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Development of One-Step, Real-Time, Quantitative Reverse Transcriptase PCR Assays for Absolute Quantitation of Human Coronaviruses OC43 and 229E

Leen Vijgen, Els Keyaerts, Elien Moës, Piet Maes, Griet Duson, and Marc Van Ranst*

Laboratory of Clinical & Epidemiological Virology, Department of Microbiology & Immunology, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium

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The clinical significance of human coronaviruses in more severe respiratory illnesses has recently been shown to be higher than was previously assumed. Rapid and reliable diagnosis of human coronavirus infections therefore becomes indispensable in a routine clinical setting. In this study, we present a very sensitive and specific TaqMan-based, real-time quantitative reverse transcriptase PCR (qRT-PCR) for the rapid detection and quantitation of human coronaviruses (HCoVs) OC43 and 229E. Absolute viral load measurement in clinical samples was achieved through the construction of in-house HCoV OC43 and 229E cRNA standards for the generation of a standard curve. The HCoV OC43 assay allows quantitation over a range from 20 to 2×10^8 RNA copies per reaction mixture (5 μl RNA extract). When this is extrapolated to clinical samples, this corresponds to a detection range of 10^3 to 10^{10} viral genome equivalents per ml. By using the HCoV 229E qRT-PCR assay, viral RNA copies ranging from 200 to 2×10^9 per reaction mixture can be detected, which corresponds to 10^4 to 10^{11} viral genome equivalents per ml sample. A total of 100 respiratory samples screened for the presence of HCoVs OC43 and 229E by using conventional RT-PCR were assessed in parallel by the qRT-PCR assays. By use of the real-time qRT-PCR techniques, the detection rate of HCoVs OC43 and 229E increased from 2.0% to 3.1% and from 0.3% to 2.5%, respectively. The real-time qRT-PCR assays described here allow the rapid, specific, and sensitive laboratory detection and quantitation of human coronaviruses OC43 and 229E.

Coronaviruses are large, enveloped, positive-stranded RNA viruses belonging to the family *Coronaviridae* in the order *Nidovirales* (2). Coronaviruses have the largest known nonsegmented viral RNA genome (27 to 32 kb), which is capped, polyadenylated, and infectious (7). Human coronaviruses (HCoVs) cause respiratory tract illnesses, but enteric and potential neurological diseases are also frequent outcomes of HCoV infection (1, 13). Until now, five types of human coronaviruses have been described: HCoV OC43, HCoV 229E, the severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV), and the recently characterized HCoVs NL63 and HKU1. HCoV OC43 and HCoV 229E are responsible for approximately one-third of the common colds during winter and early spring (8). Although HCoV OC43 and HCoV 229E infections are generally mild, there have been reports of more severe upper and lower respiratory tract infections like bronchiolitis and pneumonia, especially in infants, elderly individuals, and immunocompromised patients (4, 6, 9). Clusters of HCoV OC43 and 229E infections as a cause of pneumonia in otherwise healthy adults have also been reported (14, 18). Before the 2002–2003 SARS epidemic, coronaviruses had been somewhat neglected in human medicine. The identification of the causative agent of SARS as a new member of the *Coronaviridae* family has prompted research in the coronavirus field. Only recently, two novel human coronavirus types associated with lower respiratory tract infections have been characterized: HCoV NL63 and HCoV HKU1 (3, 16, 19). Hence, the importance of coronaviruses in more severe respiratory illnesses appears to be higher than was first assumed, leading to an increasing need of methods for the rapid and reliable detection of coronaviruses.

At present, viral culture is the "gold standard" for the laboratory diagnosis of respiratory infections. Since coronaviruses are very difficult to grow in cell culture, accurate and sensitive diagnosis by this method is not feasible. Serological methods, mainly based on enzyme-linked immunosorbent assay, are generally time-consuming and therefore have little clinical significance. To overcome the lack of sensitivity and to obtain rapid diagnostic results, more sensitive molecular methods for the detection of human coronaviruses have been developed, such as reverse transcriptase PCR (RT-PCR) (15), nested RT-PCR (11), and, recently, also real-time RT-PCR (17). A major advantage of real-time RT-PCR is that amplification and analysis are completed in a closed system, making it less time-consuming than (nested) RT-PCR methods, which still require post-PCR analysis. The risk of contamination, which can confound conventional (nested) RT-PCR protocols, is markedly reduced. In this study we present a very sensitive and specific TaqMan-based real-time quantitative RT-PCR (qRT-PCR) for the rapid detection and quantitation of human coronaviruses OC43 and 229E in clinical specimens.

