Workflow and performance of the TaqPath COVID-19 Fast PCR Combo Kit 2.0

Enabling fast, trusted COVID-19 test results from raw saliva—an ideal choice for high-frequency testing

Introduction

The Applied Biosystems™ TaqPath™ COVID-19 Fast PCR Combo Kit 2.0 (Figure 1) is a CE-IVD–marked, real-time reverse-transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in raw saliva in sterile containers from individuals suspected by their health care provider of having COVID-19 (please refer to the Instructions for Use for applicable intended use).

The TaqPath COVID-19 Fast PCR Combo Kit 2.0 utilizes an advanced assay design to compensate for SARS-CoV-2 mutations and to help ensure accurate results, even as the virus that causes COVID-19 continues to mutate.

The TaqPath COVID-19 Fast PCR Combo Kit 2.0 directly utilizes raw saliva treated with Applied Biosystems™ SalivaReady™ Solution, omitting the need for sample extraction and offering a sample-to-result turnaround time of approximately 2 hours. The use of saliva as a sample matrix not only simplifies sample collection but also reduces costs compared to using nasopharyngeal swabs for SARS-CoV-2 detection [1].

The TaqPath COVID-19 Fast PCR Combo Kit 2.0 delivers fast, trusted COVID-19 test results from raw saliva, making it ideal for widespread, high-frequency testing.

Figure 1. Components of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 (Cat. No. A51605).

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Simplified workflow enables high-frequency testing (Figure 2)

- Turnaround time of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 from sample to result is approximately 2 hours
- Applied Biosystems™ Pathogen Interpretive Software, CE-IVD edition, automatically converts genetic analysis data into a readable report, to reduce interpretation errors

Advanced assay design compensates for current and future SARS-CoV-2 mutations

- Unique fluorescence channel for each genomic region (*orf1a*, *orf1b*, and N genes) (Figure 3)
- Redundancy with multiple targets (amplicons) per genomic region
- 8 targets spanning 3 genomic regions compensate for emerging mutations
- Excludes the S gene, which has a high risk of mutation
- Human RNase P gene serves as an endogenous specimen control

Figure 2. Schematic overview of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 workflow.

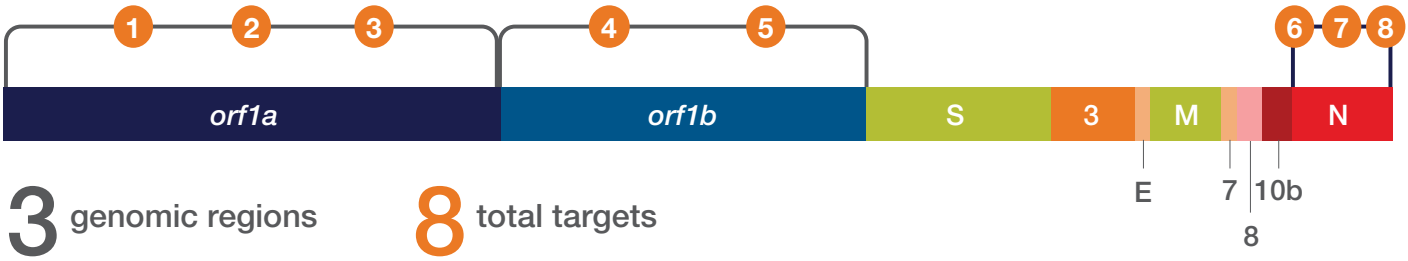

Figure 3. Schematic overview of the multi-target assay design.

Performance

Limit of detection (LOD)

The LOD study established the lowest SARS-CoV-2 viral concentrations (genomic copy equivalents, or GCE, per mL) that can be detected at least 95% of the time. Pooled raw saliva samples were spiked with gamma-irradiated SARS-CoV-2* virus at various concentrations. The LODs in Table 1 were confirmed with 20 replicates and 100% detection.

* Isolate USA-WA1/2020 (BEI Resources, Cat. No. NR-52287, Lot 70033322).

Table 1. LODs of Applied Biosystems™ QuantStudio™ PCR systems.

Real-time PCR instrument	LOD
QuantStudio 5 system (96-well, 0.2 mL)	1,000 GCE/mL
QuantStudio 7 Flex system (384-well)	750 GCE/mL

Cross-reactivity

Cross reactivity was assessed *in vitro* using microbial genomic material and *in silico* by BLAST-based sequence homology alignment to known microbial sequences (Table 2).

Table 2. Summary of cross-reactivity testing and analysis.

In vitro (wet-lab testing)	*In silico* (sequence homology)
Tested RNA or DNA from 17 organisms (4 bacteria and 13 viruses)	BLAST sequence homology to 55 organisms: (2 fungi, 27 viruses, and 26 bacteria)
No cross-reactivity detected	No cross-reactivity predicted**

** SARS-CoV showed a higher level of identity with the N gene and *orf1b* assays, but is not predicted to interfere with SARS-CoV-2 detection.

Note: SARS-CoV has not been in circulation since the 2003 outbreak.

Reactivity (inclusivity)

In silico analysis was performed using 1,802,689 complete SARS-CoV-2 genomes from the GISAID database (as of June 9, 2021).

- A positive result was called when a sample had a melting temperature higher than the annealing temperature for at least one assay per target region for at least two targets (*orf1a*, *orf1b*, and N gene).

