Workflow and performance of the TaqPath COVID-19 RNase P Combo Kit 2.0

Multiplex real-time RT-PCR test for the qualitative detection of nucleic acids from SARS-CoV-2 and RNase P internal control

Introduction

The Applied Biosystems™ TaqPath™ COVID-19 RNase P Combo Kit 2.0 contains assays and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test (Figure 1) intended for the qualitative detection of nucleic acids from SARS-CoV-2 in nasopharyngeal swabs and anterior or mid-turbinate nasal swabs from individuals suspected of having COVID-19 by their health care provider, as well as individuals without symptoms.

- Human RNase P gene serves as an endogenous control
- The multiplex RT-PCR testing solution can detect RNA from SARS-CoV-2 and RNase P in a single reaction well
- Storage temperature of –20°C for all components

Figure 1. Components of the TaqPath COVID-19 RNase P Combo Kit 2.0 (Cat. No. A51334).

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Workflow and turnaround time

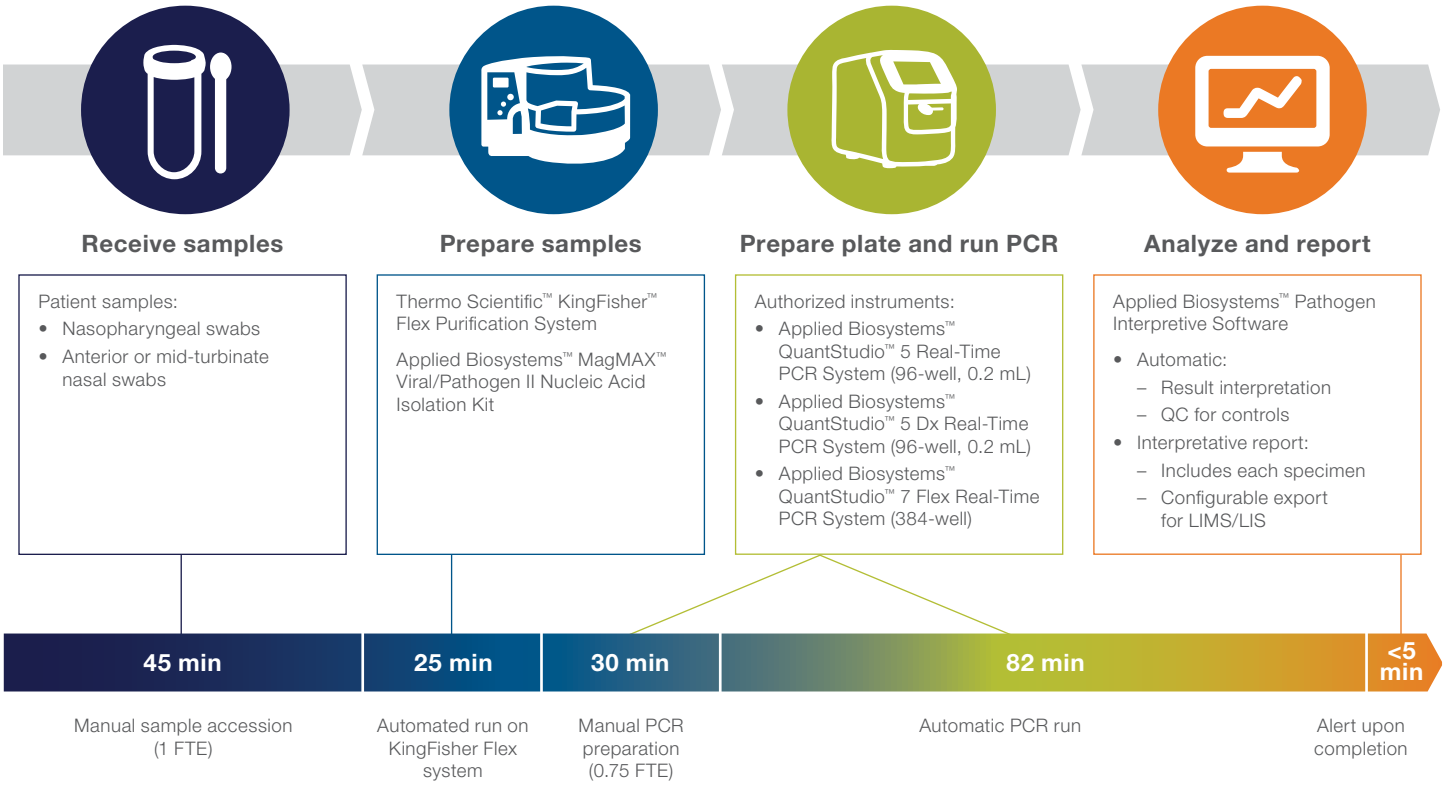

Figure 2. Workflow and turnaround time of the TaqPath COVID-19 RNase P Combo Kit 2.0.

