Reliable and accurate results using an advanced assay design with the TaqPath COVID-19 RNase P Combo Kit 2.0

Introduction

Thermo Fisher Scientific continues to provide the gold standard of COVID-19 testing with our Applied Biosystems™ TaqPath™ COVID-19 RNase P Combo Kit 2.0, authorized under an Emergency Use Authorization. The kit was developed to support new workflows and to compensate for emerging mutations. The TaqPath COVID-19 RNase P Combo Kit 2.0 supports sample testing from individuals suspected of having COVID-19 by their health care provider, as well as individuals without symptoms or with other epidemiological reasons to suspect COVID-19. Viral RNA extraction from nasopharyngeal (NP) and anterior or mid-turbinate nasal swabs (NS) are supported sample types.

The TaqPath COVID-19 RNase P Combo Kit 2.0 utilizes the RNase P gene as an internal control. This design no longer requires the addition of an external extraction control, such as the MS2 bacteriophage used in the TaqPath COVID-19 Combo Kit. Instead, RNase P demonstrates its invaluable role as an endogenous control that ensures proper sample collection by providing evidence of sample sufficiency and quality, successful nucleic acid extraction, and effective RT-PCR amplification within a reaction.

The TaqPath COVID-19 RNase P Combo Kit 2.0 provides confidence in your results with an advanced COVID-19 assay design that targets 8 sequences across 3 genomic regions (*orf1a*, *orf1b*, and N gene) to compensate for emerging SARS-CoV-2 mutations. This increased target redundancy ensures accurate results even for new mutations, with high sensitivity and specificity, providing confidence in results now and into the future.

RNase P as a control for specimen quality

RNase P is a ribonucleoprotein that performs a key processing step for protein synthesis of cleaving precursor transfer RNA (tRNA), which is conserved among all cellular life forms [1,2]. The gene encoding for the RNA subunit of RNase P is essential in all known living organisms [1], which makes it readily detectable and an ideal control for specimen quality. The sequences of RNase P are highly variable between eukaryotic taxa, differing even more significantly from bacterial and archaeal ancestral counterparts [3,4], making human RNase P an ideal candidate to identify human cells in the complex sample matrix of upper respiratory specimens. Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was successfully collected, extracted, and amplified [5].

The first-generation TaqPath COVID-19 Combo Kits utilized exogenous bacteriophage MS2 as the extraction control, which had to be spiked into all specimens prior to processing. Using endogenous human RNase P rather than exogenous MS2 has the advantage of providing an internal control for sample collection and quality, nucleic acid extraction, and RT-PCR performance. The internal RNase P control helps to reduce the risk of false-negative results caused by the presence of PCR inhibitors or inadequate sample collection, transport, and storage [5].

Widespread screening of COVID-19

The Food and Drug Administration (FDA) has defined specific requirements and use cases for both diagnostic testing and broad screening of COVID-19 [6]. Diagnostic testing is performed when a person presents signs of infection or has known or suspected recent exposure to SARS-CoV-2. Conversely, screening looks for individuals who are infected, but do not present clinical symptoms or have any reason to expect exposure to SARS-CoV-2. Examples of screening include testing all business employees returning to the workplace or testing all returning students and faculty at a school.

Notably, the TaqPath COVID-19 RNase P Combo Kit 2.0 has been authorized under the FDA Emergency Use Authorization for the qualitative detection of nucleic acid from SARS-CoV-2 from individuals suspected of COVID-19 by their healthcare provider, as well as from individuals without symptoms or other epidemiological reasons to suspect COVID-19.

Advanced assay design to compensate for SARS-CoV-2 mutations

Viruses mutate, and SARS-CoV-2 is no exception [7]. Early in the COVID-19 pandemic, SARS-CoV-2 mutated at a rate of about 1–2 mutations per month [8]. By mid- to late-2020, however, multiple SARS-CoV-2 variants emerged with significantly more accumulated mutations in short periods of time, causing global concern [9]. Scientists predict that SARS-CoV-2 variants will continue to emerge, as persistent uncontrolled transmission of SARS-CoV-2 in many parts of the world and selective pressures, such as vaccines, are creating ideal conditions for further, significant virus evolution [10].

