

Performance and Cost Evaluation of One Commercial and Six In-House Conventional and Real-Time Reverse Transcription-PCR Assays for Detection of Severe Acute Respiratory Syndrome Coronavirus

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We evaluated seven reverse transcription-PCR (RT-PCR) assays, including six in-house assays and one commercial assay for the detection of severe acute respiratory syndrome coronavirus (SARS-CoV) RNA in clinical specimens. RT-PCR assays targeted different genomic regions and included three conventional assays (one nested and two non-nested) run on a conventional heat block and four real-time assays performed in a LightCycler (LC; Roche Diagnostics). All in-house assays were optimized for assay parameters, including MgCl₂, primer, and probe concentrations. The commercial assay was the RealArt HPA CoV RT-PCR assay (Artus), which was run in the LC. Testing serial dilutions of cultured SARS-CoV showed that the analytical sensitivity of the assays ranged from 10⁻⁸ to 10⁻⁶, corresponding to 1 and 100 copies of viral RNA, respectively. Significant differences in analytical sensitivities were observed between assays ($P < 0.01$, probit regression analysis for 50% sensitivity levels for the top two assays versus the others). Testing 68 clinical specimens (including 17 respiratory tract specimens, 29 urine samples, and 22 stools or rectal swabs) demonstrated that six of the seven assays detected at least 17 of 18 positives (defined as positive in at least two assays), and two of the assays had a sensitivity of 100%. There were no significant differences in sensitivity between the assays ($P = 0.5$ [Cochran Q test, least sensitive 15 of 18 versus 18 of 18]). The specificities of the assays ranged from 94.0 to 100% without significant differences ($P = 0.25$ to 0.5 [McNemar test]). The reagent and technologist cost of performing the in-house PCR assays ranged from \$5.46 to \$9.81 Canadian dollars (CDN) per test. The commercial assay cost was considerably higher at \$40.37 per test. The results demonstrated good performance for all assays, providing laboratories that need to do SARS RNA testing with a choice of assay formats.

Severe acute respiratory syndrome (SARS) was first recognized as an atypical pneumonia in China's Guangdong province in November of 2002. Largely as a result of international air travel, SARS quickly spread to Hong Kong and the neighboring countries of Vietnam, Singapore, and Taiwan and subsequently, to North America (2, 4, 8, 10, 13). By July 31, 2003 there were 8,098 cases that had been reported to the World Health Organization (16). Through unprecedented international cooperation, the complete sequence of the SARS coronavirus (SARS-CoV) genome was deciphered in the remarkably short time of three weeks by independent teams of scientists in Canada and the United States. Genetic analysis of the SARS-CoV sequence revealed that the virus causing SARS was a newly discovered virus distantly related to other members of the *Coronaviridae* family and representing a new emerging zoonotic viral infection of humans (5, 11).

Despite certain noteworthy characteristics of SARS, namely, the absence of upper respiratory tract symptoms, the presence of dry cough, and minimal auscultatory findings, with consolidation on chest radiographs, the clinical features of SARS do not readily allow a distinction from other common causes of respiratory viral infections. For this reason and because at the onset of the outbreak the etiologic agent of the atypical pneumonia was not known, case definitions were used to identify suspect and probable cases and to assist with infection control practices in managing the epidemic (1, 15). Once the SARS-CoV was sequenced, nucleic acid amplification tests were quickly developed to identify the virus in clinical specimens, and the SARS-CoV was shown to be the etiologic agent of SARS (3). In the absence of commercially available tests, a number of in-house reverse transcription-PCR (RT-PCR) assays targeting several areas of the viral genome have been described (2, 4, 7, 9, 10). Both consensus CoV and SARS-CoV-specific primers were developed to amplify the polymerase gene by using both conventional heat block (CHB) assays and real-time PCR instruments. Despite the lack of data on the performance of these assays, they have been proven useful in identifying cases both in the hospital and at autopsy (6, 12b). In

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TABLE 1. Oligonucleotide sequences of primers and probes for SARS RT-PCR assays