Based on BLAST analysis, the TaqPath COVID-19 Fast PCR Assay Combo Kit 2.0 maps with 100% homology to 100% of SARS-CoV-2 genome sequences.

Interfering substances

The impact of potential interfering substances was tested by adding substances to saliva specimens spiked with gamma-irradiated SARS-CoV-2† virus at 3x the LOD, compared to a control with no interferents (Table 3). No false-negative or false-positive interference was observed for any interferents.

Table 3. Summary of interfering substances testing.

Interferent†	Agreement with expected results			
	Positive for SARS-CoV-2		Negative for SARS-CoV-2	
	Positive agreement	Number of positive results/ number tested	Negative agreement	Number of negative results/ number tested
Bovine mucin‡	100%	6/6	100%	6/6
Porcine mucin	100%	6/6	100%	6/6
Blood	100%	6/6	100%	6/6
Afrin™ nasal spray	100%	6/6	100%	6/6
NasoGel™ nose spray	100%	6/6	100%	6/6
Lozenge	100%	6/6	100%	6/6
Sore throat spray	100%	6/6	100%	6/6
Toothpaste	100%	6/6	100%	6/6
Mouthwash	100%	6/6	100%	6/6
Nicotine	100%	6/6	100%	6/6
hgDNA	100%	6/6	100%	6/6
No interferent	100%	6/6	100%	6/6

† Isolate USA-WA 1/2020 (BEI Resources, Cat. No. NR-52287, Lot 70039067) was used for testing all interfering substances except bovine mucin, which was tested using Cat. No. NR-52287, Lot 70033322.
‡ Bovine mucin = mucin: bovine submaxillary gland, type I-S. Porcine mucin = mucin: porcine stomach, type II. Afrin nasal spray = Afrin original nasal spray. NasoGel nose spray = NeilMed NasoGel nose spray. Lozenge = Cepacol™ benzocaine/menthol lozenge. Sore throat spray = Chloraseptic™ sore throat spray/solution. Toothpaste = Colgate™ toothpaste. Mouthwash = Crest™ mouthwash. hgDNA = human genomic DNA.

Clinical evaluation

A clinical evaluation study was performed to evaluate the performance of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 using archived, paired raw saliva and nasopharyngeal (NP) swab specimens from individuals with COVID-19 symptoms. The raw saliva specimens were tested using the TaqPath COVID-19 Fast PCR Combo Kit 2.0. The NP swab specimens were tested using an FDA-issued EUA assay from another supplier. All samples were run on both the QuantStudio 5 and 7 Flex systems.

Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated relative to the assay from the other supplier. The results are shown in Table 4. TaqPath COVID-19 Fast PCR Combo Kit 2.0 had a PPA and NPA of ≥95%.

Conclusions

The TaqPath COVID-19 Fast PCR Combo Kit 2.0 is an ideal choice for COVID-19 testing using raw saliva as a sample matrix:

- Direct-to-PCR workflow from raw saliva (no RNA extraction required)
  - Simplifies sample collection: saliva is easily self-collected, reducing both the potential exposure of virus to health care providers and the need for personal protective equipment (PPE)
  - Saliva collection can save significant amounts of money compared to using NP swabs for SARS-CoV-2 detection [1]
- Offers a simple, convenient, and efficient workflow to deliver trusted results quickly
  - Turnaround time from sample to result is only 2 hours
  - Enables widespread, high-frequency testing
- Innovative multi-target assay design compensates for emerging SARS-CoV-2 mutations
- Accurate detection provides increased confidence in results
  - Outstanding performance (LOD of 750–1,000 GCE/mL with PPA and NPA ≥95%)
  - Pathogen Interpretive Software, CE-IVD edition, helps decrease analysis and interpretation time and risk of user interpretation error

Table 4. Summary of clinical evaluation.

	Comparison with FDA-issued EUA assay from other supplier			
	PPA (%)	**95% CI**	**NPA (%)**	**95% CI**
TaqPath COVID-19 Fast PCR Combo Kit 2.0 (QuantStudio 5 system)	96.8%	83.3–99.9%	97.4%	86.5–99.9%
TaqPath COVID-19 Fast PCR Combo Kit 2.0 (QuantStudio 7 Flex system)	96.8%	83.3–99.9%	100.0%	90.9–100%

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Ordering information

Product	Components	Quantity	Cat. No.
TaqPath COVID-19 Fast PCR Combo Kit 2.0	TaqPath COVID-19 Fast PCR Assay 2.0 (1 tube)	1,000 reactions	A51605
	TaqPath COVID-19 Plus Control (10 tubes)		
	TaqPath COVID-19 Control Dilution Buffer (1 box with 10 tubes)		
	SalivaReady Solution (1 bottle with 20 mL)		
	TaqPath 1-Step Multiplex Master Mix, No ROX (1 bottle with 10 mL)		

Reference

1. Bastos ML, Perlman-Arrow S, Menzies D, Campbell JR (2021) The sensitivity and costs of testing for SARS-CoV-2 infection with saliva versus nasopharyngeal swabs. *Ann Intern Med.* 174(4):501-510. doi:10.7326/M20-6569.

Please refer to the Instructions for Use for more details and for the supported protocol. Deviations from the protocol are not permitted.

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