There are several potential implications of emerging SARS-CoV-2 variants, including increased transmission rate, changed severity of COVID-19, resistance to antiviral treatments, the ability to evade vaccine-induced immunity, and an impact on detection of the virus using molecular diagnostic tests [11]. One example of the latter is the Alpha variant (also known as B.1.1.7 or the "UK variant"), which contains a two amino acid–deletion in the S gene, that interferes with detection of the S gene (known as the S gene target failure (SGTF) or S gene dropout) when using the Applied Biosystems™ TaqPath™ COVID-19 Combo Kit. The mutations present in the Alpha variant block detection of the S gene, but do not interfere with amplification of the other two targets, *orf1ab* and N gene, resulting in overall positive detection of SARS-CoV-2 in a specimen. Due to the built-in target redundancy of amplifying 3 different viral genes, the overall performance and ability of the TaqPath COVID-19 Combo Kit to detect SARS-CoV-2 is not impacted. While the SGTF supported the surveillance of the Alpha variant spread [12,13], it also highlights the risk of SARS-CoV-2 strains evading detection by nucleic acid amplification test (NAAT)–based technologies such as RT-PCR assays.

The TaqPath COVID-19 RNase P Combo Kit 2.0 is designed to compensate for current and future mutations by incorporating increased target redundancy to continually ensure reliable and accurate detection of SARS-CoV-2. While the second-generation kit no longer targets sequences in the S gene, which is known to have a high mutation rate, it is still designed to detect three viral genes in regions expected to be more conserved as variants arise (*orf1a*, *orf1b*, and N gene). Each gene is detected with a unique dedicated fluorescence marker: *orf1a* is detected in the FAM channel, *orf1b* is detected in the ABY channel, and the N gene is detected in the VIC channel. To ensure that emerging mutations do not interfere with the detection of any of these three genes, multiple targets within each gene are amplified and detected with the same fluorescence channel. For example, the TaqPath COVID-19 RNase P Combo Kit 2.0 includes primers and probes to amplify and detect three independent sequence targets in *orf1a*, and all three amplicons are detected in the same channel (FAM). In case a new viral mutation interferes with detection of one of the three amplicons, the fluorescence signal created by the remaining two will ensure detection of *orf1a*. Detection of *orf1b* is supported by two independent amplicons in the ABY channel, and detection of the N gene includes 3 independent amplicons in the VIC channel (Table 1).

Table 1. Assay configuration for the TaqPath COVID-19 RNase P Combo Kit 2.0.

Target gene	Amplicons per target	Applied Biosystems™ dye	Quencher
orf1a	3	FAM dye	QSY
orf1b	2	ABY dye	QSY
N gene	3	VIC dye	QSY

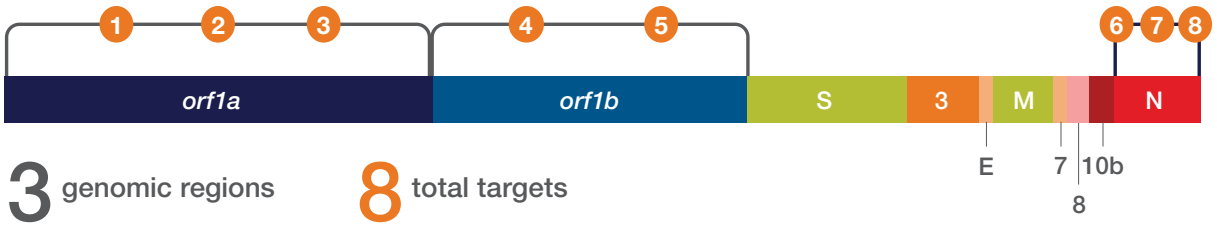


Figure 1. Overview of the advanced assay design of the TaqPath COVID-19 RNase P Combo Kit 2.0.

