

# Detection of Severe Acute Respiratory Syndrome Coronavirus in Stool Specimens by Commercially Available Real-Time Reverse Transcriptase PCR Assays<sup>∇</sup>

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**Three commercially available real-time reverse transcriptase PCR assays (the Artus RealArt HPA coronavirus LightCycler, the Artus RealArt HPA coronavirus Rotor-Gene, and the EraGen severe acute respiratory syndrome coronavirus POL assay) and three RNA extraction methodologies were evaluated for the detection of severe acute respiratory syndrome coronavirus RNA from 91 stool specimens. The assays' sensitivities were highest (58% to 75%) for specimens obtained 8 to 21 days after symptom onset. The assays were less sensitive when specimens were obtained less than 8 days or more than 21 days after the onset of symptoms. All assays were 100% specific.**

The discovery of a novel coronavirus (CoV) as the causative agent of severe acute respiratory syndrome (SARS) has led to the development of reverse transcriptase (RT) PCR-based assays for detecting this virus (1, 7, 9, 15). Although SARS is associated primarily with fever and involvement of the respiratory tract, gastrointestinal symptoms may also occur (2, 10). The presence of active viral replication within the large and small intestines, as demonstrated by biopsy samples obtained from colonoscopies or autopsies, suggests that stool may be a useful specimen for SARS-CoV detection (11). The rate of detection of SARS-CoV from stools has been at least as high as that from other clinical specimens by using conventional nested RT-PCR (16), and stool specimens have been a useful alternative to respiratory samples for the detection of SARS-CoV (14, 18). Although there have been evaluations of both conventional and real-time RT-PCR-based assays (12, 19), few studies have evaluated newer, second-generation assays for the detection of SARS-CoV RNA from stool specimens (5). In an outbreak that is short-lived and involves an emerging pathogen, the evaluation of newer diagnostic tests is difficult. While the specimens used were not ideal, we sought to overcome this problem with the use of frozen, archived stool specimens. In this study, we evaluated three RNA extraction methods and three commercially available second-generation, real-time RT-PCR-based assays for the detection of SARS-CoV by using a collection of archived stool samples. We compared these results to those obtained using a conventional nested RT-PCR assay performed at the time of receipt of these same specimens

in 2003. The results of this evaluation may be useful, should human infection due to SARS-CoV recur.

**Clinical specimens.** A total of 91 stool specimens from 57 patients were obtained between April and August 2003 during an outbreak of SARS in Toronto, Ontario, Canada. Thirty-four patients met the WHO case definition for probable or suspected SARS (17) and were seropositive for SARS-CoV antibodies during the convalescent phase of illness, as determined by the Euroimmun immunofluorescent assay (immunoglobulin G; Euroimmun, Lübeck, Germany) (8, 13, 14). Twenty-three patients with other respiratory illnesses did not meet the case definition for SARS and were seronegative for SARS-CoV antibodies. There were 56 stool specimens available for testing from the seropositive individuals and 35 specimens from those who were seronegative.

TABLE 1. Summary of SARS-CoV RT-PCR results for seven methods

No. of specimens	Test result <sup>a</sup>						
	QIAGEN viral RNA minikit			bioMérieux miniMag		Cortex MagaZorb	
	Conventional	LC Artus	EraGen	RG Artus	EraGen	RG Artus	EraGen
67	—	—	—	—	—	—	—
17	+	+	+	+	+	+	+
2	+	+	—	—	—	—	+
1	+	+	+	+	+	—	+
1	+	+	—	+	—	+	+
1	—	—	+	—	+	+	+
1	+	+	+	—	—	—	—
1	—	—	—	—	—	—	+

<sup>a</sup> Conventional, conventional nested RT-PCR; LC Artus, Artus RealArt HPA coronavirus LightCycler; EraGen, EraGen SARS coronavirus POL assay; RG Artus, Artus RealArt HPA coronavirus Rotor-Gene.

