Chapter 42

Human Coronaviruses

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42.1 Summary of Methods

Human Coronaviruses (HCoV) are recognised to be an important cause of the common cold. In 1962 HCoV-229E and HCoV-OC43 were first recognised, more recently HCoV-NL63 and HCoV-HKU-1 have been discovered in respiratory specimens from children and adults [3]. This protocol describes two real-time reverse-transcriptase polymerase chain reaction (RT-PCR) methods, a single target and triplex RT-PCR that identifies and differentiates HCoV infection.

42.2 Acceptable Specimens

Respiratory specimens (Nasopharyngeal aspirates, nose and throat swabs and bronchial samples).

42.3 Sample Extraction

Respiratory specimens (200 µl) were extracted using the QIAGEN QIAextractor, as per manufacturer's instructions giving a final volume of 50 µl.

42.4 Primer and Probe Sequences

Primers and probe targets were obtained from previously published articles [1–3] (Table 42.1).

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Table 42.1 Sequence details of primers and probes, used for the detection of human coronaviruses by real-time PCR

Oligonucleotides	Sequence (5'–3')	Target gene	Refs.
Single Target		Replicase 1b	(2)
HCoV-HKU-1-F	CCTTGCGAATGAATGTGCT		
HCoV-HKU-1-R	TTGCATCACCAGTCTAGTACCAC		
HCoV-HKU-1-R	FAM– TGTGTGGCGGTTGCTATTATGTTAAGCCTG– BHQ-1		
Triplex RT-PCR			
HCoV-229E-F	CAGTCAAATGGGCTGATGCA	N gene	(1)
HCoV-229E-R	AAAGGGCTATAAAGAGAATAAGGTATTCT		
HCoV-229E-Pr	FAM-CCCTGACGACCACGTTGTGGTTCA-BHQ		
HCoV-OC43-F	CGATGAGGCTATTCCGACTAGGT	N gene	(1)
HCoV-OC43-R	CCTTCCTGAGCCTTCAATATAGTAACC		
HCoV-OC43-Pr	QUASAR-TCCGCTGGCACGGTACTCCCT-BHQ		
HCoV-NL63-F	ACGTACTTCTATTATGAAGCATGATATTAA	1a gene	(3)
HCoV-NL63-R	AGCAGATCTAATGTTATACTTAAACTACG		
HCoV-NL63-Pr	VIC-ATTGCCAAGGCTCCTAAACGTACAGGTGTT– TAMRA (300)		

42.5 PCR Amplification and Product Detection

The first assay contains a single target for HCoV-HKU1, the conserved primer and probe sequences are found in the replicase 1b gene. The second assay detects three HCoV strains, HCoV-229E, HCoV-OC43 both contain target sequences in the N gene, and HCoV-NL63 which target sequences are in the 1a gene (Table 42.1). Both assays are performed using the QIAGEN One-step RT-PCR kit (QIAGEN, Australia) comprising 0.8 μ M of forward and reverse primers (HCoV-HKU-1-F and HCoV-HKU1-R for the single target PCR and the triplex HCoV-229E-F, HCoV-229E-R, HCoV-OC43-F, HCoV-OC43-R, HCoV-NL63-F and HCoV-NL63-R) (Table 42.1), 0.2 μ M of TaqMan probe (single target PCR, HCoV-HKU1-Pr and in the triplex HCoV-229E-Pr, HCoV-OC43-Pr and HCoV-NL63-Pr) (Table 42.1), in a total reaction volume of 25 μ l including 5 μ l of sample RNA. Both amplification reactions were performed on the RotorGene 3000 or 6000 (QIAGEN, Australia) using the following parameters; initial RT incubation of 20 min at 50°C, followed by 50 cycles of 95°C for 15 s, and 60°C for 1 min, with fluorescence acquired at the end of each 60°C step.

42.6 Quality Control and Validation Data

The single target HCoV-HKU1 assay was validated using 31 clinical specimens positive for HCoV, detected using various different techniques. No cross reaction was detected to samples positive for other respiratory pathogens. Sensitivity was measured by serial titration, 15 replicates were tested with 100% detection at 50 copies and 33.3% at 5 copies [1].

The HCoV triplex was validated by ATCC positive specimens for each strain. Each positive was tested as an individually target and in the triplex reaction with no loss of sensitivity or cross reaction between strains [2].

References

1. Dare RK, Fry AM, Chittaganpitch M et al (2007) Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis* 196:1321–1328
2. Gunson RN, Collins TC, Carman WF (2005) Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. *J Clin Virol* 33:341–344
3. van Elden LJR, van Loon AM, van Alphen F et al (2004) Frequent Detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. *J Infect Dis* 189:652–657