* Corresponding author. Mailing address: Laboratory of Clinical and Epidemiological Virology, Department of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium. Phone: 32-16-347908. Fax: 32-16-347900. E-mail: marc.vanranst@uz.kuleuven.ac.be.

MATERIALS AND METHODS

Viral propagation. Human coronaviruses OC43 and 229E were obtained from the American Type Culture Collection (VR-759 and VR-740, respectively) and were propagated in rhabdomyosarcoma cells, obtained from the European Collection of Cell Cultures (ECACC 85111502). The supernatant was harvested after 4 days of

incubation at 35°C. The SARS-CoV Frankfurt 1 (FFM-1) strain was kindly provided by H. F. Rabenau and H.W. Doerr from the Johann Wolfgang Goethe University, Frankfurt, Germany. Viral RNA was isolated by using a QIAamp viral RNA kit (QIAGEN, Westburg, Leusden, The Netherlands). HCoV NL63 RNA was kindly provided by Lia van der Hoek and Ben Berkhout from the Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

Primer and probe design. The membrane protein gene of HCoV OC43 and HCoV 229E was searched for primer and probe target sites that would be compatible with TaqMan qPCR requirements (ABI 7700 Users Manual) by using Primer express 2.0 software. Primers that spanned a target region of 68 bp for HCoV OC43 and a target region of 70 bp for HCoV 229E were selected. The primers had matched dissociation temperatures and a minimal likelihood for duplex or hairpin formation. The forward primers were OC43-FP (5′-ATGTTA GGCCGATAATTGAGGACTAT-3′; GenBank accession no. M93390; nucleotides [nt] 433 to 458) and 229E-FP (5′-TTCCGACGTGCTCGAACTTT-3′; GenBank accession no. M33560; nt 474 to 493), the TaqMan minor groove binder (MGB) probes were OC43-TP (FAM-5′-CATACTCTGACGGTCACA AT-3′-NFQ-MGB, where FAMis 6-carboxyfluorescein and NFQ is a nonfluorescent quencher; nt 459 to 478) and 229E-TP (FAM-5′-TCCTGAGGT CAATGCA-3′-NFQ-MGB; nt 506 to 521), and the reverse primers were OC43-RP (5′-AATGTAAAGATGGCCGCGTATT-3′; nt 479 to 500) and 229E-RP (5′-CCAACACGGTTGTGACAGTGA-3′; nt 523 to 543).

Construction of cRNA standards. The TaqMan HCoV OC43 and HCoV 229E forward primers, primers OC43-FPT7 (5′-TAATACGACTCACTATAGGGA GGATGTTAGGCCGATAATTGAGGACTAT-3′) and 229E-FPT7 (5′-TAA TACGACTCACTATAGGGAGGTTCCGACGTGCTCGAACTTT-3′), were modified with a T7 promoter sequence at their 5′ ends. PCR products amplified with the modified primer pairs were quantified spectrophotometrically at 260 nm. A total of 200 ng of the PCR product was used for in vitro transcription (MEGAshortscript T7 kit; Ambion, Austin, TX), performed at 37°C overnight in a 20-μl reaction mixture containing 2 μl of reaction buffer, 2 μl of each nucleoside triphosphate, and 2 μl enzyme mix. The cDNAs were removed by digestion with 2 U of RNase-free DNase I for 15 min at 37°C. The cRNAs were precipitated by adding 3 μl 3 M sodium acetate and 60 μl of 96% ethanol and a subsequent incubation at −20°C for 30 min. After 15 min of centrifugation at 13,000 rpm, the supernatant was removed and 500 μl of 70% ethanol was added. After another 5-min centrifugation at 13,000 rpm, the supernatant was removed and the pellet was dissolved in 200 μl RNase-free H_2O (Sigma-Aldrich NV, Bornem, Belgium) and stored at −80°C. Quantitation of cRNAs was performed spectrophotometrically at 260 nm. The measurement of the cRNA concentrations was performed in duplicate, and the concentration was then converted to the molecule number (5).

