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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 the reagents and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (nasopharyngeal and nasal swabs) from individuals suspected of **COVID-19.**

- **Human RNase P gene** serves as an endogenous control, helping to ensure sample integrity, quality, and extraction.
- The multiplexed RT-PCR testing solution can detect the RNA from the SARS-CoV-2 virus and RNase P in a **single reaction well**.

Figure 1

Figure 1 and Table 1. Components in the TaqPath™ COVID-19 RNase P Combo Kit 2.0, 1,000 reactions (Cat. No. A51334)

Component	Description	Contents/ Storage
TaqPath™ COVID-19 RNase P Kit 2.0	Primers and probe sets specific to: • **SARS-CoV-2** (orf1a, orf1b, & N genes) • **Rnase P** (human collection control)	1 tube (1,500 µL) –30˚ C to –10˚ C
TaqPath™ COVID-19 Plus Control	*In vitro* transcribed RNA control with templates specific to **SARS-CoV-2** and **RNase P**	10 tubes (10 µL) –30˚ C to –10˚ C
TaqPath™ COVID-19 Control Dilution Buffer	Dilution buffer for the control	10 tubes (250 µL) –30˚ C to –10˚ C
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)	Master mix	1 bottle (10 mL) –30˚ C to –10˚ C

Workflow and turnaround time

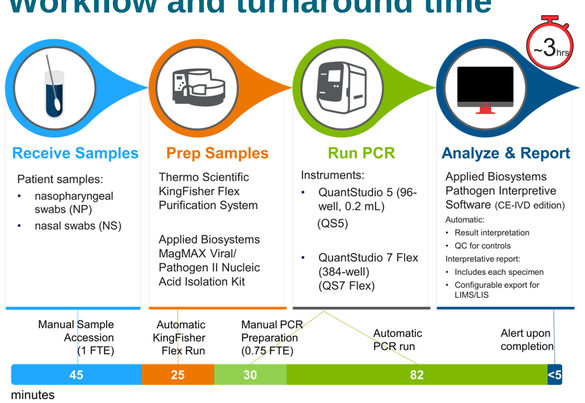

Figure 2. Workflow: TaqPath COVID-19 RNAse P Combo Kit

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

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

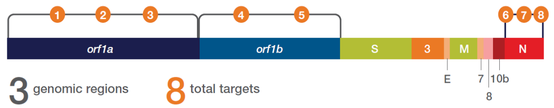

Figure 3. Schematic overview of the multi-target assay design

Performance

Limit of detection (LoD)

Study established 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 swabs (NP) spiked with gamma-irradiated SARS-CoV-2 isolate. The LoD for both specimen types was confirmed using 20 replicates each.

Table 2. Limit of detection

Real-Time PCR Instrument	Strain/Isolate	Limit of Detection
QuantStudio™ 5 96-well, 0.2-mL (QS5)	USA-WA1/2020	**75 GCE/mL NS** **75 GCE/mL NP**
QuantStudio™ 7 Flex 384-well (QS7 Flex)		

Reactivity (Inclusivity)

In Silico Analysis

In silico analysis was executed using 1,802,689 complete SARS-CoV-2 genomes in GISAID and NCBI Virus databases as of June 09, 2021. Based upon BLAST analysis, the TaqPath™ COVID-19 RNase P Combo Kit 2.0 assay maps with 100% homology to 100% of SARS-CoV-2 genome sequences in the GISAID database, including the variants of concern in Table 3.

All (100%) 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 3. *In silico* BLAST alignment to complete SARS-CoV-2 genomes

SARS-CoV-2 genomes	# 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

Pooled negative NP specimens were spiked with 10 potentially interfering substances and one no-interferent control with and without inactivated SARS-CoV-2 at 3X LoD (225 GCE/mL) for triplicate extractions.

Agreement with expected results for positive and negative NP samples on both the QS5 and QS7 Flex are shown in Table 4.

Interfering Substances, continued

No false positive or false negative results were observed for any of the substances at the concentrations tested.

Table 4. Agreement with expected results for interfering substances and concentrations tested

Interfering Substance	Concentration	Agreement
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100%
Blood (human)	1% v/v	100%
Nasal spray (Afrin™)	10% v/v	100%
Nasal corticosteroids— Fluticasone Propionate	5 µg/mL	100%
Nasal gel—NeilMed™ Nasogel™	1% w/v	100%
Homeopathic allergy relief medicine—bioAllers®	10% v/v	100%
Throat lozenges, oral anesthetic and analgesic—Dorithricin®	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

Functional testing was performed using the organisms listed in Table 5.

No false-positive SARS-CoV-2 calls obtained from any organism tested.

Table 5. *In vitro* cross-reactivity

Organisms used for *in vitro* cross-reactivity		
Human coronavirus 229E	Parainfluenza 4	*Streptococcus pneumoniae*
Human coronavirus OC43	Influenza A	*Streptococcus pyogenes*
Human coronavirus HKU1	Influenza B	*Bordetella pertussis*
Human coronavirus NL63	Enterovirus	*Mycoplasma pneumoniae*
SARS-coronavirus*	Respiratory Syncytial Virus	*Pseudomonas aeruginosa*
MERS-coronavirus	Rhinovirus	*Staphylococcus epidermidis*
Adenovirus	Epstein-Barr virus	*Streptococcus salivarius*
Human Metapneumovirus	*Chlamydophila pneumoniae*	*Candida albicans*
Parainfluenza 1	*Haemophilus influenzae*	*Pneumocystis carinii*
Parainfluenza 2	*Legionella pneumophila*	Pooled human nasal wash
Parainfluenza 3	*Mycobacterium tuberculosis*	

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

In silico

Cross-reactivity between the TaqPath™ COVID-19 RNase P Combo Kit 2.0 primer/probe sequences and 55 organisms was performed using BLAST analysis.

With the exception of SARS-CoV, 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 for the N gene and *Orf1b*.

Clinical Evaluation

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

The following specimens were tested:
• 60 positive for SARS-CoV-2 (54 NP and 6 NS)
• 60 negative for SARS-CoV-2 (45 NP and 15 NS)

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, summarized in Table 6.

Table 6. Summary of clinical evaluation for NS and NP sample types

Instrument	Positive Percent Agreement (PPA)	95% CI (LCL-UCL)	Negative Percent Agreement (NPA)	95% CI (LCL-UCL)
QS5 (96w, 0.2mL)	96.7%	88.5% - 99.6%	95.0%	86.1% - 99.0%
QS7 Flex (384w)	95.0%	86.1% - 99.0%	96.7%	88.5% - 99.6%

Conclusions

Have confidence in your results with an advanced COVID-19 test design for NP and NS specimens using RNase P for human sample confirmation.

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 (CE-IVD edition):
- Helps decrease analysis and interpretation time and risk of user interpretation error

Sensitive detection
- Detects active infections from individuals suspected of COVID-19 by their healthcare provider
- PCR-based test with excellent analytical sensitivity: 75 GCE/mL LOD

Affordable and scalable
- Increases testing throughput and lab efficiency

TRADEMARKS/ LICENSING

ThermoFisher SCIENTIFIC

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

CE IVD For *in vitro* diagnostic use.