JCM Accepted Manuscript Posted Online 27 April 2020
J. Clin. Microbiol. doi:10.1128/JCM.00708-20

Evaluation of Transport Media and Specimen Transport Conditions for the Detection of SARS-CoV-2 Using Real Time Reverse Transcription PCR

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Submitted: April 8, 2020

Revised: April 22, 2020

In Preparation for Submission to JCM as a Research Article

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Abstract: The global COVID-19 pandemic has resulted in a worldwide shortage of viral transport media and raised questions about specimen stability. The objective of this study was to determine the stability of SARS-CoV-2 virus RNA in specimen transport media under various storage conditions. Transport medium tested included: VCM, UTM®-RT, ESwab™, M4 and saline (0.9% NaCl). Specimen types tested included Nasopharyngeal/Oropharyngeal (NP/OP) swabs in the above transport media, bronchoalveolar lavage (BAL) and Sputum. A high-titer SARS-CoV-2 remnant patient specimen was spiked into pooled SARS-CoV-2 RNA-negative specimen remnants for the various media types. Aliquots of samples were stored at 18°C to 25°C, 2°C to 8°C and -10°C to -30°C and then tested at time points up to 14 days. Specimens consistently yielded amplifiable RNA with mean Ct differences of <3 over the various conditions assayed, thus supporting the use and transport of alternative collection media and specimen types under a variety of temperature storage conditions.

Introduction:

On December 31, 2019, an outbreak of respiratory disease caused by a novel coronavirus first detected in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) and has continued to expand globally(1, 2). On January 30, 2020 the United States reported the first confirmed instance of person-to-person spread of SARS-CoV-2, to an individual who had close contact to a known case(2). On March 11, 2020 the WHO declared COVID-19 a pandemic(3).

Coronaviruses are a large family of viruses that are common in many different species, including camels, cattle, cats, and bats(2). Globally, there are four common human Coronaviruses in circulation; 229E, NL63, OC43, and HKU1. Infection and spread of animal coronaviruses into people is rare. Cases of zoonotic transmission of animal coronaviruses include MERS-CoV, SARS-CoV-1, and SARS-CoV-2 (previously known as 2019-novel coronavirus, 2019-nCoV) have been described (2). Since the first reported cases of SARS-CoV-2 in December 2019 greater the 2.6 million cases have been reported globally as of April 22, 2020, according to the COVID-19 dashboard by the Center for Systems Science and Engineering at John Hopkins University (https://www.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6).

The COVID-19 pandemic has resulted in an unprecedented worldwide demand for laboratory testing. The huge increase in testing has put pressure on the laboratory supply chain and resulted in shortages of viral transport media. The Food and Drug Administration (FDA) has recommended the use of alternative viral transport media, such as liquid amies, saline and phosphate buffered saline. Further recommendations suggest specimens can be stored for up to 72 hours at 4℃, or frozen at ≤ -70℃ for longer storage(4). The objective of this study was to evaluate the detection of SARS-CoV-2 RNA by rRT-PCR, using pooled remnant respiratory specimens placed into different transport media and held under common specimen transport conditions.

Materials and Methods:

Clinical Specimens: Transport media tested at Quest Diagnostics Infectious Disease (QDID, San Juan Capistrano, CA) included VCM (Copan, Brescia, Italy), UTM®-RT (Copan, Brescia, Italy), ESwab™(Copan, Brescia, Italy), M4 media (Thermo Fisher Scientific, Waltham, MA) and Normal Saline [0.9% NaCl]. Specimen types tested included NP/OP swabs in the various transport media, bronchoalveolar lavage (BAL) and sputum. Prior to spiking, remnant sputum had been processed in 1X PBS. A known high titer SARS-CoV-2 positive specimen was utilized to spike SARS-CoV-2 RNA-negative specimen remnants for

the various media. The RNA viral load was estimated based on cycle threshold (Ct) values to be approximately 1,500 copies/mL.