Assay	Primer or probe	Sequence
1 ^a	BNIoutS2 BNIoutAs BNIinS BNIAs	5'-ATG AAT TAC CAA GTC AAT GGT TAC-3' 5'-CAT AAC CAG TCG GTA CAG CTA C-3' 5'-GAA GCT ATT CGT CAC GTT CG-3' 5'-CTG TAG AAA ATC CTA GCT GGA G-3'
2	BNIoutS2 BNIoutAs	5'-ATG AAT TAC CAA GTC AAT GGT TAC-3' 5'-CAT AAC CAG TCG GTA CAG CTA C-3'
3	Cor-p-F2 Cor-p-R1	5'-CTA ACA TGC TTA GGA TAA TGG-3' 5'-CAG GTA AGC GTA AAA CTC ATC-3'
4	BNIoutS2 BNIoutAs	5'-ATG AAT TAC CAA GTC AAT GGT TAC-3' 5'-CAT AAC CAG TCG GTA CAG CTA C-3'
5	APNF APNR	5'-TGA ATA CAC CCA AAG ACC AC-3' 5'-TGA TGA GGA GCG AGA AGA G-3'
6	APNF APNR Probe	5'-TGA ATA CAC CCA AAG ACC AC-3' 5'-TGA TGA GGA GCG AGA AGA G-3' 5'-6FAM,-CCT AAT AAC AAT GCT GCC ACC GT-TAMRA-3'
7	Proprietary	NA

^a First round, BNIoutS2 and BNIoutAs; second round, BNIinS and BNIAs.

the absence of any published comparative data on sensitivity and specificity, we evaluated the performance of seven different conventional and real-time PCR assays for the detection of SARS-CoV with a range of clinical specimens collected during the Toronto SARS outbreak of 2003 (14).

(The results of this study were presented in part at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy in Chicago, Ill., in September 2003.)

MATERIALS AND METHODS

Specimens. Specimens were collected under IRB approval obtained from the Sunnybrook and Women's College Health Sciences Centre, which is part of the University of Toronto Healthcare Network. A total of 68 specimens, including 17 respiratory tract specimens (nasopharyngeal or throat swabs), 29 urine samples, and 22 stool samples, were collected between March and April of 2003 from hospitalized patients with a probable or suspected diagnosis of SARS at Sunnybrook and Women's College Health Sciences Centre during the Toronto outbreak of SARS. A SARS-CoV lysate was prepared by infecting Vero E6 cells with the Tor2 strain of SARS-CoV and was generously provided by Martin Petric of the British Columbia Centre for Disease Control, Vancouver, British Columbia, Canada. Human CoV strain OC43 was obtained from the American Type Culture Collection. Porcine CoV and bovine CoV were provided by S. Carman, Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada, and avian CoVs (Connecticut and Massachusetts strains) were obtained from Davor Ojtkic, Animal Health Laboratory, University of Guelph.

RNA extraction. RNA was extracted by using the Qiagen RNeasy kit according to the manufacturer's instructions with the following modifications for stool and urine specimens. Approximately one gram of stool was emulsified in 5 ml of diethyl pyrocarbonate-treated water and vortexed for 10 s in a 50-ml conical centrifuge tube. The suspension was allowed to stand for 2 min. A 600- μ l aliquot was removed from the top layer of the suspension and put into a 1.5-ml microfuge tube. An equal volume of Qiagen RLT lysis buffer containing β -mercaptoethanol was added and mixed by repeat pipetting. The suspension (600 μ l) was added to a Qias shredder column and centrifuged at 14,000 rpm for 2 min in a microfuge. The column was removed, and 600 μ l of 70% ethanol was added to the filtrate. After a mixing step, 600 μ l of the mixture was added to an RNeasy column, and extraction was conducted according to the manufacturer's instructions. In the absence of optimized protocols for testing urine samples for SARS-CoV and, since Qiagen extraction kits can only handle 0.5-ml portions of specimen, 5 ml of urine was centrifuged at 3,000 rpm for 10 min (Beckman benchtop

centrifuge), and the sediment was resuspended in 600 μ l of RLT buffer. RNA was extracted with RNeasy columns. For respiratory specimens (sputum, bronchoalveolar lavage, and pleural fluid), 600 μ l of sample was added to 600 μ l of RLT buffer, and RNA was extracted by using RNeasy columns. RNA was eluted in 30 μ l of elution buffer, and an aliquot (2 to 4 μ l) was used for RT.

