

SARS-CoV Specific RT-PCR Primers

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Molecular Analyses

RNA extracts were prepared from 100 µl of each specimen (or culture supernatant) with the automated NucliSens extraction system (bioMérieux). Oligonucleotide primers used for amplification and sequencing of the SARS-related coronavirus were designed from alignments of open reading frame 1b of the coronavirus polymerase gene sequences obtained from GenBank, including human coronaviruses 229E and OC43 (accession numbers X69721 and AF124989, respectively), canine coronavirus (AF124986), feline infectious peritonitis virus (AF124987), porcine transmissible gastroenteritis virus (Z34093), porcine epidemic diarrhea virus (NC_003436), bovine coronavirus (NC_003045), porcine hemagglutinating encephalomyelitis virus (AF124988), sialodacryoadenitis virus (AF124990), mouse hepatitis virus (NC_001846), turkey coronavirus (AF124991), and avian infectious bronchitis virus (NC_001451).

Primer pair (Broadly Reactive)

IN-2 (+) 5'GGGTTGGGACTATCCTAAGTGTGA 3' and

IN-4 (-) 5'TAACACACAACICCATCATCA 3'

Previously designed to conserved regions of open reading frame 1b to achieve broad reactivity with the genus coronavirus. These primers were used to amplify DNA from SARS isolates, and the amplicon sequences obtained were used to design specific primers.

SARS-specific primers:

Cor-p-F2 (+) 5'CTAACATGCTTAGGATAATGG 3',

Cor-p-F3 (+) 5'GCCTCTCTTGTCTTGCTCGC 3', and

Cor-p-R1 (-) 5'CAGGTAAGCGTAAACTCATC 3',

Used in turn to test patient specimens. Primers used for specific amplification of human metapneumovirus have been described previously. Well-characterized primer sets for other respiratory virus pathogens (unpublished data), including human respiratory syncytial virus, parainfluenzaviruses 1, 2, and 3, influenzaviruses A and B, adenovirus, and picornavirus (rhinovirus and enterovirus), were also used to test clinical specimens in this study (primers available on request). All specimens were tested for human glyceraldehyde-3-phosphate dehydrogenase to confirm RNA integrity and control for RT-PCR inhibition.

One primer for each set was 5'-end-labeled with fluorescent dye 6-carboxyfluorescein (6-FAM) to facilitate GeneScan analysis. One-step amplification reactions were performed with the Access RT-PCR System (Promega) as previously described. Positive and negative RT-PCR controls, containing standardized viral RNA extracts, and nuclease-free water were included in each run. Amplified 6-FAM-labeled products were analyzed by capillary electrophoresis on an ABI 3100 Prism Genetic Analyzer with GeneScan software (version 3.1.2). Specimens were considered positive for SARS-associated coronavirus if the amplification products were within 1 nucleotide of the expected product size (368 nucleotides for Cor-p-F2 or Cor-p-R1 and 348 nucleotides for Cor-p-F3 or Cor-p-R1) for both specific primer sets, as confirmed by a second PCR reaction from another aliquot of RNA extract in a separate laboratory. Where DNA yield was sufficient, the amplified products were also sequenced to verify the authenticity of the amplified product.