Study Design: Samples were aliquoted and stored for up to 14 days and tested at multiple time points and storage temperatures (18°C to 25°C, 2°C to 8°C and -10°C to -30°C). Five aliquots were assayed at each condition. Molecular analysis was performed utilizing the QDID SARS-CoV-2 RNA, Qualitative Real-Time RT-PCR EUA assay according the package instructions for use (5). A positive result for SARS-CoV-2 RNA was defined as a Ct value of <40 for both the N1 and N3 detectors. Samples were tested before storage (T=0) to obtain an initial Ct result. Samples were deemed stable if the mean Ct values were within ≤ 3 amplification cycles of the mean initial Ct value.

Additional Saline Studies: Storage studies using Normal Saline [0.9% NaCl] were also performed at Quest Diagnostics Marlborough, MA, using the Quest Diagnostics and the Roche Diagnostics cobas® SARS-CoV-2 EUA tests. A high-titer SARS-CoV-2 positive patient specimen with a Ct of 18 established the Roche cobas® system was diluted 1:1,000 to obtain a calculated Ct value of 28. For each time point, samples were tested in triplicate at this concentration. A further 1:10 dilution of this material (calculated Ct = 31) was also tested in duplicate at each time point.

Statistical Analysis: Statistical analysis was performed using Analyse-it for Microsoft Excel version 5.40.2.

Results:

SARS-CoV-2 RNA was consistently detected in all transport media, specimen types, and storage conditions tested; mean Ct values obtained for SARS-Cov-2 for the various study-defined transport media and storage temperatures are shown in Table 1. Differences in average viral RNA Ct values were similar across all media and temperature storage conditions assayed (Table 1). Mean Ct differences between Day 0 and Day 7 for all media tested at room temperature were 0.6 ± 0.7 Cts. Refrigerated and frozen samples exhibited mean Ct differences of 0.5 ± 0.7 and 0.7 ± 1.1 Cts respectively. For frozen ESwab™ samples one of five replicates at day 5 did not yield detectable RNA for the N3 target (Table 1). Detection of only one of two targets in the assay is considered an inconclusive result. For saline and ESwab™ transport media there was a shift of >2 Cts in the average Ct between Day 0 and Day 7 and/or Day 14 (Table 1 & 2). These changes would not have altered the interpretation of positive results.

A further stability study performed at a second site assessed the stability of SARS-CoV-2 RNA in saline for up to 14 days using both the Quest and the Roche cobas® EUA assays. As shown in Table 2, SARS-Cov-2 RNA remained detectable for 14 days on both platforms. For the samples tested by the Quest and the cobas® EUA on frozen saline, we observed minimal variation (<1 Ct on average) in the mean Ct values over 14 days. However, at room temperature and refrigerated storage conditions, we noted a >2 Ct increase over 14 days (Table 2). The increase in the Ct values was linear over 14 days, with a slope of 0.14 and 0.15 Cts per day for the two targets (R^2: 0.79 and 0.78) at room temperature and 0.13 Cts per day (R^2: 0.83 and 0.81) while stored refrigerated. A further 1:10 dilution of the SAR-CoV-2 RNA (calculated Ct = 31) was also tested in duplicate at each time point and comparable results and trends were observed (data not shown).

Discussion:

Limited stability studies are available in the literature for SARS-CoV-2 RNA. A study of SARS-CoV-2 in aerosols and on surfaces demonstrated that culturable SAR-CoV-2 was detectable in aerosols for up to three hours, up to four hours on copper, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel (6). Given the persistence of SARS-CoV-2 in the environment, it is not surprising that RNA can be reliably amplified from viral transport media after relatively long storage times, even at room temperature.

Prior stability studies of SARS-Cov-2 RNA in various transport media and conditions are also limited. The results reported here are consistent with the findings of Druce et al (7) who examined the stability of four common viruses with different physicochemical properties in several swab and transport media and storage combinations. The authors demonstrated that influenza, enterovirus, herpes simplex virus and adenovirus were detected by PCR at 22°C and refrigerated 4°C for up to 7 days.