Real-time quantitative RT-PCR. qRT-PCR was carried out in a 25-μl reaction mixture with 5 μl extracted RNA or standard cRNA, 12.5 μl of Eurogentec One-Step Reverse Transcriptase qPCR master mix containing ROX as a passive reference dye, 0.125 μl Euroscript + RT & RNase inhibitor (Eurogentec, Seraing, Belgium), 300 nM forward and reverse primers, and 200 nM MGB probe. Amplification and detection were performed in a ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA) under the following conditions: an initial reverse transcription at 48°C for 30 min, followed by PCR activation at 95°C for 10 min and 45 cycles of amplification (15 s at 95°C and 1 min at 60°C). During amplification, the ABI PRISM sequence detector monitored real-time PCR amplification by quantitative analysis of the fluorescence emissions. The reporter dye (FAM) signal was measured against the internal reference dye (ROX) signal to normalize the signals for non-PCR-related fluorescence fluctuations that occur from well to well. The cycle threshold (C_T) represented the refraction cycle number at which a positive amplification was measured and was set at 10 times the standard deviation of the mean baseline emission calculated for PCR cycles 3 to 15.

Conventional one-step RT-PCR. To compare the sensitivity of the real-time qRT-PCR with that of a conventional RT-PCR, viral RNA extracted from infected cell culture supernatants was used. For HCoV OC43 a 334-bp fragment was amplified by one-step RT-PCR by using the MF1 and MF3 primers described by Vabret and colleagues (5′-GGCTTATGTGGCCCCTTACT-3′ as the forward primer and 5′-GGCAAATCTGCCCAAGAATA-3′ as the reverse primer) (15). The MD1 and MD3 primers described by Vabret and colleagues (5′-TGGCCCCATTAAAAATGTGT-3′ as the forward primer and 5′-CCT GAACACCTGAAGCCAAT-3′ as the reverse primer) were used to amplify a 574-bp fragment of the HCoV 229E M gene in a one-step RT-PCR (15). The RT-PCR was carried out on a Geneamp PCR System 9700 thermal cycler (Applied Biosystems). Each reaction mixture contained 0.6 μM of forward and reverse primers, 0.4 mM of each deoxynucleoside triphosphate, and 2 μl One-Step RT-PCR enzyme mix (QIAGEN). The RT-PCR conditions were as follows: an initial reverse transcription for 30 min at 50°C, followed by a PCR activation for 15 min at 95°C, 45 cycles of amplification (30 s at 94°C, 30 s at 58°C, and 1 min at 72°C), and a final extension step at 72°C for 10 min. The resulting PCR products were visualized after electrophoresis on an ethidium bromide-stained polyacrylamide gel.

Clinical specimens. Clinical samples of patients hospitalized with severe respiratory symptoms were collected in the University Hospitals of Leuven from February 2003 to February 2004. We studied 100 respiratory specimens consisting of nasopharyngeal aspirates, bronchial aspirates, bronchoalveolar lavage specimens, sputum samples, and pharyngeal swabs, all of which tested negative for the presence of respiratory syncytial virus. After sample collection, pharyngeal swab specimens were stored in viral transport medium. All samples were filtered through a 0.45-μm-pore-size filter before storage at −80°C. Viral RNA was extracted from the clinical specimens by using a QIAamp viral RNA kit (QIAGEN). The set of 100 specimens originated from patients ranging in age from 3 months to 80 years, with a mean age of 7 years. All samples were tested in parallel in the conventional one-step HCoV OC43 and HCoV 229E RT-PCRs described above and in the qRT-PCR assays, in which each sample was tested in duplicate. Positive qRT-PCR results were confirmed by retesting the sample in triplicate in a following run. Furthermore, samples additionally found to be positive by the real-time qRT-PCR assays were subjected to the one-step RT-PCR described above, followed by a second-round seminested PCR with the same reverse primer and a forward primer located in the fragment generated by the first RT-PCR (primer OC43-M-FWin [5′-GTGTATCTTGGCCTTTCTATA G-3′] and primer 229E-M-FWin [5′-AATGACAATTGTACGGGTGAC-3′]). Sequencing of the PCR products was performed by using the second-round PCR primers.

