TaqCheck SARS-CoV-2 Fast PCR Assay

Impact of storage and transport of raw saliva samples on SARS-CoV-2 detection

Introduction

The emergence of SARS-CoV-2 infections in 2019 and the resulting crisis has disrupted economic and personal activities, and continues to pose a serious risk to certain populations, including the elderly and those with certain preexisting underlying conditions. Day-to-day life has been severely impacted across the globe, requiring population-level testing for SARS-CoV-2 infection as a tool to slow the spread of the virus. This population-level testing has already been used in schools, universities, businesses, and airports. Along with other tools such as mask wearing and social distancing, population-level SARS-CoV-2 testing is being more widely implemented across communities to curb the spread of the virus.

To address the future need for high-throughput surveillance with a fast time-to-result and a simplified workflow, the Applied Biosystems™ TaqCheck™ SARS-CoV-2 Fast PCR Assay was developed. The simplified sample collection step utilizes saliva samples instead of nasal or nasopharyngeal swabs, and does not require a health care provider for the collection. With no RNA extraction step, the assay is optimized for the direct use of raw saliva samples in the PCR workflow. The workflow has been tested with five Applied Biosystems™ real-time PCR instruments, which provide more flexibility for laboratories. Finally, the faster PCR cycling conditions result in faster turnaround times, allowing for up to a 40% increase in capacity. All of these features have resulted in an assay optimized for executing high-frequency SARS-CoV-2 population testing.

The multiplex Applied Biosystems™ TaqMan® Assay targets the endogenous human RNase P gene as an indicator of sample adequacy, and also detects SARS-CoV-2 by targeting both the nucleocapsid (N) gene and the spike (S) gene. This dual-target strategy for SARS-CoV-2 detection provides an added level of confidence in the results obtained, even with emerging SARS-CoV-2 variants that may harbor mutations in the N and/or S genes. The assay configuration is shown in Table 1.

Table 1. TaqCheck SARS-CoV-2 Fast PCR Assay configuration.

Target	Dye	Quencher
SARS-CoV-2 N gene	Applied Biosystems™ VIC™ dye	Invitrogen™ QSY™ quencher
SARS-CoV-2 S gene		QSY quencher
Human RNase P *RPP30* gene*	Applied Biosystems™ FAM™ dye	QSY quencher

* Serves as an internal positive control to monitor sample adequacy.

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Two potential sources of error that can impact the efficacy of SARS-CoV-2 detection in many sample types are improper storage and shipping conditions. Two studies are presented here. The first study addresses the impact of saliva samples—that have been collected and stored, rather than processed immediately—on SARS-CoV-2 test results. In this study, samples were collected and stored at various temperatures and processed at a later time. The second study addresses the fluctuating temperatures that samples may experience when saliva samples are collected at one location but processed at another location. In this study, saliva samples were subjected to multiple temperature cycles, to see the effects on SARS-CoV-2 detection. The impact of sample storage and shipping conditions on SARS-CoV-2 detection using TaqCheck SARS-CoV-2 Fast PCR Assay are presented in this technical note.

Part 1: Saliva storage study

Methods

Three separate saliva sample pools, each derived from four subjects previously confirmed negative for SARS-CoV-2, were used as a substrate for BEI gamma-irradiated SARS-CoV-2 (BEI Resources, Cat. No. NR-52287) spike-in at two levels: low (30 copies/5 µL PCR) and high (300 copies/5 µL PCR). Assuming an analytical sensitivity for TaqCheck SARS-CoV-2 Fast PCR Assay at 6,000 GCE/mL, the low and high spike-in levels correspond to 2x limit of detection (LOD) and 10x LOD, respectively. A negative saliva sample pool with no SARS-CoV-2 spike-in was also run. Next, each of the sample pools with SARS-CoV-2 spike-in and the negative sample pool were divided into 3 sets for storage at 25°C, 4°C, and –20°C. For each time point, a 96-well plate containing 4 replicates of each sample pool and spike-in level was stored and evaluated at various time points.

