

SARS-CoV-2 research

Evaluation of SARS-CoV-2 sequencing performance using raw and stabilized saliva samples

Introduction and background

New research on viral detection, life cycles, and ancestry paves the way for scientific understanding and discoveries in infectious diseases. Screening populations for the existence and lineages of novel viruses can lead to vital discoveries pertaining to the life cycles of viruses within the community. Novel respiratory viruses have been studied through screening mechanisms within populations by cell culture techniques followed by PCR, which can take days to weeks before results are obtained. Next-generation sequencing (NGS) gives researchers an opportunity to sequence viral genomes with high-throughput sample processing and rapid turnaround time by enabling semi-automated or fully automated workflows from library preparation to variant analysis. With the Ion AmpliSeq™ SARS-CoV-2 Insight

Research Assay, full-genome SARS-CoV-2 sequencing, from sample to variant report, can be achieved in as little as 24 hours.

Saliva is used in evaluating human ancestry by NGS, but it has not been widely studied as a sample type for NGS in virology. With Thermo Scientific™ SpecIMAX™ collection kits, saliva can be easily and accurately collected and stored for viral applications. In this application note we explore the effectiveness of using raw or stabilized saliva as a sample type, for extraction of SARS-CoV-2 RNA followed by full-genome sequencing with the Ion AmpliSeq SARS-CoV-2 Insight Research Assay (Figure 1).

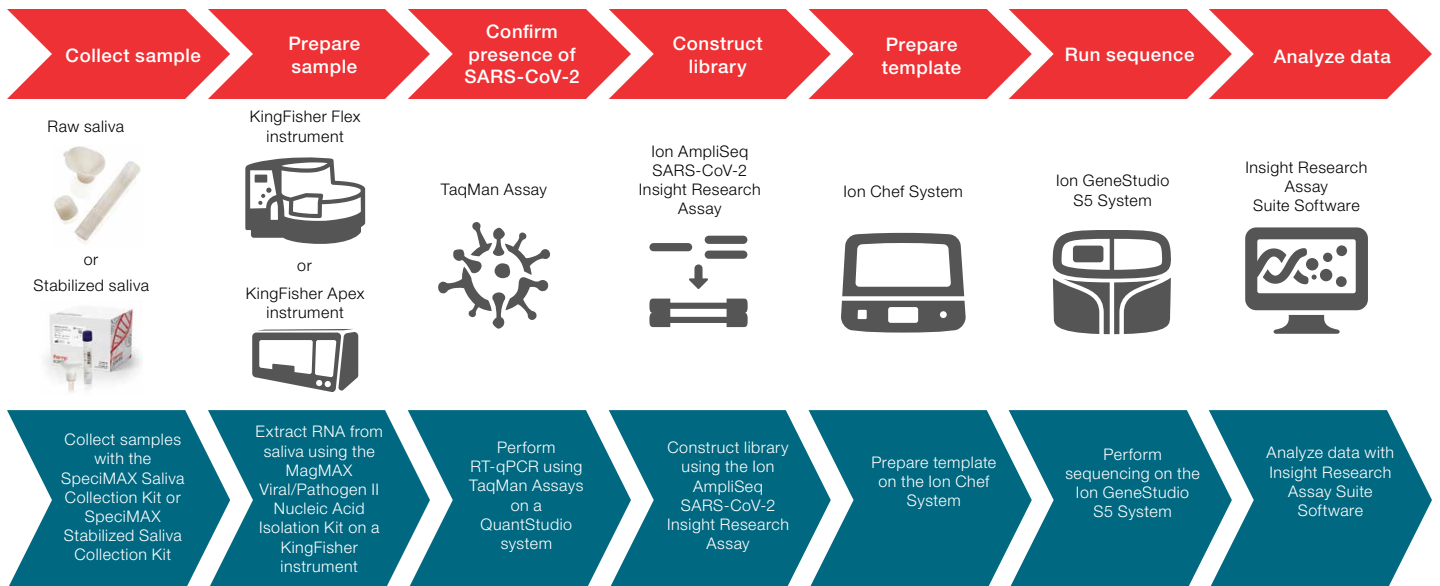


Figure 1. Overview of workflow from sample collection to variant analysis.