RESULTS

Absolute virus quantitation. To determine the absolute quantity of the viral load in patient specimens, HCoV OC43 and 229E cRNA standards were used for the generation of a standard curve (Fig. 1A and B). Tenfold serial dilutions of cRNA transcripts were used, and these corresponded to copy numbers of 2×10^2 to 2×10^7 per reaction mixture for the HCoV OC43 real-time qRT-PCR assay and 2×10^3 to 2×10^8 copies per reaction mixture for the HCoV 229E assay. Equal volumes of standard and sample were used for PCR amplification. Analysis of the copy numbers and linear regression curve was performed with Sequence Detector v1.9 software (Applied Biosystems).

Dynamic range. Tenfold dilution series of cRNA ranging from 2 to 2×10^{10} copies per reaction mixture were tested to determine the dynamic range of the assays. Results were analyzed in terms of the C_T value (the cycle in which a target sequence is first detected). The HCoV OC43 qRT-PCR was able to distinguish 10-fold differences in concentration over a range from 20 ($C_T = 38.88 \pm 0.83$) to 2×10^8 ($C_T = 15.98 \pm 0.02$) molecules per reaction mixture (Fig. 2A). The HCoV 229E real-time qRT-PCR assay, on the other hand, allowed quantitation of HCoV 229E RNA over a 7-log-unit span, ranging from 200 to 2×10^9 molecules. Corresponding C_T values of 38.09 ± 1.13 to 15.05 ± 0.02 were found (Fig. 2B).

Analytical specificity, sensitivity, and reproducibility. Experiments were undertaken to assess diagnostic criteria such as specificity, sensitivity, and reproducibility. In order to exclude nonspecific reactions with other coronaviruses, HCoV 229E, SARS-CoV, and HCoV NL63 were subjected to amplification in the real-time qRT-PCR for HCoV OC43 RNA. On the other hand, HCoV OC43, SARS-CoV, and HCoV NL63 were included in the HCoV 229E real-time qRT-PCR assay. No nonspecific amplification was obtained in either assay. The analytical detection limit was determined by assaying 10 replicates of samples containing 2, 20, 200, or 2,000 copies of the

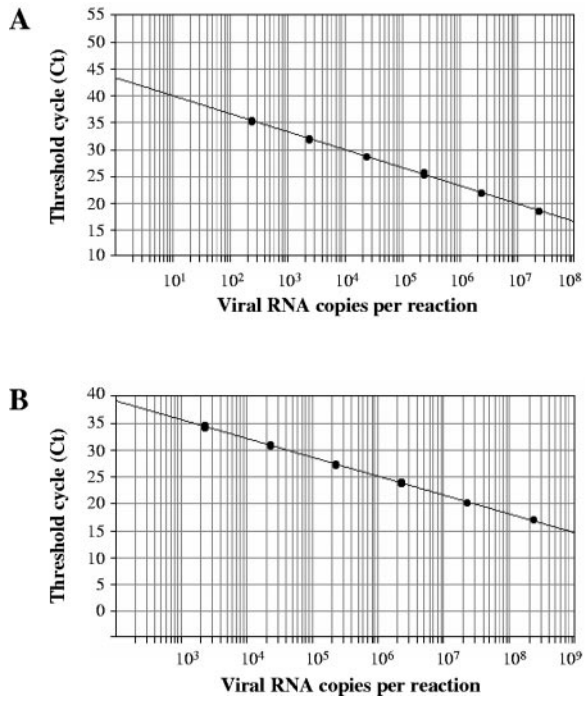

FIG. 1. Tenfold serial dilutions ranging from 10^2 to 10^7 copies of HCoV OC43 cRNA (A) and ranging from 10^3 to 10^8 copies of HCoV 229E cRNA (B) were tested in duplicate in the real-time qRT-PCR. A standard curve graph is made by plotting the C_T values on the *y* axis and the log of the input amounts on the *x* axis. The slope of the HCoV OC43 standard curve (A) is −3.54, and the correlation coefficient is 0.999. The HCoV 229E standard curve (B) displays a slope of −3.5 and a correlation coefficient of 1.000.