RT-PCR. Six in-house RT-PCR assays were evaluated for the detection of SARS-CoV RNA. These included three CHB assays (one nested and two non-nested) and three real-time assays targeting three different regions of the genome. The probes and primers for each assay are listed in Table 1, and the conditions of amplification are listed in Table 2. Assay 1 was nested with the RT step combined with the first round of PCR amplification, followed by a second round of PCR amplification. Assay 1 used the BNI outer (BNIoutS2/BNIoutAs, 190-bp product) and inner (BNIinS and BNIAs) primers and amplified a 109-bp fragment downstream of the *polB* gene. Assay 2 was two-step, non-nested assay with the BNI outer primers (BNIoutS2 and BNIoutAs) and amplified the same 190-bp fragment downstream of the *polB* gene. Assay 3 was a two-step, non-nested RT-PCR assay with Cor-p-F2 and Cor-p-R1 primers (sequence courtesy of Dean Erdman) and amplified a 368-bp fragment of the *polB* gene. For assays 1, 2 and 3, RT was performed with Moloney murine leukemia virus enzyme from Invitrogen in a 20- μ l reaction volume. Each RT reaction contained 5 μ l of sample RNA, 0.1 μ g of random hexamers as the primer, 0.625 mM deoxynucleoside triphosphates, 4 μ l of first-strand buffer, 10 mM dithiothreitol, 40 U of RNAGuard (Amersham, Mississauga, Ontario, Canada), and 200 U of Moloney murine leukemia virus reverse transcriptase. The reaction was incubated at 37°C for 1 h and then heat inactivated at 70°C for 15 min. For PCR, 4 μ l of the RT reaction was added to 46 μ l of PCR amplification master mix or 16 μ l for the LightCycler (LC; Roche Diagnostics) assays. For assay 1, RT-PCR was performed with Superscript II/Platinum *Taq* (Invitrogen/Life Technologies, Burlington, Ontario, Canada). Assays 2 and 3 were performed with AmpliTaq Gold PCR kits from Applied Biosystems, Inc. (Foster City, Calif.). Amplified product was detected by agarose gel electrophoresis with ethidium bromide staining. All in-house assays were optimized for all assay parameters, including MgCl₂ concentration, primer concentration, probe concentration, and annealing and acquisition temperatures (real-time assays).

The real-time assays included a one-step RT-PCR, with the BNI outer primers (BNIoutS2 and BNIoutAs) amplifying a 190-bp fragment downstream of the *polB* gene with SYBR Green detection (assay 4), a two-step assay amplifying a 149-bp fragment of the nucleocapsid gene with SYBR Green detection (assay 5), and a one-step assay with the same nucleocapsid primers amplifying a 149-bp fragment of the nucleocapsid gene but using a TaqMan probe (assay 6). Real-time LC PCR was performed with LightCycler-FastStart DNA Master SYBR Green or LightCycler-RNA Master hybridization probe (Roche Diagnostics,

TABLE 2. Description of SARS PCR assays^a

Assay	Amplification target	Product size (bp)	RT-PCR type	Assay format	RT-PCR vol (μl)	PCR vol (μl)	No. of amplification cycles
1	PolB	190 (1st), 109 (2nd)	One step	CHB nested	2 (1st), 1 (2nd)	20 (1st), 50 (2nd)	50 (1st), 35 (2nd)
2	PolB	190	Two step	CHB	5 (RT), 4 (PCR)	20 (RT), 50 (PCR)	50
3	PolB	368	Two step	CHB	5 (RT), 4 (PCR)	20 (RT), 50 (PCR)	50
4	PolB	190	One step	LC (SYBR Green)	2	20	50
5	Nucleocapsid	149	Two step	LC (SYBR Green)	5 (RT), 4 (PCR)	20 (RT), 50 (PCR)	50
6	Nucleocapsid	149	One step	LC (TaqMan probe)	5	20	50
7	NA ^b	80	One step	LC (TaqMan probe)	5	20	50

^a 1st, first round; 2nd, second round.