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TABLE 2. Sensitivities and specificities of seven methods for detection of SARS-CoV RNA in stool samples compared to those of conventional serology

Assay method <sup>a</sup>	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
<b>QIAGEN viral RNA minikit</b>				
Conventional	39	100	100	51
LC Artus	39	100	100	51
EraGen	36	100	100	49
<b>bioMérieux miniMag</b>				
RG Artus	34	100	100	49
EraGen	34	100	100	49
<b>Cortex MagaZorb</b>				
RG Artus	34	100	100	49
EraGen	40	100	100	51

<sup>a</sup> Conventional, conventional nested RT-PCR; LC Artus, Artus RealArt HPA coronavirus LightCycler; EraGen, EraGen SARS coronavirus POL assay; RG Artus, Artus RealArt HPA coronavirus Rotor-Gene.

**RNA extraction.** RNA was extracted from fresh stool samples upon receipt in the laboratory in 2003 using a viral RNA minikit (QIAGEN Inc., Mississauga, Ontario, Canada) according to the manufacturer's instructions, using 140  $\mu$ l of a 10% (wt/vol) stool suspension made in 5 ml of sterile diethylpyrocarbonate-treated water (Invitrogen, Burlington, Ontario, Canada). RNA was eluted in a final volume of 50  $\mu$ l of elution buffer. Stool specimens were then frozen at  $-80^{\circ}\text{C}$  until they were retested for this evaluation.

For the present evaluation, stool samples were thawed, and a 10% (wt/vol) stool suspension was made in sterile diethylpyro-

carbonate-treated water. RNA was extracted with three different extraction methods, each performed in accordance with the manufacturer's instructions: the viral RNA minikit, the miniMag (bioMérieux, St. Laurent, Quebec, Canada), and the MagaZorb RNA miniprep kit (Cortex Biochem, Inc., San Leandro, CA). A sample of 140  $\mu$ l was used for both the viral RNA minikit and the miniMag extractions, while 200  $\mu$ l was used for the MagaZorb extraction. RNA was eluted in a final volume of 50  $\mu$ l elution buffer.

**RT-PCR assays.** Conventional, nested RT-PCR was performed as described by Drosten et al. (6). Real-time RT-PCR with the RealArt HPA coronavirus (HPAC) LightCycler and RealArt HPAC Rotor-Gene kits (Artus GmbH, Hamburg, Germany) and the SARS coronavirus POL assay (EraGen Biosciences, Madison, WI) were performed according to the manufacturers' instructions. The target for the real-time RT-PCR assays was the viral polymerase gene, although details regarding the target for each assay are not available as the test kits evaluated are proprietary. All the RNA eluates were tested using the EraGen and Artus kits. The EraGen and the Artus HPAC LightCycler assays were performed with a LightCycler version 1.2 (Roche Diagnostics, Laval, Quebec), and the Artus HPAC Rotor-Gene assay was performed with a Rotor-Gene model 3000 (Corbett Life Science, Sydney, Australia). Only the Artus kits provided positive controls with which to generate a standard curve for viral load quantitation, but this feature of the assays was not evaluated.

All methods were compared, and their clinical sensitivities and specificities were calculated using serologic evidence for the presence of SARS-CoV antibodies as the gold standard.

A summary of all test results is shown in Table 1. Of the 91 specimens tested, 67 were negative and 17 were positive for