Experimental overview

Using SpecIMAX collection kits for raw and stabilized saliva, specimens were collected from 10 healthy human donors and spiked with inactivated SARS-CoV-2 (BEI Resources) at 12,500 copies/mL to evaluate the performance of matched raw and stabilized saliva specimens. An additional 10 samples known to contain SARS-CoV-2 by natural infection were collected with the SpecIMAX Stabilized Saliva Collection Kit as well as Device S, an on-market saliva collection kit that stabilizes RNA and DNA within saliva. All contrived and naturally occurring saliva samples were processed using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit, using the saliva workflow with 200 µL sample input on the Thermo Scientific™ KingFisher™ Flex Purification System. A negative extraction control, prepared with PBS and no viral material, was included in the extraction workflow. Eluates containing extracted RNA were first tested for the presence of the SARS-CoV-2 N gene using Applied Biosystems™ TaqMan™ Assays on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, and then used in the Ion AmpliSeq SARS-CoV-2 Insight Research Assay workflow with manual library preparation. The Ion AmpliSeq SARS-CoV-2 Insight Research Assay is a multiplex PCR-based method that produces sequencing-ready amplicons covering >99% of the SARS-CoV-2 genome. Templates were prepared on the Ion Chef™ System and then sequenced using Ion 540™ chips on the Ion GeneStudio™ S5 System.

Results and discussion

Matched samples were collected with the SpecIMAX Stabilized Saliva Collection Kit and Device S from donors with suspected SARS-CoV-2 infection. Samples were processed using the MagMAX Viral/Pathogen II kit on the KingFisher Flex system and evaluated by RT-qPCR. C_q values ranged from about 27 to 40, depending on the donor (Figure 2A). Contrived samples from raw and stabilized saliva collection kits were similarly processed using the MagMAX Viral/Pathogen II kit on the KingFisher Flex system. Eluates were evaluated by RT-qPCR, and a C_q of approximately 26 was observed across all contrived samples (Figure 2B).

A

Sample name	C_q (N gene)	
	SpeciMAX Stabilized Saliva Collection Kit	Device S
Positive 1	40.0	40.0
Positive 2	28.0	30.2
Positive 3	30.8	31.0
Positive 4	31.0	29.9
Positive 5	27.2	29.0

B

Sample name	C_q (N gene)	
	SpeciMAX Saliva Collection Kit	SpeciMAX Stabilized Saliva Collection Kit
Donor 11	26	26
Donor 12	26	26
Donor 13	26	26
Donor 14	26	26
Donor 15	26	26
Donor 16	26	26
Donor 17	26	26
Donor 18	26	26
Donor 19	26	26
Donor 20	26	26

Figure 2. Viral presence within extracted samples. The N gene target was detected in saliva specimen extracts using TaqMan Assays. **(A)** Donor samples with suspected SARS-CoV-2 infection, collected with the SpecIMAX kit and Device S. **(B)** Healthy donor samples (raw and stabilized) collected with SpecIMAX collection kits and spiked with inactivated SARS-CoV-2.

After N gene detection by RT-qPCR, the RNA extracts were prepared for sequencing using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay workflow to generate barcoded sample libraries for sequencing with an Ion 540 chip on the Ion GeneStudio S5 System. Read length histograms revealed similar profiles across all of the contrived raw and stabilized samples. Contrived samples prepared from stabilized saliva showed cleaner histograms compared to matched contrived

samples prepared from raw saliva (Figure 3A). Mean read length was compared across all contrived samples and used to evaluate read length across raw and stabilized samples. Figure 3B indicates variation in mean read lengths across the 10 spiked contrived samples after collection with raw and stabilized saliva collection kits. The mean read lengths show no trend from raw to stabilized samples.

