Workflow and performance of the TaqPath COVID-19, Flu A/B, RSV Combo Kit

Multiplex real-time RT-PCR test for the detection and differentiation of SARS-CoV-2, influenza A and B, and RSV RNA

Introduction

The Applied Biosystems™ TaqPath™ COVID-19, Flu A/B, RSV Combo Kit contains 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 virus, influenza A and B (flu A/B) viruses, and respiratory syncytial virus (RSV) in nasopharyngeal (NP) swabs from individuals showing symptoms of a respiratory tract infection. The components of the kit are listed in Table 1.

Workflow and turnaround time

The workflow for the TaqPath COVID-19, Flu A/B, RSV Combo Kit is shown in Figure 1. The assay offers a simple, scalable workflow:

- Up to 94 specimens can be evaluated in approximately 3 hours with the end-to-end workflow*
- Applied Biosystems™ Pathogen Interpretive Software CE-IVD Edition interprets test results, validates controls, and generates an interpretive report
- Supports high-throughput testing—with one Thermo Scientific™ KingFisher™ Flex Purification System, two RT-PCR systems, and two full-time employees, 2,726 NP swabs can be tested each day**

Table 1. Components of the TaqPath COVID-19, Flu A/B, RSV Combo Kit, 1000 reactions (Cat. No. A49867).

Component	Description
TaqPath RT-PCR COVID-19, Flu A/B, RSV Assay Kit	Multiplex RT-PCR assay for: • SARS-CoV-2 (S and N genes) • Flu A/B (matrix genes) • RSV A/B (N and M genes) • MS2
	MS2 phage control
TaqPath COVID-19, Flu A/B, RSV Control	COVID-19, influenza A/B, and RSV controls
TaqPath Control Dilution Buffer	Buffer for the viral controls
TaqPath 1-Step Multiplex Master Mix, No ROX	Optimized for multiplexing up to 4 targets without a passive reference dye

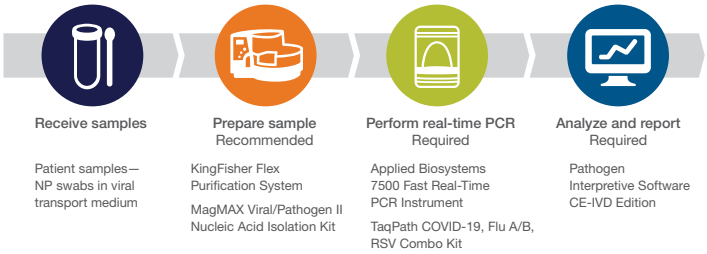

Figure 1. Overview of the TaqPath COVID-19, Flu A/B, RSV Combo Kit workflow.

* Assuming 45 min to receive samples, 25 min for KingFisher Flex instrument run, 30 min for PCR setup, 82 min for PCR run, and 5 min to generate the report.

** Assuming efficient staggering of the workflow.

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Performance of the TaqPath COVID-19, Flu A/B, RSV Combo Kit

Limit of detection (LOD)

The LOD study established the lowest viral concentrations of SARS-CoV-2, influenza A and B, and RSV A and B that can be detected at least 95% of the time. LODs are expressed as median tissue culture infectious dose per mL ($TCID_{50}$/mL) and genomic copy equivalents per mL (GCE/mL) (Table 2).

- Negative NP swab specimens were pooled and spiked with various concentrations of the respective viruses
- Each LOD was confirmed using 20 replicates

Reactivity inclusivity

In silico analysis

Sequences of the probes for each target were compared to published full-length viral genomes, and the percentage of sequences that resulted in 100% alignment were reported for each target (Table 3).

In vitro analysis

Functional testing was performed for 10 influenza A, 5 influenza B, 3 RSV A, and 3 RSV B strains (Table 4) at concentrations near the respective LODs, and positive results were produced in all replicates. The TaqPath COVID-19, Flu A/B, RSV Combo Kit detected the common influenza A subtypes H1N1 and H3N2, the major influenza B lineages B/Yamagata and B/Victoria, as well as the RSV A and RSV B subtypes.

Table 2. Limit of detection for SARS-CoV-2, influenza A and B, and RSV A and B expressed in $TCID_{50}$/mL and GCE/mL.

	Limit of detection (LOD)	
Target	$TCID_{50}$/mL	GCE/mL
SARS-CoV-2	8.2×10^{-3}	50
Influenza A/B*	1.2×10^{-3} to 1.5×10^{-1}	350–1,250
RSV A/B	1.3×10^{-2} to 1.4×10^{-2}	200

* The LODs for influenza A and influenza B varied between strains.

Table 3. *In silico* analysis and probe alignment to published database sequences.

Probe/target	Genomes for BLAST™ analysis	% sequences with 100% probe alignment
SARS-CoV-2	Full-length sequences: >76,033 (GenBank and GISAID)	100% (August 2020)
Influenza A	Full-length segment, 7 sequences: 32,460 (NCBI) 30,858 (GSAID)	88% (August 2020)
Influenza B	Full-length segment, 7 sequences: 8,660 (NCBI) 12,577 (GSAID)	41% (August 2020)
RSV A	463 human isolates (NCBI)	94% (September 2020)
RSV B	334 human isolates (NCBI)	94% (September 2020)

Table 4. Strains detected in reactivity wet-lab testing.