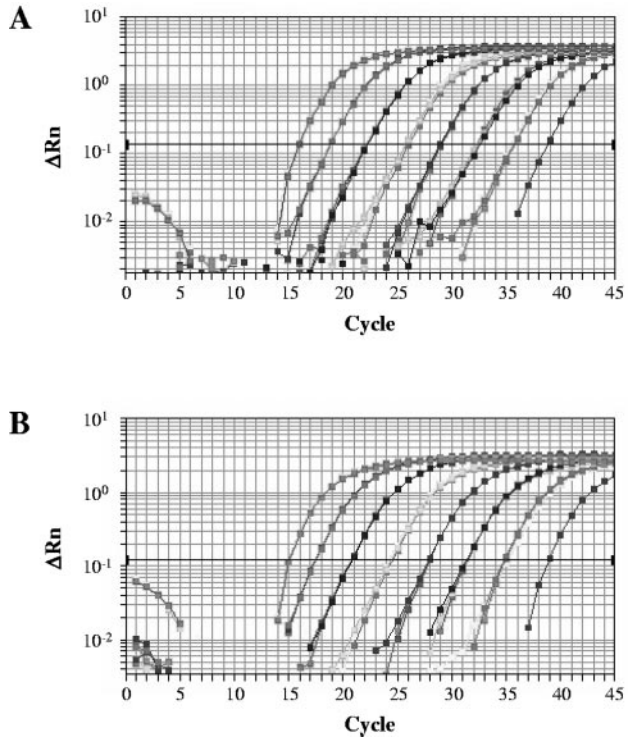

FIG. 2. The dynamic range of the assay was tested by running 10-fold serial dilutions of the cRNA standard ranging from 2 to 2×10^{12} molecules in the reaction. The amplification plot shows the ΔRn on the *y* axis (where Rn is the fluorescence emission intensity of the reporter dye normalized to a passive reference and ΔRn is the Rn of an unreacted sample minus the Rn of the reaction) against the cycle number displayed on the *x* axis. (A) The dynamic range of the HCoV OC43 assay spans 7 log units, ranging from 20 to 2×10^8 molecules of HCoV OC43 per reaction mixture, with the corresponding C_T values ranging from 38.88 ± 0.83 for 20 copies to 15.98 ± 0.02 for 2×10^8 copies. (B) For the HCoV 229E assay, a 7-log-unit dynamic range with C_T values ranging from 38.09 ± 1.13 for 2×10^2 copies per reaction mixture to 15.05 ± 0.01 for 2×10^9 copies per reaction mixture is demonstrated.

cRNA standard per reaction mixture. For each concentration level, the proportion of positive samples was calculated. Detection of 200 copies was established in all 10 replicates in the HCoV OC43 real-time assay. The use of 20 copies of HCoV OC43 cRNA still generated a good positivity rate: a C_T value lower than 45 was found for 9 of 10 replicates. The analytical detection limit of the HCoV 229E qRT-PCR assay was slightly higher: 100% positivity in 10 replicates was found for a quantity of 2,000 copies of cRNA per reaction mixture. When 200 and 20 copies of HCoV 229E cRNA were used, the detection rate decreased (60% and 30% positivity for 200 and 20 copies, respectively). To assess the intra-assay reproducibilities of both the HCoV OC43 and the HCoV 229E qRT-PCR assays, dilutions of the cRNA standards ranging from 2×10^3 to 2×10^8 copies per reaction mixture were analyzed in three replicates per run (Table 1). Mean C_T values, standard deviations, and coefficients of variation were determined. Coefficients of variation lower than 0.7% were present for both real-time assays. The interassay precision, which expresses the variability from run to run, was assessed by testing 2×10^5, 2×10^6, 2×10^7, and 2×10^8 copies of the cRNA standards in three different experiments (Table 2). The coefficients of variation of the precision from run to run were in the range of 0.08 to 0.44% and 0.13 to 0.24% for the HCoV OC43 and the HCoV 229E qRT-PCR assays, respectively.