The advanced assay design of the TaqPath COVID-19 RNase P Combo Kit 2.0 compensates for current and future SARS-CoV-2 mutations:

- Utilizes a workflow (Figure 2), which proceeds from sample to results in approximately 3 hours
- 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

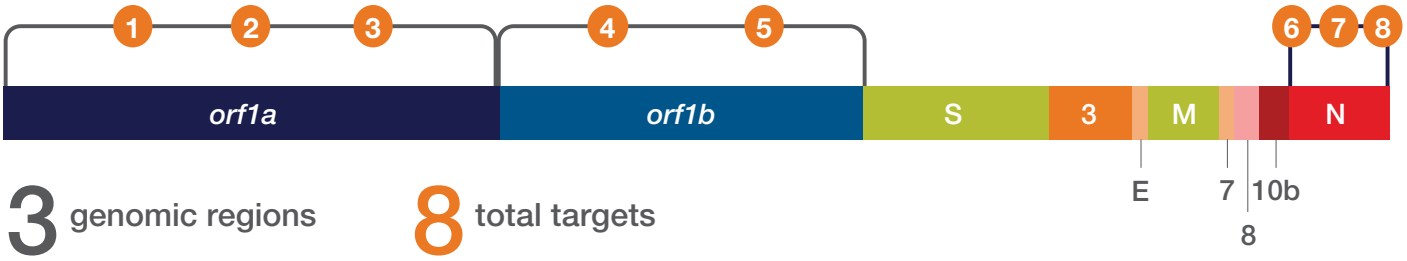

Figure 3. Overview of the multi-target assay design of the TaqPath COVID-19 RNase P Combo Kit 2.0.

Performance

Limit of detection (LOD)

The LOD study established the lowest SARS-CoV-2 viral concentrations (genomic copy equivalents or GCE/mL) that can be detected at least 95% of the time using either anterior nasal swabs (NS) or nasopharyngeal (NP) swabs spiked with a gamma-irradiated SARS-CoV-2 isolate. The LODs for both specimen types were confirmed using 20 replicates each (Table 1).

Table 1. LODs of the QuantStudio PCR systems for both NS and NP swabs spiked with gamma-irradiated SARS-CoV-2.

Real-time PCR instrument	Isolate	LOD
QuantStudio 5 system (96-well, 0.2 mL)	USA-WA1/2020	75 GCE/mL for NS, 75 GCE/mL for NP swabs
QuantStudio 5 Dx system (96-well, 0.2 mL)*		
QuantStudio 7 Flex system (384-well)		

* A bridging study yielded 100% positivity (20/20) at 1x LOD for the QuantStudio 5 Dx Real-Time PCR System.

Reactivity (inclusivity)

In silico analysis was performed using 1,802,689 complete SARS-CoV-2 genomes in the GISAID database (June 9, 2021), including the variants of concern in Table 2.

All (100%) of the SARS-CoV-2 sequences of human origin exhibited 100% homology to one or more primer and probe sets, and are thus predicted to be detected.

Table 2. *In silico* basic local alignment search tool (BLAST™) alignment to complete SARS-CoV-2 genomes.

SARS-CoV-2 genomes	Number of genomes	Alignment
Human SARS-CoV-2	1,802,689	100%
Alpha variant	819,801	
Beta variant	20,057	
Gamma variant	25,068	
Delta variant	29,149	

Interfering substances

The impact of potentially interfering substances was tested by adding 10 possible interfering substances to pooled negative NP swab specimens spiked with gamma-irradiated SARS-CoV-2 at 3x the LOD (225 GCE/mL) and comparing them to a control with no interferents. Each interferent was tested in triplicate. No false-negative or false-positive results were observed for any interferents at the concentrations listed, on both the QuantStudio 5 and QuantStudio 7 Flex systems (Table 3).

Table 3. Testing of interfering substances.

Interfering substance	Concentration	Agreement
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100%
Blood (human)	1% v/v	100%
Afrin™ nasal spray	10% v/v	100%
Nasal corticosteroid—fluticasone propionate	5 µg/mL	100%
NeilMed™ Nasogel™ nasal gel	1% w/v	100%
bioAllers™ homeopathic allergy relief medicine	10% v/v	100%
Dorithricin™ throat lozenge, oral anesthetic and analgesic	1% w/v	100%
Oseltamivir phosphate	33 µg/mL	100%
Antibiotic, nasal ointment—pseudomonic acid	5 µg/mL	100%
Antibacterial, systemic—tobramycin	0.6 mg/mL	100%

Cross-reactivity

In vitro

Cross-reactivity testing was performed using the 31 organisms listed in Table 4. No false-positive SARS-CoV-2 calls were obtained with any organism tested.

