Sample prep

Detection of SARS-CoV-2 using the SpeciMAX Dx Saliva Collection Kit paired with fast PCR

Introduction and background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to infect many people worldwide. The pandemic has led to the development of simpler, faster, and more reliable molecular diagnostic methods, such as direct quantitative PCR (qPCR) assays. An example of a qPCR assay for SARS-CoV-2 is the Applied Biosystems™ TaqPath™ COVID-19 Fast PCR Combo Kit 2.0 (CE-IVD), which eliminates the need for nucleic acid purification by use of Applied Biosystems™ SalivaReady™ Solution to disrupt virus particles. The TaqPath COVID-19 Fast PCR Combo Kit 2.0 (Cat. No. A51605) is a multiplex, highly sensitive, direct RT-PCR assay for the qualitative detection and characterization of SARS-CoV-2 N, *orf1a*, and *orf1b* genes. The kit is used for saliva collection in a container without preservatives under the supervision of a health care provider from individuals suspected of COVID-19 by their health care provider.

Saliva collection is non-invasive and has been shown to be comparable to nasopharyngeal swab specimen collection for the molecular detection of SARS-CoV-2 [1]. While nasopharyngeal swab collection is a commonly used sampling process for SARS-CoV-2 detection, it is more invasive and uncomfortable than saliva specimen collection. Additionally, the nasopharyngeal swab collection process can be an intimidating experience for individuals being tested for SARS-CoV-2. Saliva specimen collection can be accomplished using the Thermo Scientific™ SpeciMAX™ Dx Saliva Collection Kit. This kit contains a ready-to-use diagnostics (Dx) collection device with a unique design that can be used to collect and store unprocessed raw saliva under the supervision of a health care professional. The SpeciMAX saliva collection device consists of a collection tube with a screw cap and an easy-to-use funnel (Cat. No. A51022). Figure 1 includes all steps from sample collection to SARS-CoV-2 detection using the SpeciMAX Dx Saliva Collection Kit with direct PCR.

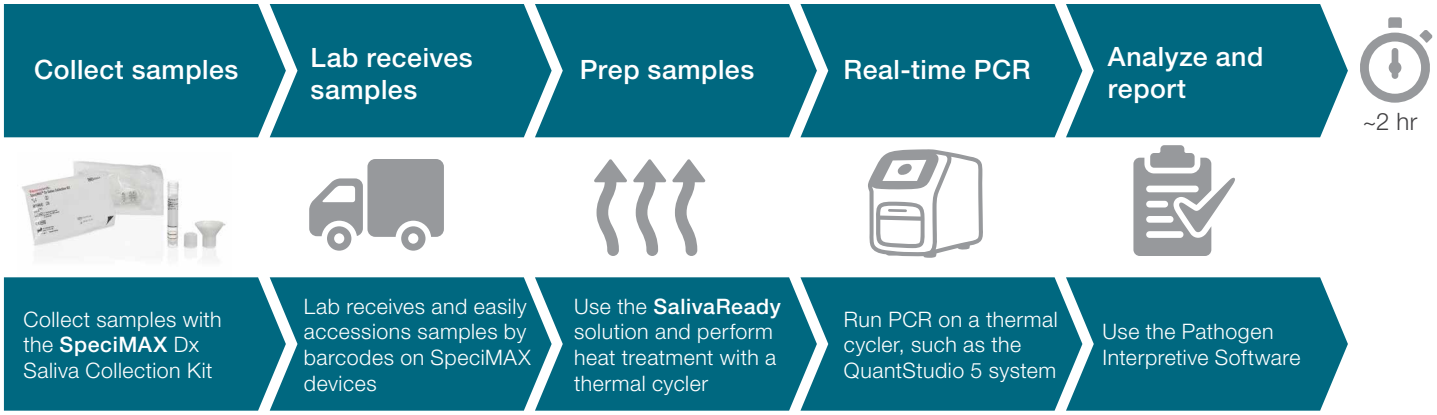

Figure 1. Workflow for the SpeciMAX Dx Saliva Collection Kit combined with the TaqPath COVID-19 Fast PCR Combo Kit 2.0.

