SCAN molecular workflow

Executive Summary

Molecular Detection of SARS-CoV-2 for SCAN the Greater Seattle Coronavirus Assessment Network

The SCAN real-time RT-PCR assay for SARS-CoV-2 contains primer/probe sets that target the Orf1b and S genes, designed against the SARS-CoV-2 genome (GenBank: MN908947.3). The primer/probe sets were analyzed *in silico* for specificity for this specific betacoronavirus by Thermo Fisher. All assays are performed on total nucleic acids extracted from upper respiratory tract swabs, including mid-turbinate and nasopharyngeal (NP) swabs stored in Universal Transport Media. The assay limit of detection of 5.6 (Orf1b) and 12.9 (S) molecules per reaction was determined from serial dilutions of synthetic DNA of the target sequences. These probes reproducibly detected SARS-CoV-2 RNA from cultured virus from USA-WA-1/2020 (BEI resources/ATCC) and from 21 specimens confirmed to be positive by the Washington State Department of health. Specificity was determined by lack of detection of SARS-CoV-2 from specimens that are known to be positive for seasonal coronavirus strains HKU1/NL63 or 229E/OC43 and/or other respiratory viruses. These specimens are further inferred to be negative by the fact that they were obtained in the greater Seattle area prior to the first known reports of SARS-CoV-2 in China.

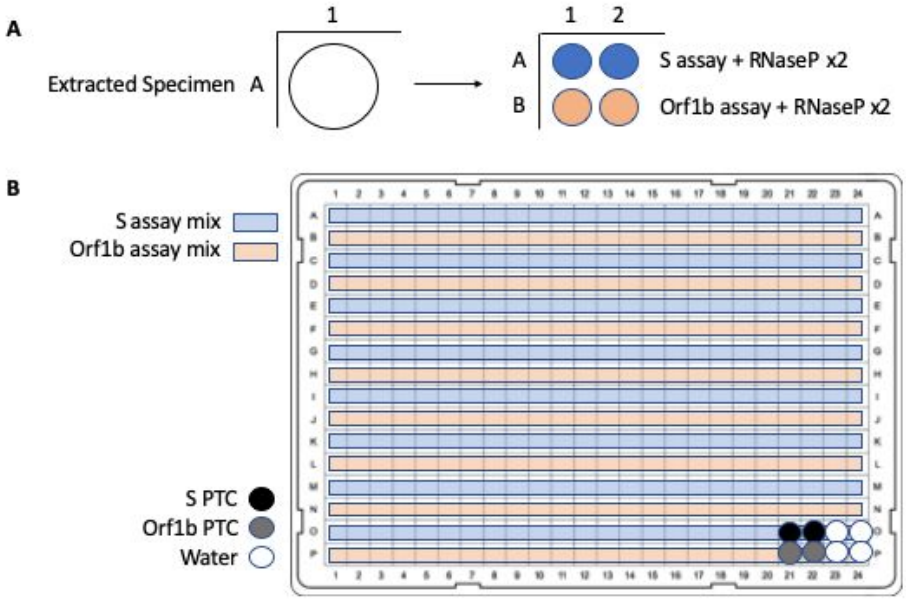
Brief summary of our protocols:

1. Samples are aliquoted from the original upper respiratory sample in Universal Transport Medium into barcoded matrix tubes (Thermo).
2. 200 ul of sample is transferred to the extraction plate. The remainder of the material is stored at -80˚C.
3. Total nucleic acids are extracted with a Magna Pure 96 automated extractor using the DNA and Viral NA Small Volume Kit (Roche).
4. Extracted nucleic acids are held on ice during all downstream steps.
5. 5 ul of extracted nucleic acids is loaded into Taqman assays using a 96-well pipettor (Liquidator, Rainin) in quadruplicate in a 384 well plate containing SARS-CoV-2 and RNaseP rtRT-PCR reactions master mixes:

1. **Master mix for S target.** (1x TaqPath 1 step RT-qPCR master mix (Life Technologies A15300), 1X SARS-CoV-2 assay with a FAM fluor (Life Technologies 4332079 assay # APXGVC4) and RNaseP assay with VIC fluor (Life Technologies A30064)) loaded into rows A, C, E, G, I, K, M, O using a Mantis liquid dispenser (Formulatrix) in a dead air hood).
2. **Master mix for Orf1B target.** (1x TaqPath 1 step RT-qPCR master mix (Life Technologies A15300), 1X SARS-CoV-2 assay with a FAM fluor (Life Technologies 4332079 assay # APGZJKF) and RNaseP assay with VIC fluor (Life Technologies A30064)) loaded into rows B, D, F, H, J, L, N, P using a Mantis liquid dispenser (Formulatrix) in a dead air hood).
3. Positive control DNA is loaded into 2 positive control wells for each target and water is added to the 4 no template control wells.
4. Plate is sealed with a clear adhesive (Bio-Rad MSB1001)

6. Real time RT-PCR is performed QuantStudio 6 (Applied Biosystems) on the Fast setting according to manufacturer instruction.

Figure 1. Layout of the plate for the assay:



MATERIALS

Equipment

MANTIS automated liquid handler, Formulatrix
Liquidator, Eppendorf
QuantStudio 6 RT-qPCR instrument, Applied Biosystems
Dead air hood

Reagents

4X TaqPath 1-Step RT qPCR master mix (Life Technologies A15300)
20X TaqMan RNase P assay with VIC fluor
(Life Technologies A30064)
60X custom Orf1B assay, with FAM fluor
(Life Technologies 4332079 assay #APGZJKF)
60X custom S assay, with FAM fluor
(Life Technologies 4332079 assay #APXGVC4)
TaqMan 2019-nCoV Control Kit v1 (Life Technologies A47533) for S and Orf1b
Nuclease-free water, Thermo

Supplies

MicroB Seal, BioRad
384-well plate, Thermo
Microfuge tubes
Pipettors and barrier tips
Centrifuge with plate adaptor

Controls we used for validation:

Positive control 1 for rtPCR:

Control RNA is from BEI Resources, NR-52285 Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WA1/2020 (lot# 70033320). LoD will be performed with this RNA.

Positive control 2 for rtPCR:

Synthesized DNA (Life Technologies A47533) of the rtRT-PCR target spiked into nucleic acids from upper respiratory samples collected prior to November, 2019. LoD will be performed with this DNA.

Positive control 3 for rtPCR:

Samples known to contain SARS-CoV-2. These samples were identified by research testing and confirmed to be positive by sequencing and additional testing by the Washington Department of Health.

Internal control for sample quality and extraction is human RNaseP.

Extraction Control 1:
50,000 cells from human cell line HAP1, these samples are positive for RNaseP by rtPCR.

Extraction Control 2:
Each respiratory specimen should be positive for human RNaseP by rtRT-PCR.