In silico

BLAST analysis was used to assess potential cross-reactivity between the TaqPath COVID-19 RNase P Combo Kit 2.0 primer/probe sequences and 55 organisms. With the exception of SARS-coronavirus, no bacterial, viral, or fungal microbe sequence aligned with ≥80% identity to more than one primer/probe set.

Note: The majority of the 285 SARS-coronavirus isolates shared ≥80% identity with more than one primer/probe set for the N gene and *orf1b*.

Clinical evaluation

A clinical study was carried out to evaluate the performance of the TaqPath COVID-19 RNase P Combo Kit 2.0 using 120 archived nasopharyngeal swab and anterior nasal swab specimens.

The following specimens were tested:

- 60 samples that were positive for SARS-CoV-2 (54 nasopharyngeal swabs and 6 nasal swabs)
- 60 samples that were negative for SARS-CoV-2 (45 nasopharyngeal swabs and 15 nasal swabs)

The samples were tested using the TaqPath COVID-19 RNase P Combo Kit 2.0 and an FDA EUA-authorized comparator test. Positive percent agreement (PPA), negative percent agreement (NPA), and confidence intervals (CI) were calculated relative to the comparator test (Table 5).

Table 4. Organisms used for *in vitro* cross-reactivity testing.

Human coronavirus 229E	Influenza A	*Streptococcus pneumoniae*
Human coronavirus OC43	Influenza B	*Streptococcus pyogenes*
Human coronavirus HKU1	Enterovirus	*Bordetella pertussis*
Human coronavirus NL63	Respiratory syncytial virus	*Mycoplasma pneumoniae*
SARS-coronavirus*	Rhinovirus	*Pseudomonas aeruginosa*
MERS-coronavirus	Epstein-Barr virus	*Staphylococcus epidermidis*
Adenovirus	*Chlamydophila pneumoniae*	*Streptococcus salivarius*
Human metapneumovirus	*Haemophilus influenzae*	*Candida albicans*
Parainfluenza 1	*Legionella pneumophila*	Pooled human nasal wash
Parainfluenza 2	*Mycobacterium tuberculosis*	
Parainfluenza 3		
Parainfluenza 4		

* An inconclusive result with the SARS-coronavirus was obtained due to amplification of the N gene. Note: SARS-coronavirus is not a common respiratory pathogen and has not been in circulation since the 2003 outbreak.

Table 5. Summary of clinical evaluation of the TaqPath COVID-19 RNase P Combo Kit 2.0 for nasal and nasopharyngeal sample types, compared to an FDA EUA-authorized assay.

Instrument	PPA	95% CI	NPA	95% CI
QuantStudio 5 system (96-well, 0.2 mL)	96.7%	88.5–99.6%	95.0%	86.1–99.0%
QuantStudio 7 Flex system (384-well)	95.0%	86.1–99.0%	96.7%	88.5–99.6%

Conclusions

The TaqPath COVID-19 RNase P Combo Kit 2.0 is an ideal choice for COVID-19 testing using nasopharyngeal or anterior or mid-turbinate nasal swabs.

- Human sample confirmation
  - The RNase P gene serves as an endogenous control, helping to ensure sample integrity, quality, and extraction
- Innovative, multi-target primer/probe design
  - Eight targets across 3 regions (*orf1a*, *orf1b*, N gene) compensate for emerging SARS-CoV-2 mutations, providing confidence in results now and into the future
- Applied Biosystems Pathogen Interpretive Software
  - Helps decrease analysis and interpretation time, reducing the risk of user interpretation error
- Sensitive detection
  - Detects active infections in individuals suspected of having COVID-19 by their health care provider, as well as individuals without symptoms or other epidemiological reasons to suspect COVID-19
  - PCR-based test with excellent analytical sensitivity: 75 GCE/mL LOD
- Affordable and scalable
  - Increases testing throughput and lab efficiency
- Storage temperature of –20°C for all components

Ordering information

Product	Components	Quantity	Cat. No.
TaqPath COVID-19 RNase P Combo Kit 2.0	TaqPath COVID-19 RNase P RT-PCR Kit 2.0 (1 tube, multiplex assay; 10 tubes, RNase P control)	1,000 rxns	A51334
	TaqPath COVID-19 Plus Control (10 tubes)		
	TaqPath COVID-19 Control Dilution Buffer (1 box with 10 tubes)		
	TaqPath 1-Step Multiplex Master Mix, No ROX (1 bottle with 10 mL)		

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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