^b NA, not available from the manufacturer (Artus).

Laval, Quebec, Canada), depending on which format of the assay was run. The criteria used for positivity in any LC assay was a positive copy number and a defined crossing threshold above baseline.

The RealArt HPA CoV RT-PCR assay from Artus (Artus GmbH, Hamburg, Germany) was performed in the LC according to the manufacturer's instructions. This assay is a non-nested, one-step RT-PCR that uses proprietary primers to amplify an 80-bp fragment of the SARS-CoV genome downstream of the *polB* gene. Amplification was detected with a TaqMan probe, and the signal acquisition was set at 55°C on the LC.

Statistical analysis. Sensitivity was calculated as the percent positive, for each test, of 18 clinical specimens from suspected SARS patients that were determined to be positive in at least two of the seven assays. Specificity was calculated as the percent negative, for each test, of 50 clinical specimens that tested positive in none or one of the seven assays. Note that if we had defined any test positive as a true positive, the specificity of all assays by definition would have been 100%.

To compare the sensitivities and specificities of the seven assays, the Cochran Q test (which compares three or more tests on the same samples) was used. Pairwise comparisons were then made by using the McNemar test, which compares two tests made on the same samples. To more precisely compare the relative sensitivities of the various assays, probit regression analysis was used to estimate the sample dilution at which each test detected 50% of samples with five replicate aliquots of log₁₀ serial dilutions of SARS-CoV RNA (SPSS 11.5; SPSS, Inc., Chicago, Ill.). A *P* value of 0.05 (two tailed) was considered statistically significant.

RESULTS

We evaluated the performance of seven different RT-PCR assays for the detection of SARS-CoV RNA in clinical specimens. At the start of the Toronto outbreak there were no commercially available assays for SARS-CoV RNA, and the genome had not been deciphered. We established six different in-house PCR assays targeting three different genes, including the *polB*, an adjacent downstream region, and the nucleocapsid gene. The first assay we developed (assay 3) used primer sequences made available to us by the Centers for Disease Control and Prevention (CDC), and subsequent assays used sequences from the Bernard Nocht Institute for Tropical Medicine and public databases based on the full sequence of the SARS-CoV Tor2 (GenBank accession no. AY274119.3) and Urbani (GenBank accession no. AY278741) strains deposited by the British Columbia Centers for Disease Control in Vancouver and the CDC. We also included in our evaluation the first commercially available RT-PCR, the RealArt HPA CoV RT-PCR assay from Artus.

The analytical sensitivity of all assays was initially compared by testing serial dilutions of a SARS-CoV RNA extracted from lung tissue and then by testing serial dilutions of SARS-CoV-infected cell lysate. All seven assays had similar analytical sensitivities with detection endpoints within 1 or 2 log₁₀ dilutions of each other between 10⁻⁸ and 10⁻⁶ (Fig. 1), corresponding

to a limit of detection of between 1 and 100 copies of viral RNA per PCR. Two assays (assay 6, the nucleocapsid TaqMan assay, and assay 7, the Artus assay) showed a trend toward a higher sensitivity with 10⁻⁷ and 10⁻⁸ endpoint dilutions. When testing was repeated with five replicates at each dilution (data not shown), these two assays were significantly more sensitive than the others; probit regression analysis for the sample dilution corresponded to 50% detection (assay 7 versus assay 2, *P* < 0.001; assay 7 versus assay 6, *P* < 0.01; and assay 6 versus assay 2, *P* < 0.05).

