. There is potential for reagent waste if processing less

TABLE 2 Logistical differences between assays

Category	Ampliseq assay	CleanPlex assay
Platform	Ion Torrent Genexus	Illumina MiSeq
Library prep	Automated	Manual
Wet lab hands-on time	10–20 mins	245 mins
Analysis hands-on time	Fully automated	95 mins
Cost per test[a]	$$$	$$
TAT[b]	1 run/24 hrs	1 run/96 hrs
Throughput	14 samples/24 hrs	144 samples/wk

[a]Includes costs for reagents and consumables only.
[b]Estimated without overnight or weekend staffing.

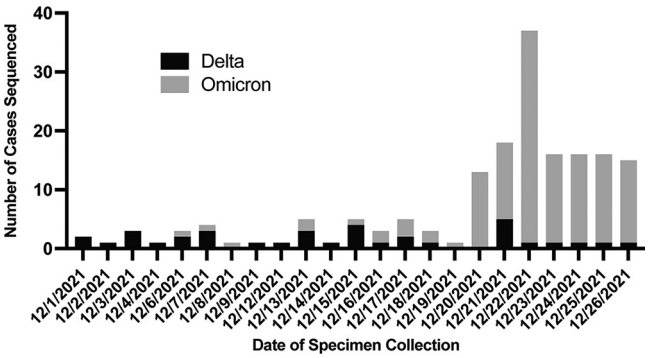

FIG 1 Number of cases of delta and omicron variants that were sequenced at our institution during the month of December 2021. Specimens included were positive by RT-PCR and had sufficient viral titer for sequencing (Ct <30) (n = 171).

than 16 samples per run, as one of four lanes of the Genexus chip is consumed for each automated run. When the omicron case was discovered, the throughput was sufficient as SARS-CoV-2 positivity at our institution was 5.47%, but as case positivity increases, the resulting backlog could increase TATs or require prioritization of samples. On the other hand, the low throughput may be more beneficial in some laboratories. There is also the flexibility to increase throughput by switching to manual library prep. While this does increase the wet lab hands-on time, it still takes advantage of the automated analysis. Sequencing data quality is reduced when loading mixed viral titer samples onto the instrument in the same run but can be improved by batching samples of similar titers together.

Overall, this methodology provides a significantly simplified workflow that makes it possible to generate real-time in-house WGS results and allows for a routine clinical laboratory to pursue genomic surveillance. These technological advances could have lasting benefit by offering clinical microbiology laboratories realistic tools to explore other infectious diseases NGS assays, from genotyping other viruses for the purposes of detecting resistance markers to inform antiviral therapy to identification of bacterial and fungal pathogens from clinical specimens.

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