Experimental procedures

Two studies were performed to evaluate the compatibility of the SpeciMAX DX Saliva Collection Kit with the TaqPath COVID-19 Fast PCR Combo Kit 2.0. In the first study, saliva was collected from 17 healthy donors using SpeciMAX Dx saliva collection devices. After collection, each sample was transferred to an empty SpeciMAX Dx saliva collection device and contrived with 1,600 genome copy equivalents (GCE) per device of gamma-irradiated SARS-CoV-2 from BEI Resources. The remaining saliva in each original collection device was then contrived with 8,000 GCE/device of the inactivated SARS-CoV-2. Total saliva volume varied from 500 µL to 1,000 µL depending on donor sample availability. Both sets of contrived saliva samples were stored at 4°C and were tested using the TaqPath COVID-19 Fast PCR Combo Kit 2.0 on days 1 through 3 and on days 6 and 7. The direct PCR assay was not run on days 4 and 5. Each sample was tested in triplicate to observe sample-to-sample variation.

In a second study, we collected a new set of raw saliva samples from 12 healthy donors using SpeciMAX Dx saliva collection devices and contrived each with 200 GCE/device of inactivated SARS-CoV-2 from BEI. The saliva samples were stored at 4°C for 3 days. The raw saliva samples were tested daily with the TaqPath COVID-19 Fast PCR workflow to detect SARS-CoV-2 RNA. Each sample was tested in triplicate to observe sample-to-sample variation.

For both studies, a positive and negative direct PCR control was used for each run. The positive control comprised one *in vitro*–transcribed (IVT) RNA that targets N, *orf1a*, and *orf1b* genes and one IVT RNA that targets the human RNase P gene. All positive controls were detected for all runs within both studies.

Results and discussion

Study 1

The results of the first study demonstrated that the SpeciMAX Dx Saliva Collection Kit is compatible with the TaqPath COVID-19 Fast PCR Combo Kit 2.0 (CE-IVD) workflow for the qualitative detection of SARS-CoV-2. Amplification of SARS-CoV-2 N, *orf1a*, and *orf1b* genes and the human RNase P gene was called as positive or negative based on the analysis parameters stated in the instructions for use for the Applied Biosystems™ Pathogen Interpretative Software. Table 1 displays the results for the detection of SARS-CoV-2 and RNase P genes for all 17 samples that were contrived with 1,600 GCE/device of SARS-CoV-2. Total percent detection was determined for all sample replicates. There was no amplification of the SARS-CoV-2 targets in a few samples. This was likely due to sample mucolytic viscosity, poor volume fill, and/or technician pipetting inconsistencies when setting up replicates. These samples were included in the data and analysis to show how sample variability can directly impact results. Fewer of the replicates from Donor 11 indicated positive results for SARS-CoV-2 as the days progressed. It is suspected that the lack of amplification was due to mucolytic viscosity of the sample that made it difficult to pipet. Replicates from Donor 7 generated similar results, which was probably due to poor fill volume of the sample and/or technical pipetting inconsistencies. SARS-CoV-2 was not detected in any replicates from Donors 9, 11, and 13 on days 6 and 7, which was suspected to be due to the degradation of viral RNA by saliva amylases beyond day 3 of the study. Table 2 displays the results for the SARS-CoV-2 and RNase P genes across all 17 evaluated specimens contrived with 8,000 GCE/device of SARS-CoV-2. One of three of each replicate set for Donor 11 on days 3 and 6 failed to amplify gene targets. Since genes were not detected for some of the replicates from Donor 11 for both SARS-CoV-2 input amounts (1,600 and 8,000 GCE), this suggests that sample consistency and pipetting errors are possible causes for failed amplification.

Table 1. Detection of RNA from 17 samples contrived with 1,600 GCE/device of SARS-CoV-2 using the TaqPath COVID-19 Fast PCR Combo Kit 2.0.

Donor	Day 1				Day 2				Day 3				Day 6				Day 7			
	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P
1	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
2	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
4	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
5	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
6	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
7	3/3	3/3	3/3	3/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3	3/3	3/3	3/3	3/3	2/3	2/3	2/3	2/3
8	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
9	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3
10	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
11	2/3	2/3	2/3	2/3	1/3	1/3	1/3	1/3	2/3	2/3	2/3	2/3	1/3	1/3	1/3	1/3	0/3	0/3	0/3	0/3
12	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
13	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3	2/3	2/3	2/3	0/3	0/3	0/3	0/3
14	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
15	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
16	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
17	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
% detection	**98%**	**98%**	**98%**	**98%**	**92%**	**92%**	**92%**	**92%**	**94%**	**94%**	**94%**	**94%**	**90%**	**90%**	**90%**	**90%**	**82%**	**82%**	**82%**	**82%**

Table 2. Detection of RNA from 17 samples contrived with 8,000 GCE/device of SARS-CoV-2 using the TaqPath COVID-19 Fast PCR Combo Kit 2.0.