The specificity of each assay was determined by testing extracted nucleic acid from a number of respiratory viruses. All of the assays were specific and none amplified RNA from porcine, bovine, or avian CoVs (Fig. 1, lanes 8 to 17) or human CoV OC43. RNA from human metapneumovirus, influenza A virus, respiratory syncytial virus, and parainfluenza virus types 1, 2, or 3 and DNA from adenovirus were also not amplified in this assay (not shown).

Testing 68 clinical specimens (17 respiratory specimens, 22 stool samples, and 29 urine samples) showed that 63 specimens had the same results in all seven assays; 46 were negative and 17 were positive, with only 5 discordant specimens. By our criteria that required a positive result in two or more assays, there were a total of 18 positives and 50 negatives. The sensitivities and specificities of the seven assays were similar, with sensitivities ranging from 83.3 to 100% and specificities ranging from 94 to 100% (Table 3). Differences in sensitivities were, however, not significant (assay 2 [18 of 18] versus assay 6 [17 of 18], *P* = 0.5; assay 2 [18 of 18] versus assay 6 [15 of 18], *P* = 0.25 [McNemar test]). The specificities of the assays ranged from 94.0 to 100% and were not significantly different (assay 2 [47 of 50] versus assay 1 [50 of 50], *P* = 0.25; assay 4 [48 of 50] versus assay 1 [50 of 50], *P* = 0.5 [McNemar test]). None of the assays was both 100% sensitive and specific. The results for the five discordant specimens are shown in Table 4. Two of the five discordant specimens were positive in two tests, and the other three were positive in a single test, suggesting that most of the discordant results were false-positive results.

The costs of the various PCR assays were calculated by determining both the reagent component cost and the salary cost by using actual purchase prices for all reagents and a technologists salary of \$35.00/h CDN (Table 5). The cost of RNA extraction (\$4.18) was the same for all assays and was not included in the PCR cost determination. The costs were based on a run size of 48 samples for a CHB assay or a run size of 32 on the LC with three controls run in each assay format. The

