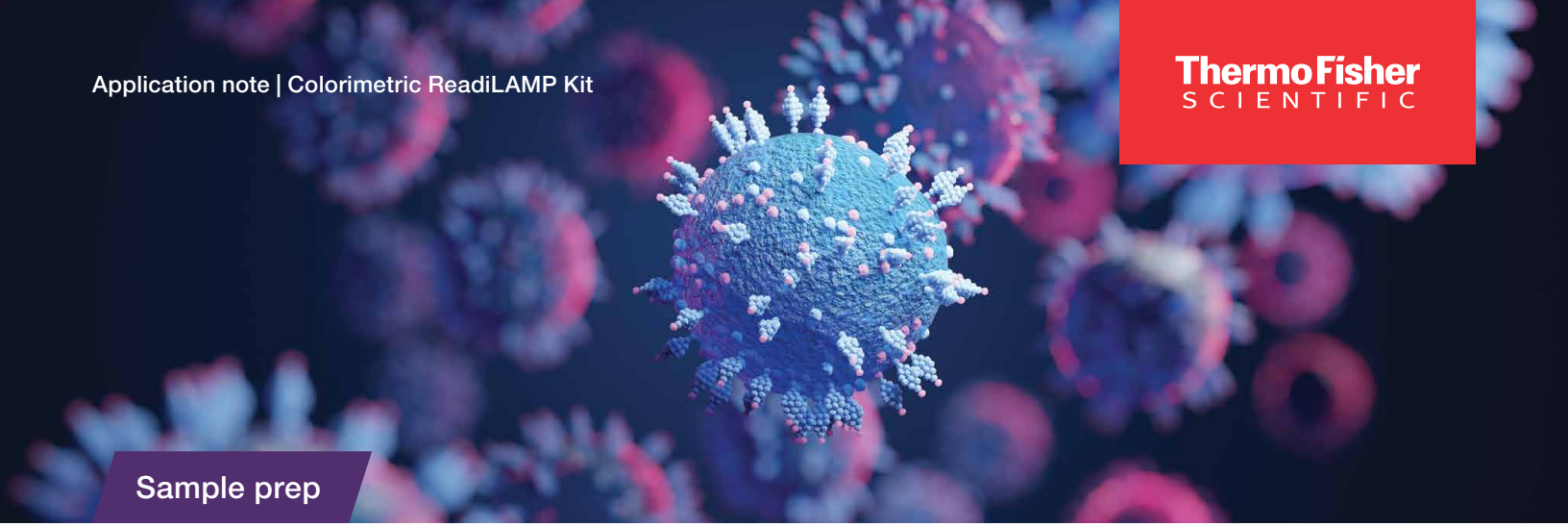

Sample prep

The Colorimetric ReadiLAMP Kit for SARS-CoV-2 demonstrates strong detection concordance when compared with qPCR

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) research and surveillance continue to be paramount, especially given the proportion of infected individuals who are asymptomatic and the emergence of viral variants. The Invitrogen™ Colorimetric ReadiLAMP™ Kit, SARS-CoV-2, enables rapid, accurate, and specific detection of the virus in field settings, requiring no specialized equipment or advanced scientific training. The loop-mediated isothermal amplification (LAMP) primers included in the kit are complementary to more than 98% of the Global Initiative on Sharing Avian Influenza Data (GISAID)-deposited SARS-CoV-2 genomes, including variants that exhibit qPCR-based S gene target failure. To assure high confidence in the Colorimetric ReadiLAMP kit's research and surveillance results, nasopharyngeal swab samples collected during February 2022 were assayed, and the results were compared to two independent qPCR-based detection approaches. The Colorimetric ReadiLAMP assay sensitivity and selectivity as well as concordance between assay types are reported.