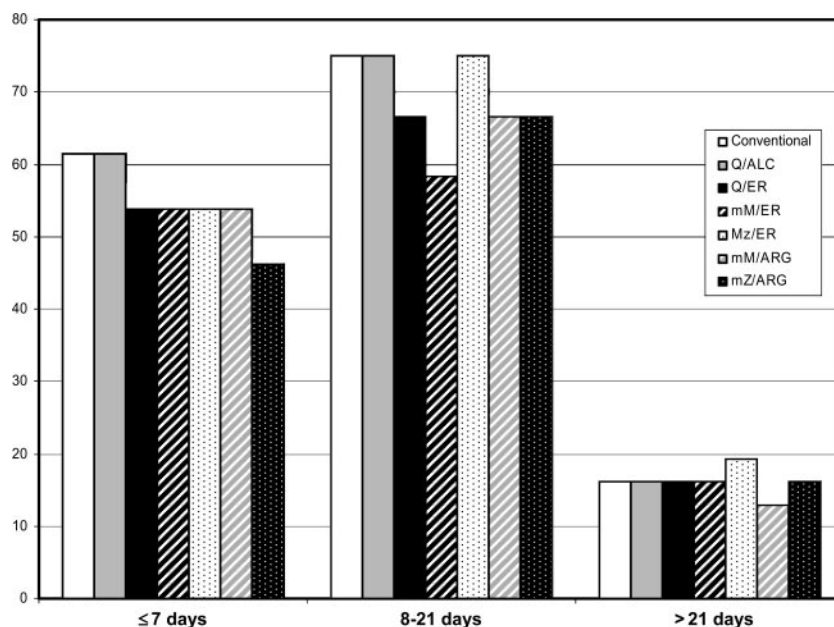


FIG. 1. SARS-CoV RT-PCR positivity rates by timing of specimen collection. Conventional, conventional nested RT-PCR; Q/ALC, QIAGEN viral RNA minikit/Artus RealArt HPA coronavirus LightCycler; Q/ER, QIAGEN viral RNA minikit/EraGen SARS coronavirus POL assay; mM/ER, bioMérieux miniMag/EraGen SARS coronavirus POL assay; Mz/ER, Cortex MagaZorb/EraGen SARS coronavirus POL assay; mM/ARG, bioMérieux miniMag/Artus RealArt HPA coronavirus Rotor-Gene; mZ/ARG, Cortex MagaZorb/Artus RealArt HPA coronavirus Rotor-Gene.

SARS-CoV RNA by all seven methods. Twenty-three specimens were found positive for viral RNA by using three or more RT-PCR assays. One specimen was positive by only one of the assay methods (EraGen). Although this outcome may have represented a false-positive test result, the patient met the case definition for SARS and was seropositive for SARS-CoV antibodies.

Although all the assays had 100% specificity, the sensitivities were low, ranging from 34 to 40% (Table 2). There were no statistically significant differences between the sensitivities or specificities of the assays evaluated. However, when the assays were analyzed according to the time of specimen collection from the onset of symptoms, there were certain times that were associated with an improved ability to detect viral RNA (Fig. 1). The sensitivities of RT-PCR for the 13 specimens collected  $\leq 7$  days from the onset of symptoms ranged from 46 to 62%, whereas between 8 and 21 days after symptom onset, the sensitivities ranged from 58 to 75% (12 specimens). For the 31 specimens obtained  $>21$  days after symptom onset, the sensitivities were only 13 to 19%. When individual RT-PCR assay results were compared to a modified gold standard of three or more assays yielding a positive result, the sensitivities improved (range, 83% to 96%; data not shown). None of the 35 specimens collected from the 23 non-SARS patient samples was positive for SARS-CoV RNA.

The newer assays performed as well as a conventional nested RT-PCR assay for the detection of SARS-CoV RNA from stool samples, even after sample storage at  $-80^{\circ}\text{C}$  for approximately 2 years. This is consistent with a previous report showing that SARS-CoV could withstand freezing and thawing and was stable for many weeks in clinical specimens maintained at  $4^{\circ}\text{C}$  or at  $-70^{\circ}\text{C}$  (3). The newer real-time PCR assays produced more rapid results than the conventional method, which was labor-intensive and required 5 h to complete.

The results of our RT-PCR testing for SARS-CoV in stool samples are similar to those previously reported (4, 14, 16). The negative RT-PCR results for serologically confirmed SARS patients obtained in this study could have been due to suboptimal timing of specimen collection (4, 14). Stool samples collected during the first week of illness or more than 21 days after symptom onset often yielded false-negative results, possibly due to a decreased viral load. More than half (55%) of the specimens from SARS patients that were available for testing had been collected more than 21 days after the onset of symptoms. An additional limitation of this study was the lack of availability of multiple samples from a large number of patients collected over an extended period of time during the course of illness. In the majority of cases, only one stool sample was available for testing. The use of archived stool samples may also have affected the yield, although our data do not appear to suggest that freezing affected RT-PCR assay results.

The results of this evaluation indicate that these commercially available real-time RT-PCR assays with newer extraction methodologies are highly specific and at least as sensitive as conventional nested RT-PCR assays for the detection of SARS-CoV. Major advantages of the newer tests are the ease with which they are performed and their rapidity. Any of these assays would be suitable for use, should a need to detect SARS-CoV arise in the future. However, it is clear that should SARS recur in humans, there is still a need for

more knowledge about optimal diagnostic test strategies (appropriate specimens and their timing) and for improved diagnostic assays.

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