At each time point, the 96-well plate was processed according to the TaqCheck SARS-CoV-2 Fast PCR Assay protocol. The 96-well plate was incubated at 95°C for 30 minutes in the thermal cycler for heat inactivation. Next, 100 µL of the sample was added to 100 µL of premixed Thermo Scientific™ Pierce™ 2X TBE buffer with 2% Pierce™ Tween™ 20 solution. After mixing, 5 µL of the solution was used for the real-time PCR assay.

Analysis parameters and acceptance criteria

The C_t cutoff values were based on our previous internal verification of the TaqCheck SARS-CoV-2 Fast PCR Assay protocol described in the document "Evaluating analysis settings and assay verification for the TaqCheck SARS-CoV-2 Fast PCR Assay", and are shown in Table 2.

Table 2. C_t cutoff values for secondary analysis of SARS-CoV-2 assay samples.

C_t cutoff value		
SARS-CoV-2 N and S gene (VIC dye)	RNase P (FAM dye)	Results
≤37	≤32	Present
≤37	>32	Present
>37	≤32	Absent
>37	>32	Inconclusive; retest

The RNase P target is an endogenous control and is only used as an indicator of sample adequacy and successful assay performance. As long as the RNase P target produces a positive result, the storage or shipping condition is considered acceptable.

In order for the storage condition to be considered qualified for use with the TaqCheck assay, the following acceptance criteria must be met for the SARS-CoV-2 target:

1. 100% of contrived sample replicates must produce a positive result (i.e., 100% of replicates at the following levels: 2x LOD and 10x LOD)
2. ΔC_t (calculated using mean C_t for replicates at each level) <3 for SARS-CoV-2 genes at 2x LOD and 10x LOD

Results

Representative data are shown for the 10x LOD samples in Figures 1 and 2. For saliva sample pool 1, the SARS-CoV-2 target is generally stable across all temperatures and storage times tested. The C_t value is well below the C_t cutoff value of 37 for SARS-CoV-2 target at all storage times and temperatures. In contrast, the RNase P C_t value increases over time for the room temperature samples, although all samples are well below the C_t cutoff value of 32 for RNase P target.

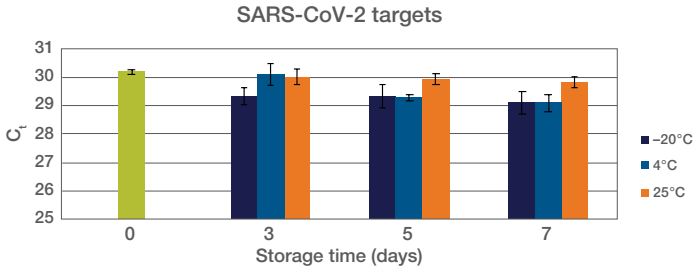

Figure 1. Effect of saliva sample storage on SARS-CoV-2 assay performance. Results are from saliva sample pool 1 with 10x LOD spike-in samples.

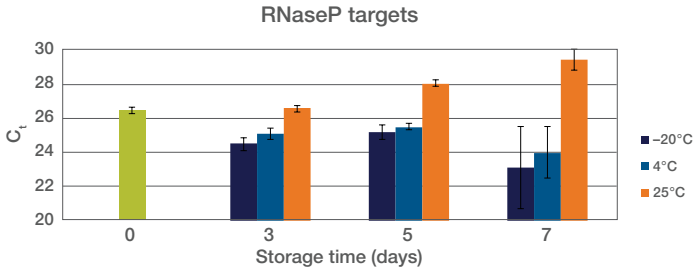

Figure 2. Effect of saliva sample storage on RNase P assay performance. Results are from saliva sample pool 1 with 10x LOD spike-in samples.

All samples produced positive samples (C_t <37) for SARS-CoV-2 at both high (10x LOD) and low (2x LOD) spike-in levels. The differences in C_t values for all storage conditions at high and low spike-in levels compared to fresh samples are shown in Figures 3 and 4, respectively. The maximum increase in C_t value with any condition was 1.3, far below our ΔC_t acceptance criterion of 3.