Methods

Experimental samples

Nasopharyngeal swab samples eluted in viral transport media (NPVTM) were evaluated. Thirty-five pooled NPVTM samples were obtained from Precision for Medicine. These samples were characterized as SARS-CoV-2 negative by the vendor using the Applied Biosystems™ TaqPath™ COVID-19 Combo Kit assay. An additional thirty-five NPVTM samples collected from individual donors on February 1–2, 2022, were obtained from iSpecimen. This second sample set was characterized as SARS-CoV-2 positive by the vendor using the Cepheid™ GeneXpert™ platform. All samples were stored and transported as recommended by the assay manufacturers.

Total RNA isolation

Nucleic acid was isolated from NPVTM samples according to the protocol for the Applied Biosystems™ MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit and using the Thermo Scientific™ KingFisher™ Flex Purification System with 96 Deep-Well Head. Isolated RNA was stored at –20°C for a maximum of three weeks and was discarded after three freeze/thaw cycles.

TaqPath COVID-19 Combo Kit assay

The presence or absence of SARS-CoV-2 in each respective NPVTM sample was confirmed in-house following the manufacturer's protocol for the TaqPath COVID-19 Combo Kit assay. Briefly, 10 µL of isolated nucleic acid from each sample was combined with 15 µL of the TaqPath COVID-19 Combo Kit reagents. Assays were performed on an Applied Biosystems™ QuantStudio™ 7 Pro Real-Time PCR System with resultant data analyzed using the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument SDS Software, v1.4.

Colorimetric ReadiLAMP Kit, SARS-CoV-2 assay

The Colorimetric ReadiLAMP assay was performed according to the kit instructions using the purified RNA sample workflow. Nuclease-free water was selected as a negative control. Positive controls contained 200 copies of Thermo Scientific™ AcroMetrix™ SARS-CoV-2 Control genomic RNA per 25 µL reaction. For all experimental samples, 5 µL of isolated RNA derived from each respective NPVTM sample was added per 25 µL reaction. The presence or absence of SARS-CoV-2 RNA was ascertained based on final reaction color as determined by visual inspection with the unaided eye.

Results and discussion

NPVTM samples were screened by external vendors for the presence or absence of SARS-CoV-2. For SARS-CoV-2 negative samples (Table 1, Donors 1–35), no amplification was recorded by the vendor for at least two of the TaqPath COVID-19 Combo Kit targets (*orf1ab*, N gene, and S gene). Samples positive for SARS-CoV-2 contained a wide viral load range, with C_t values between 14.1 and 34.6 (Table 1, Donors 36–70, see vendor-provided Cepheid GeneXpert data). These samples were distributed continuously along a range from high (C_t = 14.1) to low (C_t = 34.6) viral load where ranked samples of increasing viral load resulted in only a 0.60 ± 0.46 increase in C_t value between consecutive samples.

Table 1. SARS-CoV-2 detection by the Colorimetric ReadiLAMP kit and qPCR-based methods.