Donor	Day 1				Day 2				Day 3				Day 6				Day 7			
	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P
1	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
2	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
4	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
5	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
6	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
7	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
8	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
9	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
10	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
11	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	3/3	3/3	3/3	3/3
12	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3	2/3	2/3	2/3	3/3	3/3	3/3	3/3
13	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
14	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
15	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
16	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
17	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
% detection	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**98%**	**98%**	**98%**	**98%**	**98%**	**98%**	**98%**	**98%**	**100%**	**100%**	**100%**	**100%**

Study 2

The second study further confirmed that the SpeciMAX Dx Saliva Collection Kit is compatible with the TaqPath COVID-19 Fast PCR kit workflow for qualitative detection of SARS-CoV-2 by evaluating samples contrived with even lower GCE. Samples were tested for the presence of SARS-CoV-2 N, *orf1a*, and *orf1b* genes and the human RNase P gene. Tests were considered positive or negative based on the analysis parameters stated in the instructions for use for the Pathogen Interpretative Software. SARS-CoV-2 and RNase P genes were detected in all saliva samples from 12 healthy donors that were contrived with 200 GCE/device of SARS-CoV-2 (Table 3). Amplification of the SARS-CoV-2 targets was unsuccessful for a few replicates. As noted in the first study, the observation that these samples were difficult to consistently pipet suggests that the failed amplification was due to sample mucolytic viscosity, poor volume fill, and/or technician pipetting inconsistencies when the replicates were set up. Reactions that failed were included in the data and analysis to show how sample-to-sample variability can affect results.

Table 3. Detection of RNA from 12 samples contrived with 200 GCE/device of SARS-CoV-2 using the TaqPath COVID-19 Fast PCR Combo Kit 2.0.

Donor	Day 1				Day 2				Day 3			
	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P	N gene	*orf1a*	*orf1b*	RNase P
1	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
2	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
3	3/3	3/3	3/3	3/3	2/3	2/3	2/3	2/3	3/3	3/3	3/3	3/3
4	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
5	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
6	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
7	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
8	2/3	2/3	2/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
9	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
10	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
11	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3	2/3	2/3	2/3
12	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
% detection	**97%**	**97%**	**97%**	**97%**	**86%**	**86%**	**86%**	**86%**	**89%**	**89%**	**89%**	**89%**

Conclusions

Samples were considered to be positive or negative for the SARS-CoV-2 N, *orf1*, and *orf2* genes as well as the human RNase P gene based on the parameters stated in the instructions for use for the Pathogen Interpretative Software. Our results indicate that the SpeciMAX Dx Saliva Collection Kit is compatible from day 1 to day 3 with the TaqPath COVID-19 Fast PCR Combo Kit 2.0 (CE-IVD) workflow. Thermo Fisher Scientific has developed a complete workflow, from specimen collection to detection of SARS-CoV-2, that is both fast and accurate using raw saliva as the specimen type. Even though raw saliva specimens demonstrated sample-to-sample variability, we were able to detect SARS-CoV-2 virus at 200 GCE/device, 1,600 GCE/device, and 8,000 GCE/device from contrived samples. The results presented here demonstrate that the combination of the SpeciMAX Dx Saliva Collection Kit and the TaqPath COVID-19 Fast PCR Combo Kit 2.0 can be used for rapid and reliable detection of SARS-CoV-2.

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Ordering information

Product	Cat. No.
SpeciMAX Dx Saliva Collection Kit	A51022
TaqPath COVID-19 Fast PCR Combo Kit 2.0 (CE-IVD)	A51605
QuantStudio 5 Dx Real-Time PCR System, laptop	A47326

Reference

1. Callahan C., Ditelberg S., et al. (2021) Saliva is comparable to nasopharyngeal swabs for molecular detection of SARS-CoV-2. *Microbiology Spectrum* 9(1):e00162-21. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8552668.

CE IVD

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