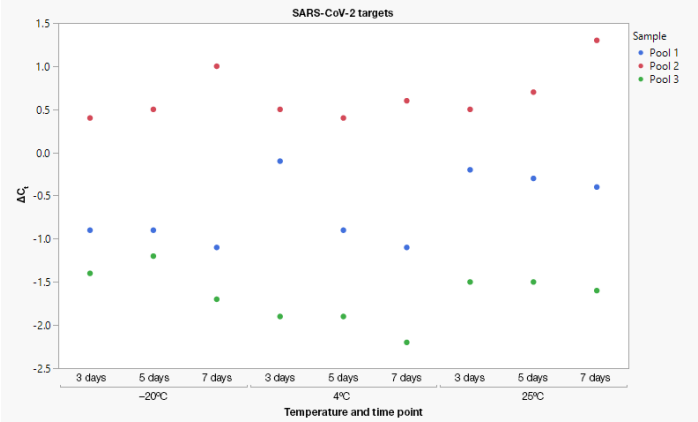

Figure 3. ΔC_t for all stored samples compared to fresh samples for SARS-CoV-2 target for high spike-in samples (10x LOD). All samples, regardless of storage temperature and duration, had ΔC_t far below the acceptance criterion of 3.

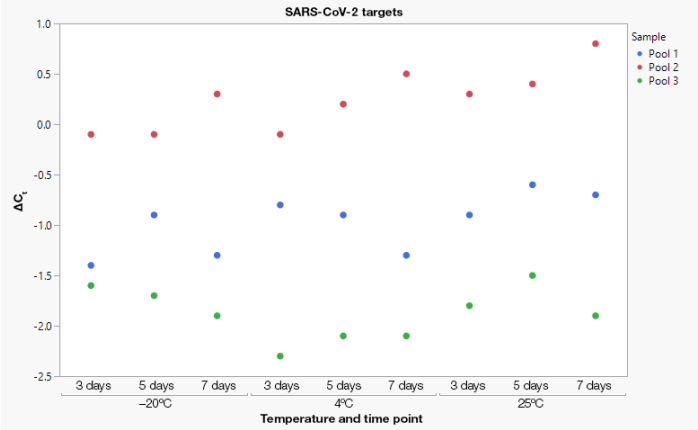

Figure 4. ΔC_t for all stored samples compared to fresh samples for SARS-CoV-2 target for low spike-in samples (2x LOD). All samples, regardless of storage temperature and duration, had ΔC_t far below the acceptance criterion of 3.

All stored samples produced positive results (C_t <32) for RNase P at both low (2x) and high (10x) spike-in levels as shown in Figures 5 and 6, respectively. For saliva sample pool 2, an upwards shift in C_t value was observed. Also, as expected, storage at room temperature resulted in higher C_t values, indicating poorer sample quality.

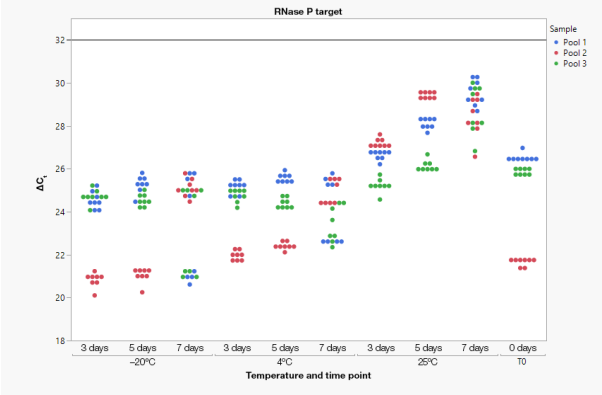

Figure 5. C_t values for all high spike-in samples (10x LOD) under post–storage conditions, compared to fresh samples, for the RNase P target.