Stability using various viral transport media performed with in-house laboratory developed tests for Influenza and Rubeola viruses (both enveloped RNA viruses) are also consistent with viral RNA detection at 18 to 26°C after 7 days, 2 to 8°C after 14 days and -10 to -30°C after 30 days (data not shown). Rodino et al (8) demonstrated reliable detection of SARS-CoV-2 RNA in swab stored in MEM, PBS, Saline and VTM after 7 days at 2-8°C and frozen at -20°C using an in-house EUA as well as the Roche cobas® EUA.

Qualitative detection of SARS-CoV-2 RNA was unchanged over the various combinations of transport media and conditions tested at two study sites regardless of molecular platform utilized. While stability in saline stored at room temperature and under refrigerated conditions exhibited a linear trend with increasing Ct values over time, these trends did not impact the qualitative interpretation of positive results. Additionally, specimen stability was assessed in this study for a significantly longer duration than would be deemed acceptable for routine clinical testing, further reducing the probability of clinical impact. The greatest theoretical impact of this linear trend would be for specimens with low titers of virus. In our experience with clinical specimens, however, the majority of positive specimens tested exhibited low Ct values (<30) which correlate to specimens with higher titers of virus. Viral titers may be impacted by the clinical course at time of sample acquisition and the quality of the specimen collection. These data provide additional supporting evidence for the use of alternative viral transport media and temperature storage conditions for the detection SARS-CoV-2 RNA using sensitive rRT-PCR assays.

Table 1. Stability of SARS-COV-2 RNA detected by the Quest EUA rRT-PCR[1]

		Room Temperature (18 to 26°C) N=5; mean (sd)				
Media/sample type	Detector[2]	Day 0	Day 2	Day 3	Day 5	Day 7
VCM	N1	31.8 (0.2)	31.6 (0.4)	31.6 (0.3)	31.6 (0.4)	31.5 (0.8)
	N3	31.3 (0.4)	30.8 (0.2)	30.8 (0.5)	30.8 (0.4)	30.7 (0.7)
UTM®-R	N1	31.8 (0.3)	31.6 (0.3)	31.3 (0.2)	31.9 (0.4)	33.1 (0.4)
	N3	31.2 (0.4)	30.8 (0.3)	31.0 (0.2)	31.1 (0.3)	32.2 (0.4)
ESwab™	N1	31.7 (0.4)	32.0 (0.4)	31.9 (0.5)	31.9 (0.4)	32.6 (1.0)
	N3	31.3 (0.3)	31.1 (0.2)	30.9 (0.3)	31.0 (0.3)	31.6 (0.7)
M4	N1	31.8 (0.2)	32.1 (0.4)	31.5 (0.2)	32.4 (0.5)	32.6 (0.6)
	N3	31.3 (0.4)	31.2 (0.4)	31.0 (0.1)	31.2 (0.2)	31.6 (0.4)
Saline	N1	29.2 (0.7)	29.9 (0.3)	30.3 (0.1)	30.7 (0.3)	31.1 (0.3)
	N3	28.4 (0.6)	29.1 (0.2)	29.5 (0.1)	29.9 (0.5)	30.0 (0.2)
BAL	N1	31.8 (0.2)	31.5 (0.2)	32.2 (0.5)	31.4 (0.3)	32.4 (0.4)
	N3	31.1 (0.2)	30.8 (0.2)	31.2 (0.4)	30.6 (0.3)	31.2 (0.4)
Sputum	N1	31.4 (0.5)	31.8 (0.6)	32.1 (0.4)	31.8 (0.4)	32.1 (0.3)
	N3	30.8 (0.6)	31.1 (0.7)	31.1 (0.5)	30.8 (0.3)	31.1 (0.3)