Figure 1 shows an overview of the assay design and targeted genes. To ensure sufficient redundancy, the TaqPath COVID-19 RNase P Combo Kit 2.0 supports amplification of 8 total targets (amplicons) across 3 genomic regions (*orf1a*, *orf1b*, and N gene) to compensate for emerging mutations.

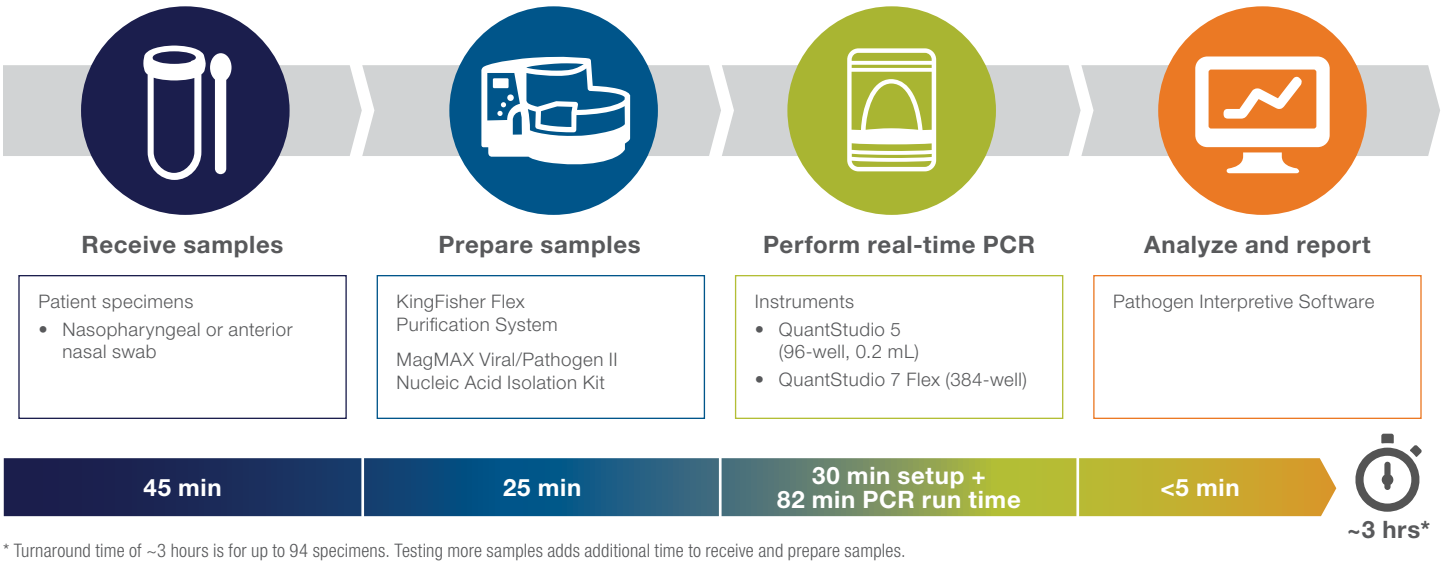
The TaqPath COVID-19 RNase P Combo Kit 2.0 maps with 100% homology to 100% of SARS-CoV-2 genome sequences found in the GISAID database, including 4 known variants (Table 2).

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

After specimens are collected and received by the laboratory, viral nucleic acid is purified using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit on the Thermo Scientific™ KingFisher™ Flex Purification System. The extracted nucleic acid is then reverse-transcribed into cDNA and amplified using the TaqPath™ 1-Step Multiplex Master Mix (No ROX™ dye) on one of the supported Applied Biosystems™ QuantStudio™ real-time PCR instruments. Notably, all contents included in the TaqPath COVID-19 RNase P Combo Kit 2.0 have a storage temperature of –30°C to –10°C, which differs from the first-generation kit that required the TaqPath COVID-19 control to be stored at ≤–70°C. The assay utilizes an RT-qPCR program that has a running time of 82 minutes. The data are automatically analyzed and interpreted, and a report is provided by the Applied Biosystems™ Pathogen Interpretive Software. The turnaround time of the TaqPath COVID-19 RNase P Combo Kit 2.0 from sample to result (for up to 94 samples) is approximately 3 hours. The workflow and turnaround times are shown in Figure 2.