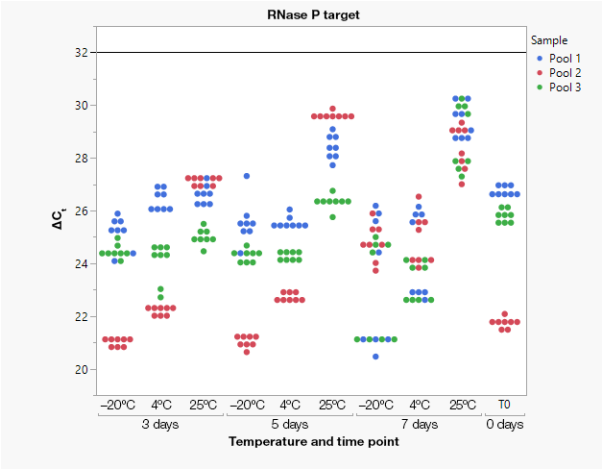

Figure 6. C_t values for all low spike-in samples (2x LOD) under post–storage conditions, compared to fresh samples, for the RNase P target.

Part 2: Saliva transport study

Methods

Four separate saliva sample pools, each derived from three subjects previously confirmed negative for SARS-CoV-2, were used as a substrate for BEI gamma-irradiated SARS-CoV-2 (BEI Resources, Cat. No. NR-52287) spike-in at two levels: low (30 copies/5 µL PCR) and high (300 copies/5 µL PCR). As the LOD for TaqCheck SARS-CoV-2 Fast PCR Assay is 6,000 GCE/mL, the low and high spike-in levels correspond to 2x LOD and 10x LOD, respectively. A negative saliva pool with no SARS-CoV-2 spike-in was also run. As with the saliva storage study, for each time point, a 96-well plate containing 4 replicates of each sample pool and spike-in level was stored and evaluated.

Each plate was stored at a range of temperatures simulating winter and summer conditions as summarized in Table 3.

At each time point, the 96-well plate was processed according to the TaqCheck SARS-CoV-2 Fast PCR Assay protocol as described in the previous Methods section.

Table 3. Temperature conditions for simulated winter and summer transport conditions.

Cycle	Cycle period	Total cycling time	Temperature profile		Replicating step	Start day and time
			Summer	Winter		Jan 27, 2021 Wednesday 6 a.m.
1	8 hours	8 hours	40°C	–10°C	After collection, before shipping	Wednesday 2 p.m.
2	4 hours	12 hours	22°C	18°C	48-hour shipping cycle	Wednesday 6 p.m.
3	2 hours	14 hours	40°C	–10°C		Wednesday 8 p.m.
4	36 hours	50 hours	30°C	10°C		Friday 8 a.m.
5	6 hours	56 hours	40°C	–10°C		Friday 2 p.m.
6	72 hours	128 hours	30°C		After arrival to lab, before testing	Feb 1, 2021 Monday 2 p.m.

Results

As with the saliva storage study, all samples post–weather simulation produced positive results (C_t <37) for SARS-CoV-2 at both low (2x LOD) and high (10x LOD) spike-in levels (data not shown). The differences in C_t values for each experimental conditions at high and low spike-in levels compared to fresh samples are shown in Figures 7 and 8, respectively. The maximum increase in C_t values with any condition was <1, far below our ΔC_t acceptance criterion of 3.

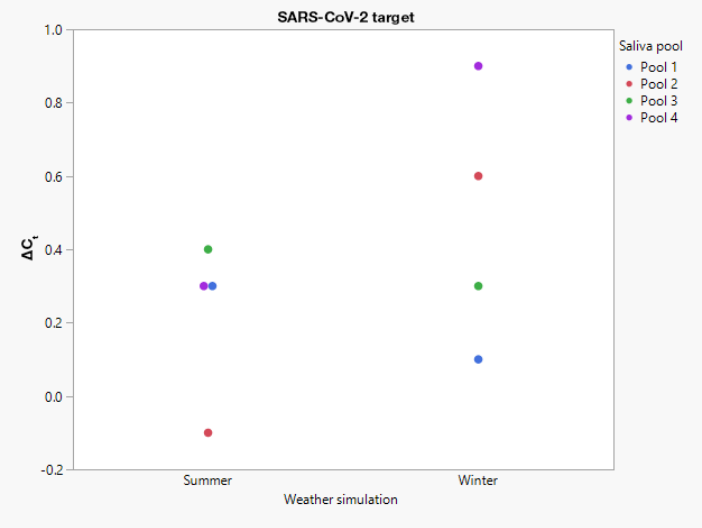

Figure 7. ΔC_t for all samples post–weather simulation compared to fresh samples, for SARS-CoV-2 target, for high spike-in samples (10x LOD). All samples, regardless of weather simulation, had ΔC_t far below the acceptance criterion of 3.

