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 real-time reverse-transcription polymerase chain reaction (RT-PCR) test with Emergency Use Authorization (EUA), 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. A51606). All kit components can be stored in the same –20°C freezer. The Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix (No ROX) (Cat. No. A28523) is also required but sold separately.

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, EUA 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

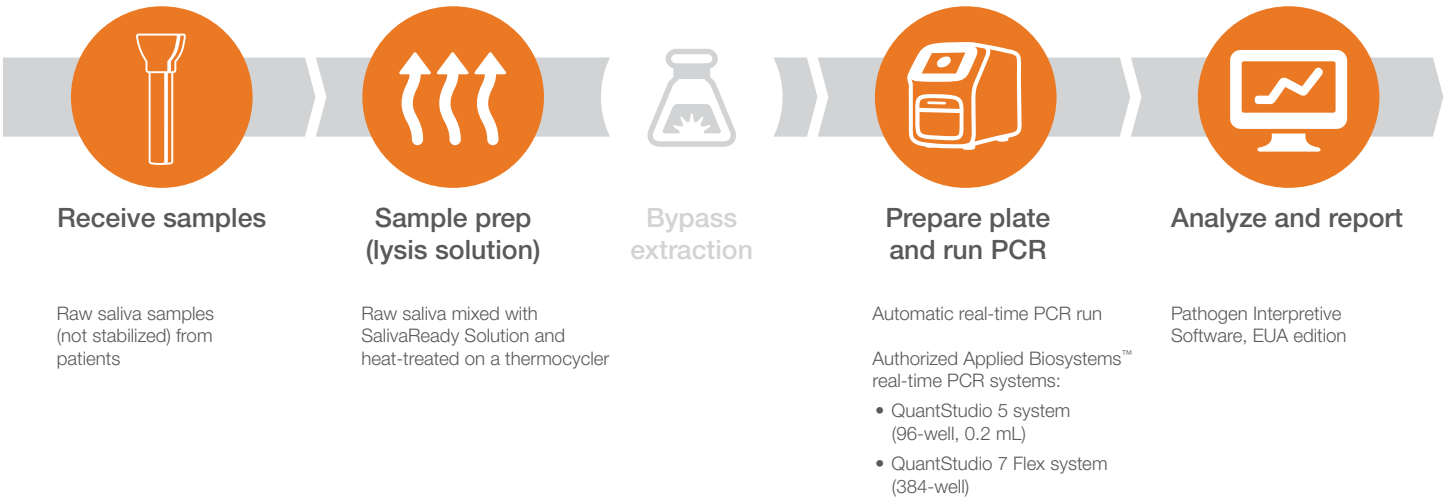

Figure 2. Schematic overview of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 workflow.

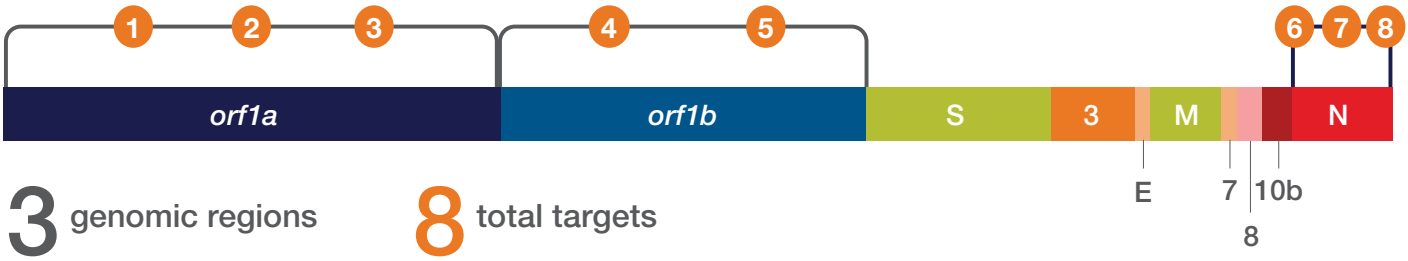

Figure 3. Schematic overview of the multi-target assay design.

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).

Cross-reactivity

Cross reactivity was assessed *in silico* by BLAST™ sequence homology alignment to 55 known microbial sequences (Table 2).

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 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

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

In silico (sequence homology)
BLAST sequence homology to 55 organisms: 2 fungi, 27 viruses, and 26 bacteria
No cross-reactivity predicted**

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

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

Table 3. Summary of interfering substances testing.

Interferent†	Final concentration in sample	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	2.5 mg/mL	100%	6/6	100%	6/6
Whole blood	1% v/v	100%	6/6	100%	6/6
Nasal spray	15% v/v	100%	6/6	100%	6/6
Nasal gel	1.25%	100%	6/6	100%	6/6
Lozenge	3 mg/mL	100%	6/6	100%	6/6
Throat spray	5% v/v	100%	6/6	100%	6/6
Toothpaste	0.5% v/v	100%	6/6	100%	6/6
Mouthwash	5% v/v	100%	6/6	100%	6/6
Nicotine	0.03 mg/mL	100%	6/6	100%	6/6
hgDNA	10 ng/µL	100%	6/6	100%	6/6
No interferent	NA	100%	6/6	100%	6/6

† Bovine mucin: mucin, bovine submaxillary gland, type I-S. Nasal spray: Afrin™ original nasal spray. Nasal gel: NeilMed™ NasoGel™ nasal gel. Lozenge: Cepacol™ benzocaine/menthol lozenges. 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 ≥97%.

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 both PPA and NPA >97%)
  - Pathogen Interpretive Software, EUA 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)	97.1%	84.6–99.9%	97.6%	87.4–99.9%
TaqPath COVID-19 Fast PCR Combo Kit 2.0 (QuantStudio 7 Flex system)	97.1%	84.6–99.9%	100.0%	91.5–100%

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	A51606
	TaqPath COVID-19 Plus Control (10 tubes)		
	TaqPath COVID-19 Control Dilution Buffer (1 box; 10 tubes)		
	SalivaReady Solution (1 bottle; 20 mL)		
TaqPath 1-Step Multiplex Master Mix (No ROX)*	4X RT-qPCR master mix (1 tube; 10 mL)	2,000 reactions	A28523

* Required but not included in the TaqPath COVID-19 Fast PCR Combo Kit 2.0.

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.

Find out more at **thermofisher.com/covid19evolved**

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