

SYNOPSIS

02/03/2020

Review of “Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR”

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One-minute summary

- Evaluation of a real-time RT-PCR assay for the detection of 2019-nCoV. This is a two-step protocol that recommends **screening** using an assay that detects the **E (envelope) gene**, followed by **confirmation** with an assay that detects the **RdRp (RNA-dependent RNA polymerase) gene**.
 - The E gene screening assay detects a broad range of human and bat coronaviruses.
 - The RdRp confirmation assay uses two probes. One probe is **specific for 2019-nCoV and does not cross-react with SARS-CoV, MERS-CoV, or the seasonal human coronaviruses (HKU1, OC43, NL63, 229E)**. The other detects **both 2019-nCoV AND human and bat-related SARS CoVs**, and can be used as a positive control, or omitted.
- The assay is highly sensitive for 2019-nCoV, with a limit of detection (LOD) of 3.9 copies per reaction and 3.6 copies per reaction for the E gene assay and RdRp gene assay, respectively.
- The assay is highly specific for 2019-nCoV with no false positive reactions or cross-reactions with other respiratory pathogens.
- Diagnostic sensitivity and specificity was not determined due to a lack of positive clinical specimens and viral isolates of 2019-nCoV.

Additional information

- In addition to the above two targets, an N gene target was also tested but was found to be less analytically sensitive.
- Primer and probe design was based on alignment of 375 sequences of SARS-CoV and 6 sequences of 2019-nCoV.
 - The primers and probe for the E gene assay matches the 2019-nCoV target sequence exactly.

- There is a 1 base pair mismatch in the reverse primer for the RdRp gene assay.
- Validation specimens included: typed coronavirus isolates grown in cell culture, previously tested respiratory patient specimens (including sputum, nose and throat specimens, with and without viral transport media) that were positive for a variety of respiratory pathogens, bat-derived fecal samples containing bat SARS-related coronavirus, and synthetic 2019-nCoV nucleic acid.
- 297 clinical samples containing a variety of respiratory viruses, 13 spiked sputum samples, and water samples were used to determine analytical specificity.
 - No false positives were observed.
 - No cross-reactivity was observed against: seasonal human coronaviruses (HKU1, OC43, NL63, 229E), MERS-CoV, Influenza A (H1N1, N3N2, H5N1, H7N9, untyped), Influenza B (Victoria or Yamagata), Rhinovirus/Enterovirus, Respiratory Syncytial Virus (A/B), Parainfluenza virus (1-4), Adenovirus, human Bocavirus, *Legionella* spp., and *Mycoplasma* spp.

Citation

Ontario Agency for Health Protection and Promotion (Public Health Ontario). Review of “Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR”. Toronto, ON: Queens’s Printer for Ontario; 2020.

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