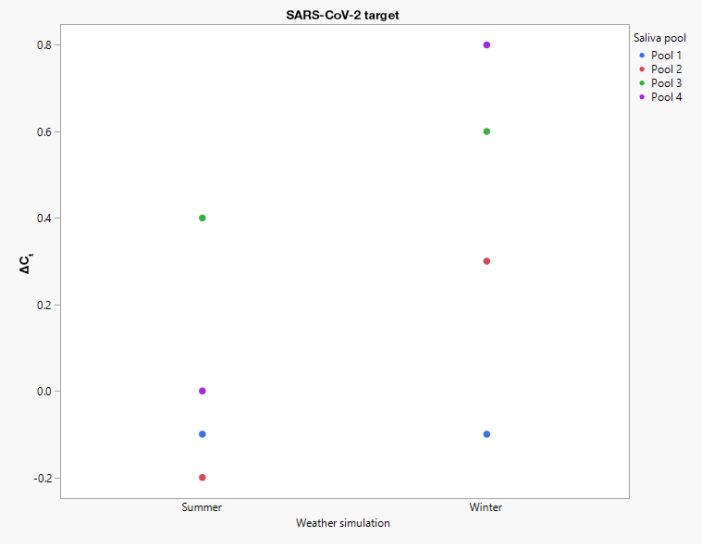

Figure 8. ΔC_t for all samples post–weather simulation compared to fresh samples, for SARS-CoV-2 target, for low spike-in samples (2x LOD). All samples, regardless of weather simulation, had ΔC_t far below the acceptance criterion of 3.

All samples post–weather simulation produced positive results (C_t <32) for RNase P at both low (2x) and high (10x) spike-in levels, as shown in Figure 9. Shipping simulation conditions for both summer and winter resulted in higher C_t values. Also as expected, summer shipping simulation resulted in higher C_t values, indicating poorer sample quality.

For the negative samples, we expected C_t <37 for SARS-CoV-2 target because no inactivated virus was added. Out of the 104 reactions run (13 time points, 4 saliva sample pools, 2 replicates), only one sample without weather simulation from sample pool 3 came close to the cutoff value, with C_t = 36.62.

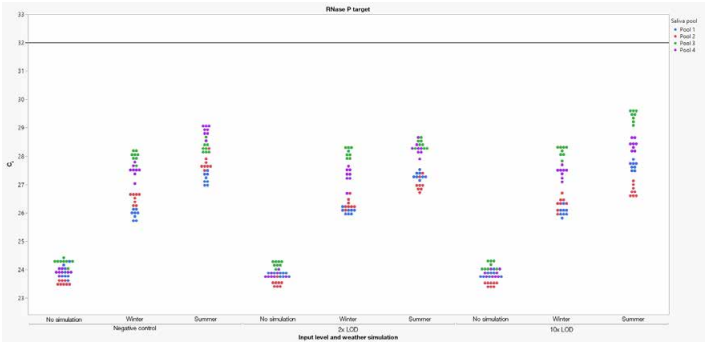

Figure 9. C_t values for all high (10x LOD) and low (2x LOD) spike-in samples and negative control (T0) samples post–weather simulation, for the RNase P target.

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Conclusions

The experiments outlined in this study show the impact of storage and shipping of raw saliva samples to be used for the TaqCheck SARS-CoV-2 Fast PCR Assay, accounting for common methods of storing and shipping saliva samples before use. These studies demonstrated the efficacy of the TaqCheck SARS-CoV-2 Fast PCR Assay in successfully detecting SARS-CoV-2 in positive samples, regardless of the tested storage and shipping conditions, thus providing a reference for any labs looking to study saliva sample storage and shipping conditions.

Find out more at **thermofisher.com/taqcheck**

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