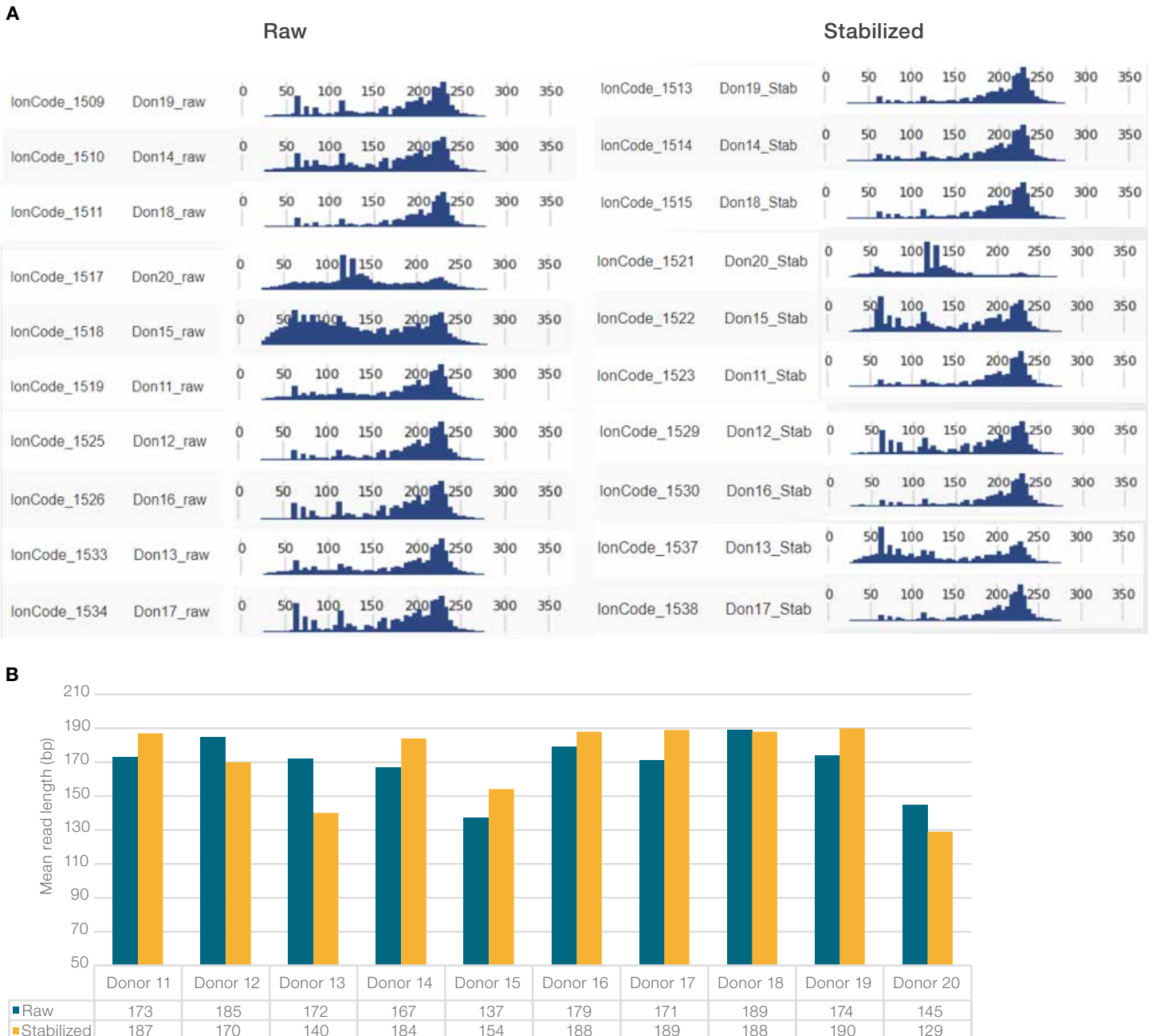


Figure 3. Sequencing data for matched raw and stabilized saliva collected in SpecIMAX devices and spiked with inactivated SARS-CoV-2. Data for histograms (A) and mean read lengths (B) were obtained from sequencing on an Ion 540 chip using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay.

Panel base uniformity and amplicon mean read depth with contrived samples prepared after collection with SpecIMAX raw and stabilized saliva kits were analyzed by the software (Figure 4). A spiked virus control in PBS with no human RNA background showed very high uniformity and mean read depth similar to or better than a spiked library control at 100 and 300 copies (Thermo Scientific™ AcroMetrix™ Coronavirus 2019 RNA Control). Stabilized saliva yielded higher base uniformity and mean read depth across the 10 specimens collected from healthy donors.

A

Base uniformity

Donor	SpeciMAX Saliva Collection Kit	SpeciMAX Stabilized Saliva Collection Kit
11	97.96%	98.84%
12	98.94%	97.67%
13	98.97%	96.33%
14	97.97%	99.36%
15	97.63%	98.52%
16	98.47%	99.19%
17	97.90%	99.19%
18	98.45%	99.63%
19	97.77%	99.42%
20	96.84%	95.12%
Controls		
Spiked virus in PBS	99.25%	NA
Spiked library, 100 cp	99.07%	NA
Spiked library, 300 cp	98.04%	NA



Figure 4. Base uniformity (A) and mean read depths (B) for 10 contrived samples collected using SpecIMAX raw and stabilized saliva collection kits. cp: copies.

Suspected infected samples collected with the SpecIMAX Stabilized Saliva Collection Kit and Device S were also evaluated using read length histograms and mean read length data. Overall, the SpecIMAX kit had cleaner read length histograms and longer mean read lengths (Figure 5).