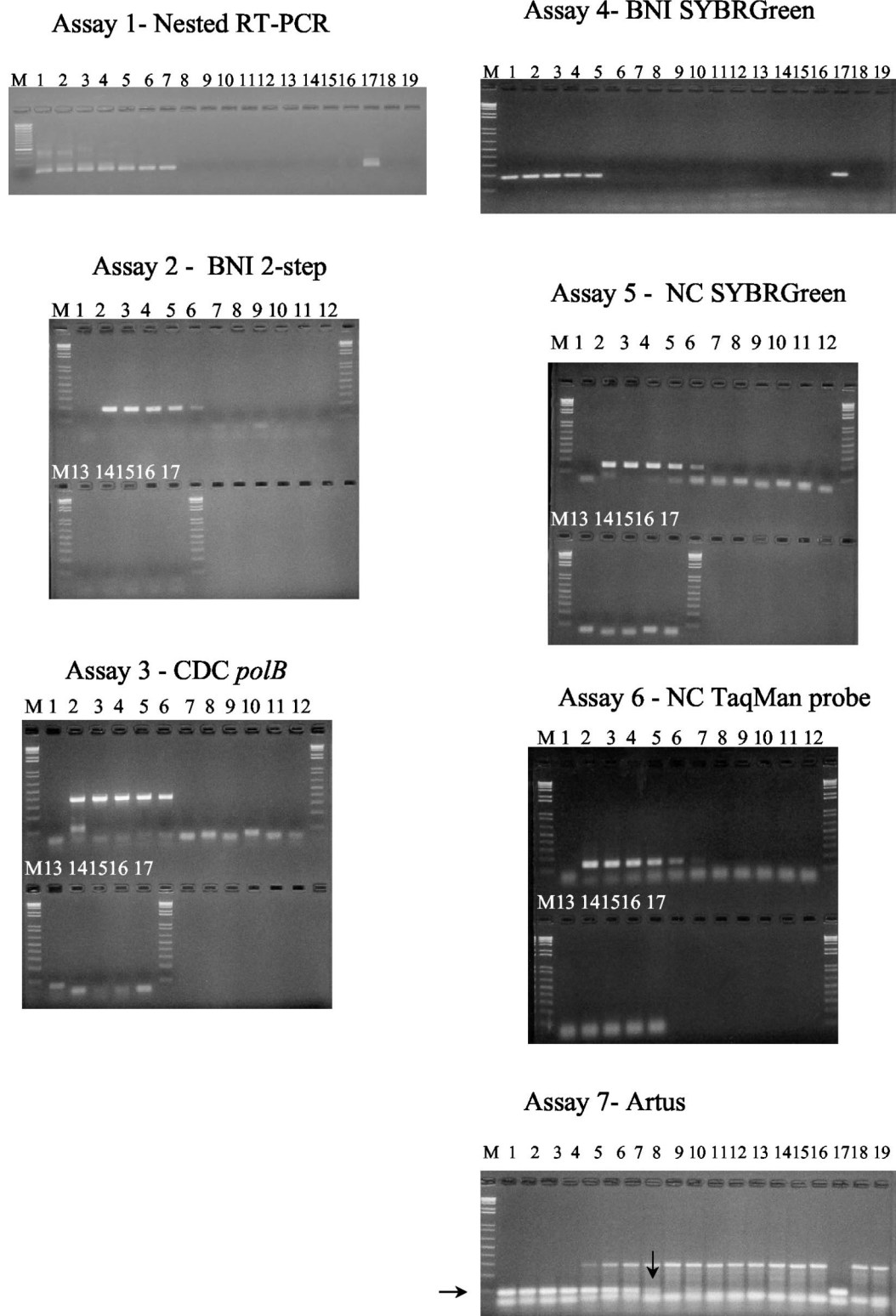


FIG. 1. Analysis of amplification products of seven RT-PCR assays for SARS-CoV RNA. Amplification products of both CHB and real-time PCR assays were analyzed by agarose gel electrophoresis and ethidium bromide staining. Outside lanes (M) contain molecular weight markers. The sizes of the amplification products for the various assays are as follows: 109 bp for assay 1, 190 bp for assays 2 and 4, 368 bp for assay 3, 149 bp for assays 5 and 6, and 80 bp for assay 7. Serial dilutions of SARS-CoV RNA from 10^{-2} to 10^{-12} were run in each gel. For assays 1, 4, and 7, the order of the lanes is as follows: SARS RNA dilutions of 10^{-2} to 10^{-12} are in lanes 1 through 11, swine CoV RNA is in lane 12, bovine CoV is in lane 13, human respiratory CoV OC43 is in lane 14, avian CoV (Connecticut strain) in lane 15, avian CoV (Massachusetts strain) is in lane 16, a SARS-CoV RNA-positive control is in lane 17, and negative controls are in lanes 18 and 19. For assays 2, 3, 5, and 6, the order of the lanes is as follows: lane 1, no template control; lanes 2 to 12, SARS RNA dilutions of 10^{-2} to 10^{-12} ; lane 13, swine CoV RNA; lane 14, bovine CoV; lane 15, human respiratory CoV OC43; lane 16, avian CoV (Connecticut strain); and lane 17, avian CoV (Massachusetts strain). The last dilution giving an amplification product was 10^{-6} for assays 2, 3, 4, and 5; 10^{-7} for assay 6; and 10^{-8} for assays 1 and 7.

TABLE 3. Sensitivity, specificity, and predictive values for SARS PCR assays^a

Assay	% Sensitivity	% Specificity	% Positive predictive value	% Negative predictive value
1	94.4 (17/18)	100 (50/50)	100 (17/17)	98.0 (50/51)
2	100 (18/18)	94.0 (47/50)	85.7 (18/21)	100 (47/47)
3	94.4 (17/18)	100 (50/50)	100 (17/17)	98.0 (50/51)
4	100 (18/18)	96.0 (48/50)	90.0 (18/20)	100 (48/48)
5	94.4 (17/18)	100 (50/50)	100 (17/17)	98.0 (50/51)
6	83.3 (15/18)	100 (50/50)	100 (15/15)	94.3 (50/53)
7	94.4 (17/18)	100 (50/50)	100 (17/17)	98.0 (50/51)