	Cepheid GeneXpert	TaqPath COVID-19 Combo Kit				Colorimetric ReadiLAMP Kit, SARS-CoV-2
Donor(s)	C_t value	C_q value (10 µL input)			Result	Result (5 µL input)
		orf1ab	N gene	S gene		
1–35	Not applicable	> 37.0	> 37.0	> 37.0	Negative	Negative
36	18.9	15.0	15.9	> 37.0	Positive	Positive
37	16.5	14.7	15.2	> 37.0	Positive	Positive
38	17.2	14.5	15.1	> 37.0	Positive	Positive
39	20.1	15.0	15.9	> 37.0	Positive	Positive
40	17.6	13.9	14.1	> 37.0	Positive	Positive
41	25.0	18.8	20.2	> 37.0	Positive	Positive
42	28.6	19.6	21.5	23.3	Positive	Positive
43	22.9	16.7	17.5	> 37.0	Positive	Positive
44	18.2	14.5	15.1	> 37.0	Positive	Positive
45	14.1	13.7	13.8	> 37.0	Positive	Positive
46	32.9	29.2	29.2	> 37.0	Positive	Positive
47	23.5	16.8	17.9	> 37.0	Positive	Positive
48	20.3	15.2	16.1	> 37.0	Positive	Positive
49	29.8	24.0	24.9	> 37.0	Positive	Positive
50	24.0	19.2	20.2	> 37.0	Positive	Positive
51	25.5	21.3	22.1	> 37.0	Positive	Positive
52	34.6	30.9	31.9	> 37.0	Positive	Positive
53	16.2	13.7	13.9	> 37.0	Positive	Positive
54	27.6	20.1	21.4	> 37.0	Positive	Positive
55	22.0	16.2	17.6	17.1	Positive	Positive
56	22.6	18.4	18.2	> 37.0	Positive	Positive
57	29.4	20.6	22.4	23.7	Positive	Positive
58	29.5	27.4	27.1	> 37.0	Positive	Positive
59	16.4	14.1	14.3	> 37.0	Positive	Positive
60	29.4	21.8	23.8	> 37.0	Positive	Positive
61	19.9	14.6	15.0	> 37.0	Positive	Positive
62	26.7	22.6	23.6	> 37.0	Positive	Positive
63	23.7	16.3	18.1	19.2	Positive	Positive
64	20.7	16.2	16.9	> 37.0	Positive	Positive
65	25.1	19.9	20.8	> 37.0	Positive	Positive
66	33.6	28.2	29.7	> 37.0	Positive	Positive
67	32.2	25.4	25.6	> 37.0	Positive	Positive
68	19.3	14.4	14.9	> 37.0	Positive	Positive
69	17.5	14.5	14.9	> 37.0	Positive	Positive
70	30.8	26.5	26.3	> 37.0	Positive	Positive

When NPVTM samples were received, RNA was isolated from each respective sample using the MagMAX MVP II kit. The presence or absence of SARS-CoV-2 was confirmed in-house using the TaqPath COVID-19 Combo Kit assay (Table 1). Comparison of GeneXpert C_t values with internally collected C_q values resulted in highly correlative detection of SARS-CoV-2 RNA. Pearson correlation coefficients between the GeneXpert C_t values and TaqPath C_q values for *orf1ab* or N gene were calculated as 0.94 and 0.96, respectively. Based on the ranges of C_t and C_q values provided by the independent qPCR-based detection assays, these NPVTM samples likely have viral loads that vary over 5–6 orders of magnitude. Furthermore, negative samples were confirmed to lack SARS-CoV-2 RNA as defined by the TaqPath COVID-19 Combo Kit assay.

NPVTM samples that were confirmed to be positive or negative for SARS-CoV-2 were evaluated using the Colorimetric ReadiLAMP Kit. Using the kit's RNA isolation protocol, the recommended sample input of 5 µL (one-half of that used in the TaqPath COVID-19 Combo Kit assay) was included in the LAMP reaction. The Colorimetric ReadiLAMP assay resulted in detection of SARS-CoV-2 in 100% of the positive samples and no detection of SARS-CoV-2 in 100% of the negative samples (Table 1, Figure 1). No false-negative or false-positive detection was observed. Therefore, the Colorimetric ReadiLAMP assay sensitivity, defined as the proportion of true positives to all positive reactions (35/35), was 100%; while the specificity, defined as the proportion of true negatives to all negative reactions (35/35), was also 100% for these NPVTM samples (Table 2).

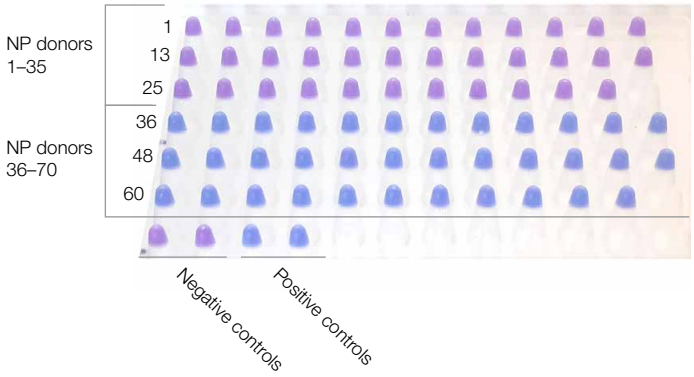

Figure 1. The Colorimetric ReadiLAMP Kit assay accurately and specifically detects SARS-CoV-2 RNA isolated from NPVTM samples.