Table 2. *In silico* BLAST™ alignment to complete SARS-CoV-2 genomes as of June 2021.

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	



* Turnaround time of ~3 hours is for up to 94 specimens. Testing more samples adds additional time to receive and prepare samples.

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

Performance of the TaqPath COVID-19 RNase P Combo Kit 2.0

The analytical performance of the TaqPath COVID-19 RNase P Combo Kit 2.0 was evaluated by performing a series of studies including, but not limited to, limit of detection (LOD), impact of interfering substances, reactivity, and cross-reactivity. In addition, a clinical evaluation study was carried out to further evaluate the performance of the TaqPath COVID-19 RNase P Combo Kit 2.0.

Here we highlight the LOD and clinical evaluation study performance of the TaqPath COVID-19 RNase P Combo Kit 2.0, and then compare performance of the advanced assay design against the original design that relied on a single target for each of the three targeted genes (*orf1ab*, N gene, and S gene).

Limit of detection (LOD)

The LOD is the lowest SARS-CoV-2 viral concentration (expressed as genomic copy equivalents/mL or GCE/mL) that can be detected at least 95% of the time using either 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 on two supported QuantStudio PCR instruments (Table 3).

Clinical evaluation

A clinical evaluation study for the TaqPath COVID-19 RNase P Combo Kit 2.0 was performed using archived NS and NP swab samples. The following specimens were tested:

- 60 samples that were positive for SARS-CoV-2 (54 NP and 6 NS)
- 60 samples that were negative for SARS-CoV-2 (45 NP and 15 NS)

The samples were tested for SARS-CoV-2 using the TaqPath COVID-19 RNase P Combo Kit 2.0 and a comparator assay with FDA EUA. Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated relative to the comparator assay. The results are shown in Table 4.

Table 3. LODs of the TaqPath COVID-19 RNase P Combo Kit 2.0.

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 7 Flex system (384-well)		

Table 4. PPA and NPA of the TaqPath COVID-19 RNase P Combo Kit 2.0 relative to a comparator assay.

	Comparator method			
	PPA	95% CI	NPA	95% CI
QuantStudio 5 system	96.7%	88.5–99.6%	95.0%	86.1–99.0%
QuantStudio 7 Flex system	95.0%	86.1–99.0%	96.7%	88.5–99.6%

Performance of the advanced assay design in detecting variants

To demonstrate the ability of the advanced assay design to amplify and detect viral RNA from different strains, we compared the performance of the advanced assay design to the first-generation assay design. We tested serial dilutions of four different isolates (Table 5), including a representative for the Alpha variant of concern (also known as B.1.1.7 lineage). Testing was conducted on the QuantStudio 5 Real-Time PCR Instrument (96-well, 0.2 mL block), and all samples were run in duplicate.

Viral RNAs from either the B.1.1.7 or B.1.1.298 lineage include a deletion of two amino acids in the S gene, showing the SGTF or S gene dropout with the first-generation assay design. The *orf1ab* and N gene targets were detected, resulting in the detection of SARS-CoV-2.

The advanced assay design no longer targets the S gene and is thus not affected by the SGTF. All three genetic targets (*orf1a*, *orf1b*, and N gene) for all four isolates were successfully detected.

Performance evaluation of the advanced assay design by independent laboratories

Three independent laboratories (designated as Laboratory A, B, or C) evaluated the performance of the Applied Biosystems™ TaqMan® SARS-CoV-2 RNase P Combo Kit 2.0 (RUO) by testing retrospective archived samples that had previously been tested for SARS-CoV-2 using the first-generation TaqPath assay kit as comparator.

Laboratory A tested a total of 186 retrospective samples, including 22 specimens that were positive and 164 specimens that were negative for SARS-CoV-2. The TaqMan SARS-CoV-2 RNase P Combo Kit 2.0 (RUO), run on the QuantStudio 5 Real-Time PCR System (96-well configuration), confirmed all 22/22 positive and all 164/164 negative specimens (Table 6).