Comparison of real-time quantitative RT-PCR and conventional RT-PCR. Tenfold dilutions of HCoV OC43 and 229E RNA, extracted from the cell culture supernatants, were tested in parallel in the qRT-PCR assays described here and in the conventional RT-PCR, which amplifies 334-bp and 574-bp fragments located in the M genes of HCoVs OC43 and 229E, respectively. The detection limits of both assays were com-

TABLE 1. Intra-assay variability of the HCoV OC43 and HCoV 229E qRT-PCR[a]

Sample	cRNA copies	Mean C_T	SD	CV (%)
HCoV OC43 cRNA	2.35×10^8	15.98	0.05	0.33
	2.35×10^7	18.75	0.05	0.27
	2.35×10^6	22.24	0.03	0.13
	2.35×10^5	25.27	0.01	0.05
	2.35×10^4	28.82	0.06	0.21
	2.35×10^3	32.13	0.16	0.49
HCoV 229E cRNA	2.26×10^8	17.35	0.03	0.18
	2.26×10^7	20.79	0.03	0.13
	2.26×10^6	24.12	0.08	0.34
	2.26×10^5	27.82	0.18	0.63
	2.26×10^4	31.12	0.17	0.55
	2.26×10^3	34.25	0.15	0.43

[a] Each of the dilutions was assayed three times per run. The mean values of C_T, standard deviations (SDs), and coefficients of variation (CVs) were calculated.

TABLE 2. Interassay reproducibilities of the HCoV OC43 and HCoV 229E qRT-PCRs[a]

Sample	Dilution	cRNA copies	Mean C_T	SD	CV (%)
HCoV OC43 cRNA	1×10^{-5}	2.35×10^{8}	15.96	0.02	0.14
	1×10^{-6}	2.35×10^{7}	18.67	0.08	0.44
	1×10^{-7}	2.35×10^{6}	22.22	0.02	0.08
	1×10^{-8}	2.35×10^{5}	25.18	0.11	0.44
HCoV 229E cRNA	1×10^{-5}	2.26×10^{8}	17.35	0.03	0.18
	1×10^{-6}	2.26×10^{7}	20.82	0.03	0.13
	1×10^{-7}	2.26×10^{6}	24.19	0.06	0.24
	1×10^{-8}	2.26×10^{5}	27.76	0.07	0.24

[a] The mean values of C_T, standard deviations (SDs), and coefficients of variation (CVs) were determined for three assay runs.

pared, and a 1-log-unit increase in sensitivity for HCoV OC43 as well as for 229E was observed.

Detection in clinical specimens. To evaluate the use of the qRT-PCR for the detection of HCoVs OC43 and 229E in clinical specimens, we analyzed 100 respiratory samples which are part of a collection of 342 samples from patients hospitalized with severe respiratory symptoms. In the collection of 342 specimens, conventional RT-PCR assays identified a total of seven samples positive for HCoV OC43 (2.0%), while only one sample was found to be positive for the presence of HCoV 229E (0.3%) (10). All samples positive by conventional RT-PCR were confirmed by the real-time qRT-PCR assays, and viral loads in the range of 10^4 to 10^7 copies per ml sample were revealed for the HCoV OC43-positive samples. The HCoV 229E-infected sample was found to contain a viral load on the order of 10^4 copies per ml sample. Moreover, among the set of 100 samples, 1 additional sample tested positive for HCoV OC43 by the qRT-PCR assay, and two additional samples were found to be positive for HCoV 229E by the qRT-PCR assay. The viral loads in these additional coronavirus-positive samples were determined to be in the range of 10^2 genome equivalents per reaction mixture, which corresponds to 10^4 HCoV OC43 and 229E copies per ml sample. These samples were confirmed to be positive by sequencing after a one-step RT-PCR followed by a second-round seminested PCR. The three HCoV 229E-positive samples were obtained from one patient aged 4 months, one patient aged 3 years, and one patient aged 12 years. The eight HCoV OC43-positive specimens were obtained from four patients younger than 1 year of age (3, 6, 7, and 9 months old), one patient who was 2 years old, two patients aged 5 years, and one patient who was 11 years old. The patient files showed that all subjects had respiratory tract illnesses and that some had underlying diseases (Table 3).