It is interesting to note that of the samples positive for SARS-CoV-2, nearly 90% exhibited S gene dropout in the TaqPath COVID-19 Combo Kit assay (Table 1). Some SARS-CoV-2 strains, including some Alpha and Omicron variants, lack histidine 69 and valine 70 in the S gene (commonly referred to as 69-70del), resulting in the characteristic S gene dropout [1-4]. It has been suggested that the amplification of *orf1ab* and N gene, but not the amplification of the S gene, in a single sample may therefore be used as a marker or proxy for 69-70del variants prior to genome sequencing [1,4]. According to the Centers for Disease Control and Prevention COVID Data Tracker, approximately 89.1% of reported COVID-19 cases during the week of February 1, 2022, were attributed to the Omicron variant (B.1.1.529 or BA.1.1), with an additional 10.1% of cases attributed to the Delta variant (B.1.617.2) [5]. Based on the high prevalence of the Omicron variant and the observation of S gene dropout in the NPVTM samples examined herein, the majority of positive samples detected by the Colorimetric ReadiLAMP Kit in this report may have been the Omicron variant. The potential use of this kit for the detection of the Omicron variant and its subvariants is further supported by the observation that the Colorimetric ReadiLAMP Kit detects synthetic SARS-CoV-2 Omicron and Delta variant RNA controls (available as an application note at thermofisher.com/readilamp).

Table 2. The Colorimetric ReadiLAMP Kit and TaqPath COVID-19 Combo Kit demonstrate high concordance for SARS-CoV-2 RNA detection.

		TaqPath COVID-19 Combo Kit (10µL input)		
		Positive detection	Negative detection	Total
Colorimetric ReadiLAMP Kit, SARS-CoV-2 (5 µL input)	Positive detection	35	0	35
	Negative detection	0	35	35
	Total	35	35	70

Conclusions

The detection of SARS-CoV-2 using the Colorimetric ReadiLAMP Kit is highly concordant with pathogen detection using two independent qPCR-based approaches. By evaluating 70 NPVTM samples collected during February 2022, the Colorimetric ReadiLAMP Kit assay performance was measured as 100% sensitive and 100% specific for SARS-CoV-2 concentrations spanning five orders of magnitude.

Furthermore, based on observed S gene dropout and the SARS-CoV-2 variant prevalence, it is likely that the Colorimetric ReadiLAMP Kit assay performance reported here corresponds predominantly to Omicron variant detection. These results suggest that the Colorimetric ReadiLAMP Kit assay is a sensitive and specific test for the detection of SARS-CoV-2 strains, including Omicron and Alpha variants.

Ordering information

Product	Cat. No.
Colorimetric ReadiLAMP Kit, SARS-CoV-2	A52539/A52544
MicroAmp Optical 96-Well Reaction Plate	4316813
MicroAmp Clear Adhesive Film	4306311
VeritiPro Thermal Cycler, 96 well	A48141
MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	A48383
TaqPath COVID-19 Combo Kit	A47814
QuantStudio 7 Pro Real-Time PCR System, 96-well, 0.2 mL	A43162
KingFisher Flex Purification System, with 96 Deep-Well Head	5400630

References

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3. United States Food and Drug Administration (December 28, 2021) SARS-CoV-2 viral mutations: impact on COVID-19 tests. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests.
4. Thermo Fisher Scientific (January 7, 2022) TaqPath COVID-19 tests support detection of SARS-CoV-2 in samples containing the Omicron variant. https://www.thermofisher.com/blog/clinical-conversations/taqpath-covid-19-tests-support-detection-of-sars-cov-2-in-samples-containing-the-omicron-variant-3.
5. Centers for Disease Control and Prevention (March 10, 2022) COVID data tracker. Atlanta, GA: US Department of Health and Human Services, CDC. https://covid.cdc.gov/covid-data-tracker.

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