Influenza A	Influenza B	
H1N1/Georgia/M5081/2012	Unknown lineage/Taiwan/2/62	
H1N1/New Caledonia/20/99	Mixed lineage/Malaysia/2506/2004	
H1N1/Puerto Rico/08/1934	Victoria/Colorado/06/2017	
H1N1/Solomon Islands/3/2006	Yamagata/Massachusetts/02/2012	
H1N1/California/04/2009	Yamagata/Brisbane/03/2007	
H3N2/Wisconsin/15/2009		
H3N2/Switzerland/9715293/2013	RSV A	RSV B
H3N2/Wisconsin/67/2005	Long	18537
H3N2/Aichi/2/68	A2	ATCC-2012-11
H3N2/Hong Kong/8/68	2013 Isolate	12/2014 Isolate #1

Competitive interference

Negative NP specimens were pooled and spiked with SARS-CoV-2, influenza A, influenza B, RSV A, and RSV B in combinations in which one virus was present at a high concentration (≥10^5 $TCID_{50}$/mL) and at least one of the other viruses was present at a low concentration near the respective LOD. Results indicate that the TaqPath COVID-19, Flu A/B, RSV Combo Kit provides highly accurate results for co-infections (Figure 2).

Cross-reactivity

No cross-reactivity between the viruses and the organisms listed in Table 5 was observed in *in vitro* functional tests or in pooled human nasal wash (14% v/v), indicating a low propensity for false-positive results.

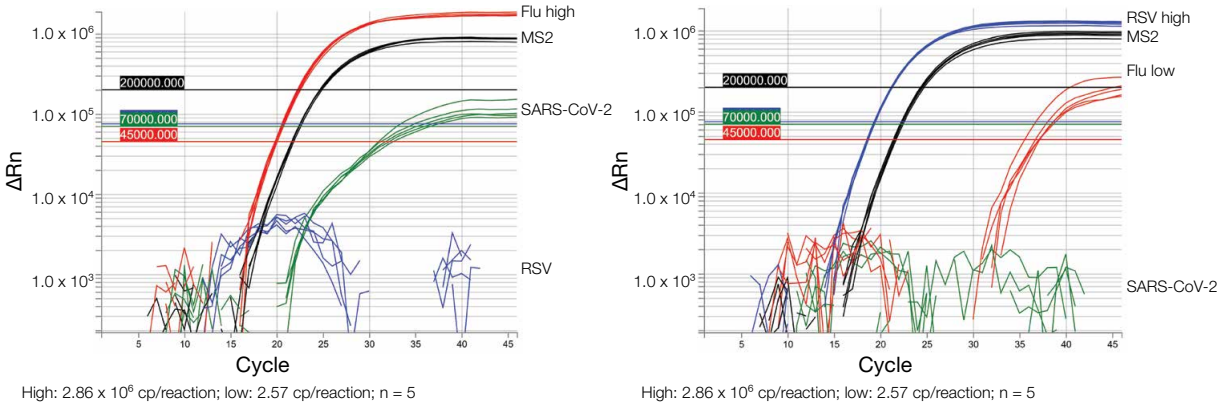

Figure 2. Analysis of competitive interference between SARS-CoV-2, influenza A and B, and RSV A and B.

Table 5. Organisms functionally tested for cross-reactivity.

Haemophilus influenzae	*Streptococcus pneumoniae*	Rhinovirus
Haemophilus parainfluenzae	*Streptococcus pyogenes*	SARS coronavirus
Legionella pneumophila	*Streptococcus salivarius*	*Candida albicans*
Moraxella catarrhalis	Adenovirus	*Pneumocystis jirovecii*
Mycobacterium tuberculosis	Enterovirus	Coronaviruses 229E, HKU1, NL63, and OC43
Mycoplasma pneumoniae	Human metapneumovirus	*Staphylococcus aureus* (MRSA)
Neisseria meningitidis	Measles	*Staphylococcus epidermidis*
Pseudomonas aeruginosa	MERS coronavirus	Parainfluenza 1, 2, 3, and 4

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Clinical evaluation

A clinical evaluation study was carried out to assess the performance of the TaqPath COVID-19, Flu A/B, RSV Combo Kit using the following archived NP specimens:

- 55 positive and 65 negative samples for SARS-CoV-2
- 55 positive and 114 negative samples for influenza A and B
- 55 positive and 125 negative samples for RSV

Samples were tested using the TaqPath COVID-19, Flu A/B, RSV Combo Kit and a comparator test for each of the viruses listed in the footnotes of Table 6. Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated relative to the comparator test. The results are shown in Table 6.

Table 6. Summary of a clinical evaluation of the TaqPath COVID-19, Flu A/B, RSV Combo Kit performed using the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument.

Target	Positive percent agreement (PPA)	Negative percent agreement (NPA)
SARS-CoV-2*	98.2%	100%
Influenza A/B**	100%	96.5%
RSV A/B†	98.2%	92.8%

* SARS-CoV-2 compared using the Applied Biosystems™ TaqPath™ COVID-19 Combo Kit Advanced.
** Influenza A and B compared using the Quidel™ Lyra™ Influenza A+B Assay.
† RSV A and B compared using the Quidel™ Lyra™ RSV + hMPV Assay.

Conclusions

The TaqPath COVID-19, Flu A/B, RSV Combo Kit is an all-in-one, real-time RT-PCR respiratory test that detects and differentiates SARS-CoV-2, influenza A and B, and RSV.

- Simultaneously differentiates three respiratory viruses
- Helps identify cases of co-infection‡
- Affordable and scalable
  - Helps increase testing throughput and lab efficiency
- Sensitive and specific RT-PCR detection
  - Robust performance for detecting SARS-CoV-2, influenza A and B (undifferentiated), and RSV
- Automated data analysis with Pathogen Interpretive Software CE-IVD Edition
  - Quickly interprets results to expedite reporting
  - Helps reduce risk of interpretation error

‡ Based on *in vitro* competitive interference studies.

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