^a True positives are defined as positive in at least two assays (*n* = 18). Values in parentheses represent the numbers of specimens positive or negative for the specified assay/the total number of true positives (sensitivity) or true negatives (specificity).

cost of the in-house assays ranged from \$5.46 to \$9.81 per test; the least expensive was assay 2 (two-step CHB) and the most expensive was assay 6 (two-step LC). This cost was compared to a cost of \$40.37 per test for the commercial test (Table 5).

DISCUSSION

We developed six different RT-PCR assays for the detection of SARS-CoV RNA and evaluated their performance and that of a commercial test for analytical sensitivity and also determined their clinical performance with clinical specimens from SARS patients. All seven assays were highly specific, and none of them amplified RNA from animal CoVs or human respiratory viruses, including CoV OC43, human metapneumovirus, influenza A virus, respiratory syncytial virus, parainfluenza virus types 1, 2, and 3, or DNA from adenovirus. Five assays showed similar analytical sensitivity with the same RNA endpoint dilutions, whereas two assays had detection endpoints at one or two higher dilutions. When the dilutions were tested in replicates of five and the results were analyzed by probit regression analysis, the assays demonstrated significant differences in sensitivity. Assays 7 and 6 showed the highest sensitivities, with 50% detection at dilutions containing 4.53×10^{11} and 7.18×10^{10} copies, respectively, compared to 7.19×10^9 copies for assay 2. Assays 7 and 6 were 166 and 16 times more sensitive, respectively, than assay 2 (dilution for 50% detection by probit analysis).

Our evaluation of seven different PCR assays for SARS-CoV revealed that, despite their different formats, the seven different assays performed similarly. The clinical sensitivities

ranged from 83.3 to 100%; however, the differences were not significant (*P* = 0.25 for assay 6, with 15 of 18 isolates versus 18 of 18 isolates [McNemar test], and *P* = 1.0 for assays 1, 3, 5, and 7, with 17 of 18 versus 18 of 18 isolates). The differences in specificities between the seven assays (94 to 100%) were also not significant (*P* = 0.25 for the lowest versus the highest). This lack of significant differences in sensitivity with clinical specimens was surprising given the differences in the analytical sensitivities of the assays. Despite the most sensitive assays having analytical sensitivities 16 to 166 times higher than the other assays (dilution for 50% detection by probit analysis), they did not show significant improvements in clinical performance. One reason may be that clinical specimens contain amplification inhibitors that copurify with RNA and adversely affect different assays. Alternatively, the similar sensitivity that we observed for the different assays could have been due to the high viral load of SARS-CoV in the clinical specimens used in our study. One might expect a reduced sensitivity of some of these assays when specimens with lower viral loads are tested. This possibility could be examined in future studies by correlating viral loads with PCR results for various assays. An improved understanding of the natural history of SARS-CoV infection, in particular, of which clinical specimens contain the most virus, will assist clinicians and laboratories in diagnosing SARS cases. Analysis of serially collected specimens from SARS cases in the Toronto outbreak has indicated that stool specimens contain a large amount of virus, are positive early in the course of infection, and may be the preferred specimen for diagnosis (12b). If assay sensitivity becomes a problem, then improvements in RNA extraction and/or recovery, together with testing of replicate aliquots of extracted RNA (12), should increase assay sensitivity. Another explanation could be that there were real differences in the performance of the assays that were not detected here due to the small number of specimens. Our evaluation was limited in size to 68 clinical specimens, and additional evaluations with larger numbers of clinical specimens may be required to determine whether there are significant differences in the performance of various assays for the detection SARS-CoV in clinical specimens.