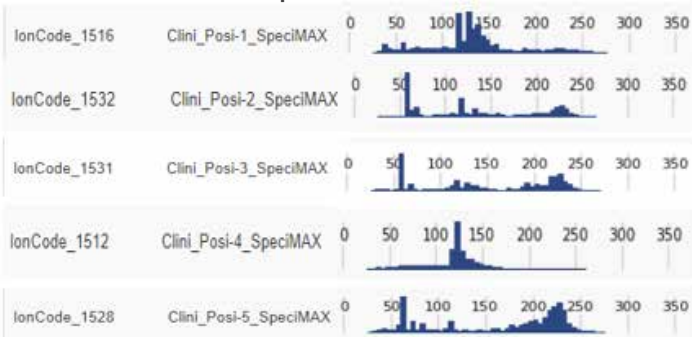
B

Mean read depth

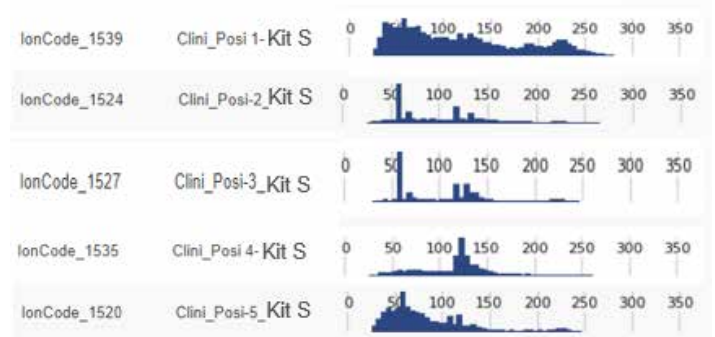
Donor	SpeciMAX Saliva Collection Kit	SpeciMAX Stabilized Saliva Collection Kit
11	6,652	10,113
12	10,931	3,757
13	8,728	613
14	6,709	10,751
15	2,252	1,853
16	7,328	13,007
17	4,256	12,591
18	10,462	10,674
19	6,666	12,411
20	1,133	28
Controls		
Spiked virus in PBS	11,012	NA
Spiked library, 100 cp	6,273	NA
Spiked library, 300 cp	5,071	NA

A

SpeciMAX kit



Device S



B

Samples containing SARS-CoV-2 collected with SpecIMAX kit and Device S

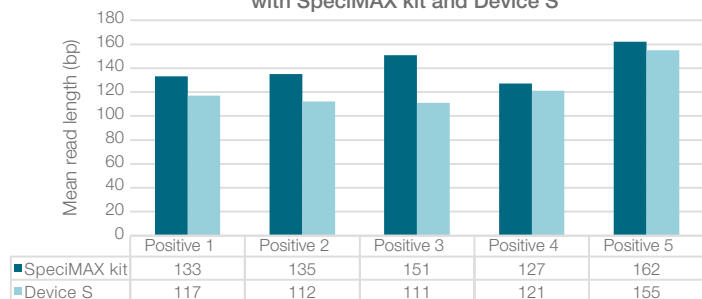


Figure 5. Sequencing data for suspected positive samples collected with stabilized saliva collection devices. Read length histograms (A) and mean read lengths (B) of suspected positive specimens collected using the SpecIMAX Stabilized Saliva Collection Kit and Device S.

Conclusions

Both raw and stabilized saliva samples indicated good-quality sequencing data on an Ion 540 chip using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay. Results from this study indicate high-quality sequencing metrics with stabilized and raw saliva, with the highest quality obtained using SpecIMAX Stabilized Saliva Collection Kits. When compared to another on-market collection kit (Device S) used to collect specimens with naturally occurring virus, the SpecIMAX Stabilized Saliva Collection Kit performed better overall in the Ion AmpliSeq SARS-CoV-2 Insight Research Assay.

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Ordering information

Product	Cat. No.
Reagents and consumables	
Ion AmpliSeq SARS-CoV-2 Insight Research Assay – GS Manual	A51305
Ion AmpliSeq SARS-CoV-2 Insight Research Assay – GS Chef Ready	A51306
Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX	A51307
SpeciMAX Saliva Collection Kit	A50696
SpeciMAX Stabilized Saliva Collection Kit	A50697
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (RUO)	A48383R
TaqMan 2019-nCoV Assay Kit v1	A47532
TaqPath 1-Step RT-qPCR Master Mix, CG	A15299
Instruments	
KingFisher Flex Purification System, KingFisher with 96 Deep-Well Head	5400630
QuantStudio 5 Real-Time PCR System, 384-Well	A28575
Ion Chef Instrument	4484177
Ion GeneStudio S5 System	A38194

Acknowledgment

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