DISCUSSION

Human coronaviruses are only seldom detected in clinical laboratories. Although human coronavirus OC43 and 229E infections are generally mild, there have been reports of more severe upper and lower respiratory tract diseases, like bronchiolitis and pneumonia (14, 18), supporting the need for rapid and reliable techniques for the detection of these respiratory pathogens.

Conventional RT-PCR is a reasonably time-consuming method that requires additional post-PCR analysis for the detection of PCR products. Real-time qRT-PCR, on the other hand, generates complete results within 3 h. The reaction is performed in a closed-tube system and requires no additional manipulations. Another major advantage of real-time qRT-PCR is the ability to quantitate the viral load in clinical specimens, whereas conventional RT-PCR allows only qualitative analysis. In the real-time HCoV OC43 and 229E RT-PCR assays developed by van Elden and colleagues (17), virus quantity is expressed as relative units of HCoV and so does not provide an absolute quantitation of the viral load. As has been described for infection with the SARS-associated coronavirus, the amount of virus particles can be indicative of the severity and the outcome of the disease (12). The qRT-PCR assays described here allow absolute viral load measurement and can be used as rapid diagnostic and prognostic tools. Compared to the sensitivity of the conventional RT-PCR, a 1-log-unit increase in sensitivity is obtained in the real-time assays. In both qRT-PCR assays, quantitation over a wide dynamic range (a 7-log-unit span) with low intra- and interrun variabilities is feasible. A high specificity is demonstrated, as no amplification was observed when HCoV 229E RNA was tested in the HCoV OC43 assay and vice versa. Also, no positive results were obtained for HCoV NL63 and SARS-CoV RNA in either assay.

TABLE 3. Patients hospitalized with respiratory tract illness associated with HCoV OC43 and HCoV 229E infections

HCoV	Age	Sex	Symptom(s)	Underlying disease	Specimen[a]
229E	3 yr	Female	Fever, coughing, rhinorrhea	Asthma	PS
	4 mo	Female	Bronchitis, coughing	None	NPA
	12 yr	Male	Fever, diarrhea	Ewing's sarcoma	PS
OC43	11 yr	Male	Fever, coughing	None	NPA[b]
	9 mo	Male	Fever, diarrhea, coughing	None	NPA
	2 yr	Female	Fever, bronchiolitis	None	PS
	5 yr	Male	Fever	Renal insufficiency	PS[c]
	5 yr	Male	Chronic bronchitis	Tetralogy of Fallot	BAL
	6 mo	Female	Fever, bronchitis	Epilepsy	BA
	7 mo	Female	Fever, wheezing	Epilepsy	PS
	3 mo	Male	Bronchiolitis, rhinitis, coughing	None	NPA

[a] PS, pharyngeal swab; NPA, nasopharyngeal aspirate; BAL, bronchoalveolar lavage; BA, bronchial aspirate.
[b] Positive for influenza B virus.
[c] Positive for adenovirus.

Conventional RT-PCR screening of a collection of 342 respiratory samples from patients hospitalized with relatively severe respiratory tract infections revealed seven HCoV OC43-infected samples (2.0%) and one HCoV 229E-positive sample (0.3%) (10). In addition to the eight samples found to be positive by conventional RT-PCR, a total of 92 samples considered negative for HCoV were included in the real-time qRT-PCR assays. By use of these real-time techniques, the rates of positivity for HCoV OC43 and HCoV 229E increased from 2.0% to 3.1% and from 0.3% to 2.5%, respectively. The amount of virus particles present in these isolates is less than the detection limit of conventional RT-PCR but fell in the dynamic range of the real-time assays. For use for clinical screening and diagnosis, the HCoV OC43 and 229E qRT-PCRs described here allow the detection of virus over a concentration range where conventional RT-PCR seems to fail. Therefore, the real-time qRT-PCR assays outperform conventional RT-PCR not only because of their speed, lower contamination risk, and ability for quantitation but also because of their higher sensitivities for the screening of clinical specimens.

As coronaviruses no longer seem to be as harmless as was previously assumed and can be associated with more severe respiratory disorders, rapid and accurate identification of coronaviruses, even when only traces of the virus are present, becomes an important issue in clinical virology.

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