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		Refrigerated (2 to 8°C) N=5; mean (sd)					
Media/sample type	Detector[2]	Day 0	Day 3	Day 5	Day 7	Day 10	Day 14
VCM	N1	31.8 (0.2)	31.8 (0.2)	31.8 (0.4)	31.9 (0.6)	32.2 (0.4)	32.1 (0.3)
	N3	31.3 (0.4)	31.3 (0.4)	31.1 (0.5)	31.0 (0.5)	31.6 (0.4)	31.4 (0.4)
UTM®-R	N1	31.8 (0.3)	31.4 (0.3)	32.1 (0.1)	31.1 (1.8)	32.5 (0.2)	32.1 (0.3)
	N3	31.2 (0.4)	30.8 (0.4)	31.3 (0.2)	30.4 (1.7)	31.6 (0.1)	31.4 (0.3)
ESwab™	N1	31.7 (0.4)	31.9 (0.3)	31.7 (0.2)	31.8 (0.3)	31.7 (0.4)	31.8 (0.4)
	N3	31.3 (0.3)	31.0 (0.4)	30.7 (0.2)	30.7 (0.3)	30.8 (0.4)	31.2 (0.4)
M4	N1	31.8 (0.2)	31.7 (0.3)	32.0 (0.4)	31.5 (1.5)	31.8 (0.5)	31.9 (0.2)
	N3	31.3 (0.4)	31.0 (0.3)	31.1 (0.3)	30.7 (1.2)	31.3 (0.6)	31.3 (0.3)
Saline	N1	29.2 (0.7)	30.1 (0.2)	30.0 (0.4)	30.1 (1.7)	30.4 (1.2)	31.3 (0.4)[3]
	N3	28.4 (0.6)	28.9 (0.2)	29.0 (0.5)	28.9 (1.4)	29.4 (1.0)	30.5 (0.4)[3]
BAL	N1	31.8 (0.2)	32.2 (0.6)	31.0 (1.4)	32.5 (0.1)	32.1 (0.6)	32.4 (0.7)
	N3	31.8 (0.2)	32.2 (0.6)	31.0 (1.4)	32.5 (0.1)	32.1 (0.6)	31.5 (0.5)
Sputum	N1	31.4 (0.5)	31.4 (0.5)	31.8 (0.6)	32.1 (0.5)	32.1 (0.4)	32.2 (0.7)
	N3	30.8 (0.6)	30.7 (0.9)	31.0 (0.4)	31.0 (0.4)	31.8 (0.6)	31.6 (0.8)

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		Frozen (-10-to -20°C) N=5; mean (sd)				
Media/sample type	Detector[3]	Day 0	Day 3	Day 5	Day 7	Day 14
VCM	N1	31.8 (0.2)	31.9 (0.4)	31.5 (0.3)	32.0 (0.4)	32.0 (0.4)
	N3	31.3 (0.4)	31.1 (0.3)	30.6 (0.4)	31.1 (0.3)	31.2 (0.4)
UTM®-R	N1	31.8 (0.3)	31.7 (0.4)	31.9 (0.4)	32.3 (0.2)	31.7 (0.4)
	N3	31.2 (0.4)	31.2 (0.3)	31.0 (0.4)	31.4 (0.4)	31.0 (0.2)
ESwab™	N1	31.7 (0.4)	34.3 (1.1)	34.8 (0.4)	31.8 (0.3)	33.5 (0.6)
	N3	31.3 (0.3)	33.7 (0.9)	34.5 (0.4)*	31.7 (0.4)	33.8 (0.6)[3]
M4	N1	31.8 (0.2)	32.1 (0.7)	32.0 (0.5)	31.0 (3.0)	31.5 (0.2)
	N3	31.3 (0.4)	31.6 (0.7)	31.1 (0.5)	30.1 (3.0)	31.0 (0.3)
Saline	N1	29.2 (0.7)	30.6 (0.5)	31.4 (0.3)	31.7 (0.4)[3]	31.6 (0.9)[3]
	N3	28.4 (0.6)	29.6 (0.4)	30.3 (0.3)	30.6 (0.4)[3]	30.7 (0.9)[3]
BAL	N1	31.8 (0.2)	32.4 (0.6)	32.2 (0.4)	32.5 (0.3)	32.5 (0.1)
	N3	31.1 (0.2)	31.3 (0.4)	31.3 (0.3)	31.8 (0.3)	31.6 (0.2)
Sputum	N1	31.4 (0.5)	31.8 (1.0)	31.6 (0.3)	31.8 (0.5)	31.8 (0.3)
	N3	30.8 (0.6)	30.9 (1.0)	30.7 (0.1)	30.8 (0.5)	31.0 (0.4)

