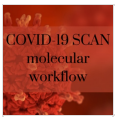

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COVID-19 SCAN molecular workflow

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1 Works for me dx.doi.org/10.17504/protocols.io.bebkjakw

Coronavirus Method Development Community

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ABSTRACT

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.

ATTACHMENTS

SCAN_molecular_workflow.pdf

GUIDELINES

Controls for validation:

Positive control 1 for RT-PCR:
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 RT-PCR:
Synthesized DNA (Life Technologies A47533) of the RT-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 RT-PCR:
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 RT-PCR.

Citation: Lea Starita (04/01/2020). COVID-19 SCAN molecular workflow. https://dx.doi.org/10.17504/protocols.io.bebkjakw

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

MATERIALS

NAME	CATALOG #	VENDOR
Nuclease-Free Water	AM9939	Thermo Fisher Scientific
TaqPath™ 1-Step RT-qPCR Master Mix, CG	A15300	Thermo Fisher
TaqMan™ RNase P Assay, VIC™ dye/QSY™ probe	A30064	Thermo Fisher
TaqMan™ 2019nCoV Control Kit v1	A47533	Thermo Fisher Scientific
60X custom Orf1B assay with FAM fluor (#APGZJKF)	4332079	Thermo Fisher Scientific
60X custom S assay with FAM fluor (#APXGVC4)	4332079	Thermo Fisher Scientific
MagNA Pure 96 DNA and Viral NA Small Volume Kit	06543588001	Roche

MATERIALS TEXT

Equipment

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

Supplies

- MicroB Seal (BioRad MSB1001)
- 384-well plate (Thermo)
- Microfuge tubes
- Pipettors and barrier tips
- Centrifuge with plate adaptor

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

1 Aliquot samples from the original upper respiratory sample found in Universal Transport Medium and transfer into barcoded matrix tubes (Thermo).

2 Transfer **200 µl** of sample to the extraction plate and store the remainder of sample in **-80 °C** .

3 Extract total nucleic acids using the Magna Pure 96 automated extractor using the DNA and Viral NA Small Volume Kit (Roche).

4 Place and hold extracted nucleic acids **On ice** for the remainder of steps in the protocol.

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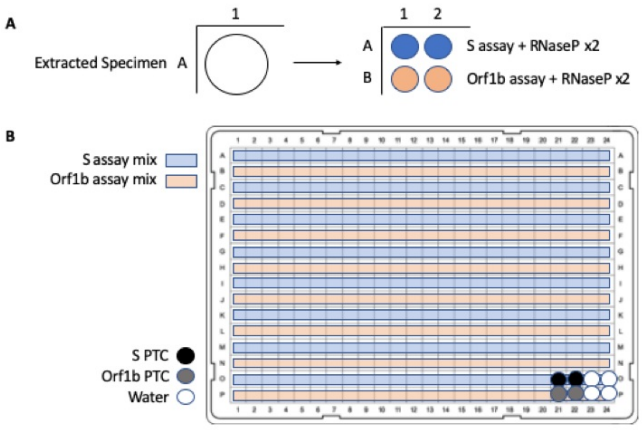
5 Using a 96-well pipettor (Liquidator, Rainin), load **5 µl** of extracted nucleic acids into Taqman assays in quadruplicate into a 384 well plate containing SARS-CoV-2 and RNaseP RT-PCR reactions master mixes:



Layout of the plate for the assay

5.1 **Master mix for S target:** load into rows A, C, E, G, I, K, M, O using a Mantis liquid dispenser (Formulatrix) in a dead air hood:
- Master Mix Component 1: 1x TaqPath 1 step RT-qPCR master mix (A15300)
- Master Mix Component 2: 1x SARS-CoV-2 assay with a FAM fluor (4332079 assay # APXGVC4)
- Master Mix Component 3: RNaseP assay with VIC fluor (A30064)

5.2 **Master mix for Orf1B target:** load into rows B, D, F, H, J, L, N, P using a Mantis liquid dispenser (Formulatrix) in a dead air hood:
- Master Mix Component 1: 1x TaqPath 1 step RT-qPCR master mix (A15300)
- Master Mix Component 2: 1X SARS-CoV-2 assay with a FAM fluor (4332079 assay # APGZJKF)
- Master MIx Component 3: RNaseP assay with VIC fluor (A30064)

5.3 Load positive control DNA into 2 positive control wells for each target and add water to the 4 no template control wells.

5.4 Seal plate with a clear adhesive (Bio-Rad MSB1001).

6 Perform RT-PCR on QuantStudio 6 (Applied Biosystems) on the Fast setting according to manufacturer instruction.

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