Our decision to use as a “gold standard” for defining true positives, specimens that were positive in at least two tests, was arbitrary but was guided by the fact that, if we had defined a positive specimen as one that was positive in a single test, then

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TABLE 4. RT-PCR results for five discordant specimens^a

Specimen no.	Specimen type	Assay result						
		1	2	3	4	5	6	7
008	Urine	–	–	–	+	–	–	–
011	Urine	–	–	–	+	–	–	–
029	Stool	–	+*	–*	+	–*	–	–
043	Sputum	–	+*	–*	–	–*	–	+
048	Urine	–	+*	–*	–	–	–	–

^a There were 5 discordant specimens and 63 concordant specimens. Of the concordant specimens, 17 were positive and 46 were negative in all seven assays. Eight discordant results (marked with an asterisks) were reevaluated by retesting specimens in the same assay and yielded a negative result in the repeat test.

TABLE 5. Relative costs of various SARS PCR assays^a

Assay	No. of tests/run ^b	Reagent cost/run (\$)	Hands-on time (h)	Labor cost/run ^c (\$)	Cost/test (\$)	Total cost per test ^d (\$)
1	48	176.34	3	105.00	6.25	10.43
2	48	140.86	3	105.00	5.46	9.64
3	48	140.86	3	105.00	5.46	9.64
4	32	207.78	1	35.00	8.37	12.55
5	32	199.60	2	70.00	9.30	13.48
6	32	249.47	1	35.00	9.81	13.99
7	32	1,135.61	1	35.00	40.37	44.55

^a All dollar figures are in Canadian dollars (= 0.72 U.S. dollars).

^b Three controls included in each run of 48 or 32 samples.

^c Based on a technologist salary of \$35.00/h.

^d Includes an amount of \$4.18 per specimen for RNA extraction by using a Qiagen Viral RNA kit.

the specificity of all assays would have been 100%, which would have been unrealistic. Assay performances could change if a different reference standard was used. For example, if we had defined a positive as being positive in any single test, then assays 2 and 4 would have been the most sensitive. We felt, however, that being positive in two different assays was a more rigorous way to define true positives and to detect false positives. This was, in fact, borne out by repeat testing of three specimens that were positive initially in assay 2 that repeated as negative (Table 4). Future studies with larger numbers of specimens could examine the role of choosing various reference standards.

A comparison of the cost of performing each assay, including the costs of both reagents and the technologist's time, indicated that the cost of the in-house assays ranged from \$5.46 to \$9.81 CDN per test, the least expensive being the two-step CHB assays and the most expensive being the two-step LC assay. By comparison, the cost of the commercial assay was \$40.37 per test (this might be reduced with contract purchasing). Given that most laboratories faced with a SARS test request would not wait to batch specimens, cost comparisons for a single test per run may also be useful. Additional costs for in-house quality controls that are included in the commercial test would bring the prices closer together. When the cost of RNA extraction was added to each assay the actual costs of testing one specimen ranged from \$9.64 to \$13.99 for the in-house assays and \$44.55 for the commercial assay. For laboratories setting up SARS testing for the first time without pedigreed specimens and controls, the commercial test may offer a quick start-up.

Laboratories setting up SARS-CoV PCR testing can therefore choose between various PCR assay formats and have an assay suited to their specific needs and instrumentation that will provide good sensitivity and specificity. The use of a second confirmatory PCR with a different amplification target will provide laboratories with some assurance that specimens giving positive PCR results are true positives. With this in mind, we developed an LC assay (assay 6) that targets the nucleocapsid gene and uses a TaqMan probe that can be used to confirm positive results obtained with the commercial RealArt HPA assay that targets a region downstream of the *polB* gene. The performance of the nucleocapsid LC assay has recently been validated in a multicenter evaluation involving nine different laboratories (J. B. Mahony et al., unpublished data). Since, in some SARS patients, seroconversion may take as long as 28 days postinfection (1, 12b), the laboratory diagnosis of SARS will continue to rely heavily on the detection of viral RNA by PCR. Given the unknown specificity of available SARS PCR tests in current use and the obvious consequences of reporting a SARS false-positive result, laboratories would be wise to confirm PCR-positive specimens by using a second assay that targets a different part of the genome.

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