* 1/5 not detected

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155 [1] Testing was performed at QDID. Five replicates were assayed by rRT-PCR at each of the indicated conditions
156 [2] N1 and N3 Taqman® probes as described in the Quest Diagnostics EUA package insert
157 [3] Increase of >2 Cts from Day 0
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159 Table 2. Additional Stability of SARS-COV-2 RNA in Saline detected by by the Quest EUA and Roche cobas EUA[1]

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Room Temperature (18 to 26°C)
N=3; mean (sd)

Platform	Detector[2]	Day 0	Day 1	Day 2	Day 3	Day 5	Day 7	Day 14
Quest EUA	N1	26.2 (0.1)	26.3 (0.0)	26.1 (0.5)	26.8 (0.1)	27.2 (0.1)	26.4 (0.1)	26.8 (0.4)
	N3	30.2 (0.1)	30.3 (0.1)	30.0 (0.4)	30.7 (0.1)	31.5 (0.1)	29.3 (0.1)	29.3 (0.3)
Cobas	ORF 1a/b	27.9 (0.3)	28.4 (0.2)	28.5 (0.1)	29.1 (0.1)	29.4 (0.2)	29.5 (0.0)	30.0 (0.2)[3]
	E gene	28.1 (0.3)	28.6 (0.2)	28.7 (0.1)	29.3 (0.2)	29.7 (0.2)	29.9 (0.2)	30.4 (0.2)[3]

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Refrigerated (2 to 8°C)
N=3; mean (sd)

Platform	Detector[2]	Day 0	Day 1	Day 2	Day 3	Day 5	Day 7	Day 14
Quest EUA	N1	26.2 (0.1)	26.4 (0.1)	26.0 (0.2)	26.5 (0.0)	27.0 (0.1)	26.3 (0.2)	26.5 (0.2)
	N3	30.2 (0.1)	30.2 (0.1)	29.8 (0.2)	30.6 (0.0)	31.3 (0.1)	29.0 (0.2)	29.1 (0.1)
Cobas	ORF 1a/b	27.9 (0.3)	28.5 (0.1)	28.5 (0.1)	28.8 (0.1)	29.2 (0.1)	29.3 (0.1)	29.9 (0.1)
	E gene	28.1 (0.3)	28.8 (0.1)	28.7 (0.1)	29.1 (0.1)	29.5 (0.1)	29.5 (0.2)	30.2 (0.2)[3]

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Frozen (-70 to -90°C)
N=3; mean (sd)

Platform	Detector[2]	Day 0	Day 1	Day 2	Day 3	Day 5	Day 7	Day 14
Quest EUA	N1	26.2 (0.1)	26.3 (0.0)	26.1 (0.5)	26.8 (0.1)	27.2 (0.1)	26.4 (0.1)	26.8 (0.4)
	N3	30.2 (0.1)	30.3 (0.1)	30.0 (0.4)	30.7 (0.1)	31.5 (0.1)	29.3 (0.1)	29.3 (0.3)
Cobas	ORF 1a/b	27.9 (0.3)	28.4 (0.1)	28.2 (0.2)	28.7 (0.2)	28.5 (0.2)	28.3 (0.1)	28.4 (0.1)
	E gene	28.1 (0.3)	28.7 (0.2)	28.4 (0.3)	28.7 (0.1)	28.8 (0.1)	28.6 (0.1)	28.7 (0.0)

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[1] Testing was performed at Quest Diagnostics Marlborough, MA. Three replicates were assayed by rRT-PCR at each of the indicated conditions

[2] N1 and N3 Taqman® probes as described in the Quest Diagnostics EUA package insert, and ORF 1a/1b and E gene probes as described in the Roche Diagnostics EUA package insert

[3] Increase of >2 Cts from Day 0

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