Table 5. Overview of analyzed SARS-CoV-2 isolates.

Isolate*	Lineage	GISAID clade
hCoV-19/England/204820464/2020	B.1.1.7	GR
hCoV-19/Denmark/DCGC-3024/2020	B.1.1.298	GR
hCoV-19/South Africa/KRISP-EC-K005321/2020	B.1.351	GH
Italy-INMI1	None	O

* Note: SARS-CoV-2 variant genomic RNAs were isolated from infected culture cells and additional mutations were identified in the S gene of isolates Italy-INMI1 (T21784G), hCoV-19/Denmark/DCGC-3024/2020 (C24374T), and hCoV-19/South Africa/KRISP-EC-K005321/2020 (A21759G) that are not present in the original published sequence of the indicated isolate.

Table 6. Performance of the advanced assay design reported by three independent laboratories.

	Laboratory A		Laboratory B		Laboratory C	
	Number of samples confirmed	Agreement	Number of samples confirmed	Agreement	Number of samples confirmed	Agreement
Positive samples	22/22*	100%*	89/89**	100%**	23/23	100%
Negative samples	164/164*	100%*	93/93†	100%†	67/68‡	98.5%‡

* One sample was a false positive with the comparator and was moved to the negative sample pool.

** Four samples had inconclusive results with the TaqMan SARS-CoV-2 RNase P Combo Kit 2.0 (RUO) and were removed from the calculation.

† Viral transport media (VTM) was used as substitution for negative specimens.

‡ One sample had an invalid testing result with the TaqMan SARS-CoV-2 RNase P Combo Kit 2.0 (RUO) (RNase P not detected) and was removed from the performance assessment as the laboratory was unable to repeat testing.

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Laboratory B assessed the performance of the TaqMan SARS-CoV-2 RNase P Combo Kit 2.0 (RUO) on the QuantStudio 5 Real-Time PCR System 5 (384-well configuration) using retrospective specimens that were positive for SARS-CoV-2 and samples containing only viral transport media (VTM) as a substitute for negative specimens. Note that samples containing only VTM produced invalid test results with the TaqMan SARS-CoV-2 RNase P Combo Kit 2.0 (RUO) due to the absence of endogenous RNase P control. The TaqMan SARS-CoV-2 RNase P Combo Kit 2.0 (RUO) confirmed 89/89 positive samples and none of the VTM samples had a signal for SARS-CoV-2 (Table 6).

Laboratory C tested the TaqMan SARS-CoV-2 RNase P Combo Kit 2.0 (RUO) on the Applied Biosystems™ 7500 Fast Real-Time PCR System, and confirmed all 23/23 positive and 67/68 negative retrospective samples (Table 6). One sample tested positive with the TaqMan SARS-CoV-2 RNase P Combo Kit 2.0 (RUO) with a C_t value close to the cutoff, which could be indicative of a low viral titer, possibly explaining why the specimen had tested negative for SARS-CoV-2 with the first-generation kit.

Conclusions

The TaqPath COVID-19 RNase P Combo Kit 2.0 utilizes an advanced assay design to detect known and emerging SARS-CoV-2 variants. The kit can be used with NS and NP swabs from individuals suspected of having COVID-19 by their health care provider, as well as from individuals without symptoms or with other epidemiological reasons to suspect COVID-19.

The TaqPath COVID-19 RNase P Combo Kit 2.0 uses the endogenous RNase P gene to control for sample integrity, quality, and extraction efficiency. The Pathogen Interpretive Software decreases analysis and interpretation time and helps reduce the risk of user interpretation error.

With a LOD of 75 GCE/mL, the TaqPath COVID-19 RNase P Combo Kit 2.0 reports excellent analytical sensitivity. The clinical evaluation demonstrated a PPA and NPA of ≥95%. The outstanding performance of the advanced assay design was further demonstrated by 3 independent laboratories that tested retrospective samples.

The TaqPath COVID-19 RNase P Combo Kit 2.0 is a highly accurate and reliable diagnostic SARS-CoV-2 test with an advanced assay design that provides confidence in results.

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