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Fecal sample pooling for SARS-CoV-2 real-time RT-PCR screening

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DISCLAIMER

Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

ABSTRACT

This document includes the information and procedures to do sample pooling for fecal samples and real-time RT-PCR for SARS- CoV-2 using CDC based primers and probes.

Validation data for 5 sample pools (in-house and by an independent laboratory via collaborative study such as Blinded Method Test) are available upon request.

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Protocol status: Working
We use this protocol and it's working

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MATERIALS

A. Kits and Reagents

- CDC-based SARS-CoV-2 N1 primers and probe
  2019-nCoV_N1 Cimbined Primer/Probe Mix at 22.5 nmol
- MagMAX™ Pathogen RNA/DNA Kit, **Catalog number:** 4462359.
- ThermoFisher Scientific. AgPath-ID™ One-Step RT-PCR Reagents, **Catalog number:** AM1005.

B. Equipment

- KingFisher 96 magnetic particle processor, or equivalent (Thermo Electron Corp. Cat#5400050)
- Benchtop centrifuge-Eppendorf (Model# 5415 D) or equivalent
- BioRad CFX96, BioRad Opus real-time PCR machine or equivalent
- Vortex Mixer or equivalent
- Mini Plate Spinner, equivalent, or larger centrifuge capable of centrifuging 96-well plates

C. Supplies

- 1000 µl, 200 µl, and 20 µl pipets
- 1000 µl, 200 µl and 20 µl aerosol pipet tips
- Serological pipettor
- 50 ml reservoirs
- Sterile 1.5 ml microcentrifuge tubes
- Sterile PCR reaction tubes, strip tubes, or 96-well plates
- Optically clear adhesive seals for plates
- Gloves
- 10 ml, 5 ml serological pipets

SAFETY WARNINGS

SARS-CoV-2 is a Biosafety Level 3 agent and samples should be handled as approved by each insitution. Proper PPE including double gloves, disposable or autoclavable gown, N95 with face shield or CAPR unit is required and must be used when working in the BSL-3. Gloves and lab coats are required for extraction and PCR setup. Gloves must be worn at any time when touching extraction or PCR plates or reagents.

Prepare fecal samples

1 Add 1 mL phosphate buffered saline (PBS, pH 7.4) to an Eppendorf tube.

2 Use cotton tipped wooden swabs to wipe a fecal sample in four places.

> **Note**
>
> Consistence of fecal samples can vary. For drier samples cotton tipped can be dipped into PBS prior to wiping the fecal samples.

3 Place the cotton swab with fecal material into the PBS in the Eppendorf tube.

3.1 Move the swab up and down within the PBS three time to remove as much feces as possible.

3.2 While removing the swab, let swab contact the top inside of Eppendorf tube with slight pressure to avoid losing solution.

> **Note**
>
> Some PBS will remain on the swab after removal.

4 Add additional amount of PBS to make 1 mL fecal suspension solution for each sample.

5 Vortex the tube for 00:00:15 and 8000 rpm centrifuge 00:02:00. 2m 15s

6 Sample can be stored at -20/-80C

Note

-80C is recommended to prevent viral degradation

Sample pooling

7 Based on the manual procedure of MagMAX™ Pathogen RNA/DNA Kit, 200 µl amount of fecal suspension supernatant is used for extraction of nucleic acid.

8 For a 5-sample pooling: 40 µL of supernatant from each sample will be mixed in a new Eppendorf tube, for a total of 200 µL

Note

Critical point: Make sure all material is transferred into micro plate

Note

If samples were stored after step 5 make sure to thaw on ice and repeat step 5

9 For a 10-sample pooling, 20 µL of supernatant from each sample will be mixed in a new Eppendorf tube, for a total of 200 µL.

Note

Critical point: Make sure all material is transferred into micro plate

RNA extraction using MagMAX™ Pathogen RNA/DNA Kit

10 Prepare lysis/binding solution and mix well by vortexing

Note

Amount needed for each sample are given below. Make a master mix by combining all elements into one solution for total samples being processed.

10.1 Lysis/Binding Solution Concentrate: 200 µL

10.2 Carrier RNA (µg/µL): 2 µL

10.3 100% Isopropanol: 200 µL

11 Prepare the Bead Mix and mix well by vortexing

Note

Amount needed for each sample are given below. Make a master mix by combining all elements into one solution for total samples being processed.

11.1 Nucleic Acid Binding Beads: 10 µL

11.2 Lysis ENHANCER: 10 µL

12 Set up plates.

12.1 Lable a MME-96 Deep Well Plate "First Wash 1" and add 300 µL into each well with Wash Soultion 1

12.2 Lable a MME-96 Deep Well Plate "Second Wash 1" and add 300 µL into each well with Wash Soultion 1

12.3 Lable a MME-96 Deep Well Plate "First Wash 2" and add 450 µL into each well with Wash Soultion 2

12.4 Lable a MME-96 Deep Well Plate "Second Wash 2" and add 450 µL into each well with Wash Soultion 2

12.5 Lable a MME-96 Deep Well Plate "Elution". Add 60 µL Elution buffer to each well.

12.6 Put Tip comb plate to a MME-96 Standard Plate.

13 Set up sample plate in a MME-96 Deep Well Plate.

13.1 Add 20 µL of prepared Bead Mix to each well.

13.2 add 200 µL of fecal suspension supernatant mixtures to each well.

13.3 add 400 µL lysis/binding solution to each well.

13.4 Vortex sample plate on shaker for 00:05:00 5m

14 Load all plates to KingFisher Flex for extraction of nucleic acid using the MagMAX™ Pathogen RNA/DNA Kit procedure.

14.1 Select the program: MagMax_Pathogen_Hight_Volume 96DW on the instrument (4462359_DW_50)

15 Once extraction is complete, take the elution plate out.

15.1 Store the purified nucleic acid on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

Real-time RT-PCR

16 AgPath-ID™ One-Step RT-PCR Reagents is used in the real-time RT-PCR along with the CDC-based SARS-CoV-2 N1 primer assay

Note

Amount needed for each sample are given below. Make a "master mix' by combining all elements into one solution for total samples being processed.

16.1 2X RT-PCR Buffer: 12.5 µL

16.2 25X RT-PCR Enzyme Mix: 1 µL

16.3 CDC-based SARS-CoV-2 N1 primers and probe (FAM dye) are used: 2 µL

16.4 The VetMAX™ Xeno™ Internal Positive Control (IPC) - LIZ™ Assay (Cy5): 1 µL

16.5 VetMAX™ Xeno™ Internal Positive Control DNA: 1 µL

16.6 DNase/RNase-free distilled water: 2.5 µL

17 Add 20 µL of Master Mix to each well/tube used for RT-PCR

18 Add 5 µL RNA templates pooled samples, Negative template control (NTC) such as, DNase/RNase-free distilled Water, and positive control.

19 Program real time thermocycler

19.1 48^{O}C for 10 minutes.

95 ^{O}C for 10 minutes.

40 cycles of

95^{O}C for 15 seconds.

60^{O}C for 45 seconds.

8. Data analysis.

20 Check Ct value of Xeno spiked control for each sample to see if any inhibition occurs