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Detection of Sars-Cov2 Using Droplet Digital PCR

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.be8tjhwn

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ABSTRACT

Assay sensitivity is an important part of any lab test, this becomes a more critical issue when dealing with matters involving public health. The potential of pre- or asymptomatic carriers, as well as convalescents which have not completely cleared the virus are a public health risk. PCR based tests, specifically quantitative PCR is the gold standard to test for the novel coronavirus (SARS-Cov2), however, it is limited by its detection capabilities, and the results are based off of an amplification curve, rather than a real number or concentration. The limit of detection with quantitative PCR could easily yield a false negative for contagious individuals. In comparison, droplet digital PCR (ddPCR) is estimated to be 500 times more sensitive than quantitative PCR, and results are displayed as an absolute number (copies/uL). In this method our group adapted from our usual studies related to Parkinson's disease, we have been able to confirm that ddPCR is more sensitive than quantitative PCR, detect SARS-Cov2 RNA-spiked into human samples, and detect SARS-Cov2 in known positive cases of COVID-19.

GUIDELINES

Prior to testing human samples, dilution series of a control plasmid, such as 2019-nCoV_N_ Positive control template (IDT 10006625), and control RNA such as SARS-Cov2 RNA spike control (ATCC VR-3276T) should be performed to determine the limit of detection of the equipment.

It is important to note that the 2019-nCoV_N_ Positive control template (IDT 10006625) is a circular plasmid. As such, the plasmid is prone to super-coiling, which can cause the ddPCR to read fewer copies than would be expected.

MATERIALS TEXT

- DNase/RNase free molecular grade water
- PCR tube strips (Sterile and DNase/RNase free)
- Microcentrifuge tubes (Sterile and DNase/RNase free)
- 2019-nCoV RUO Kit, primer/probe set for N1, N2, and human RNaseP (IDT 10006713)
- 2019-nCoV_N_ Positive control template (IDT 10006625)
- SARS-Cov2 RNA spike control (ATCC VR-3276T)
- iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad 1708841)
- ddPCR Supermix for Probes (no dUTP) (Bio-Rad 186-3026)
- Droplet generation oil (Bio-Rad 1863005)
- DG8 droplet generator cartridge (Bio-Rad 1864008)
- Droplet generator gaskets (Bio-Rad 1863009)
- 200 ul XTIP4 Filtered Pipet tips for transferring droplets (Fisher 12111362)
- 96 well plate (Bio-Rad 12001925)
- Heat seal pierceable foil (Bio-Rad 181-4040)
- Thermocycler (Bio-Rad C1000)
- QX droplet generator (Bio-Rad 186-4002)
- Plate sealer (Bio-Rad 181-4000)
- BioRad droplet reader
- Droplet Reader Oil (Bio-Rad 1863004)

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cDNA Synthesis

1 Add 11 µL of Master mix to each PCR tube used.
•4.4 µL iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad 1708841).
•6.6 µL DNase/RNase free water.

2 Add 11 µL of sample/diluted RNA to each tube (identify optimal dilution prior to performing the real experiment).

3 Close tubes and shake by hand to mix.

4 Centrifuge to collection samples at the bottom of the tubes.

5 Perform cDNA synthesis reaction in a Thermocycler (~30 min. total).
•Constant lid temperature of 105ºC.
•5 min. at 25ºC.
•20 min. at 46ºC.
•1 min. 95ºC
• Hold at 4ºC.

Droplet Digital PCR

6 Dispense 11 µL master mix to each PCR tube.
•9 parts ddPCR Supermix for Probes (no dUTP) (Bio-Rad 186-3026).
•1 part Probe

6.1 A human RNaseP, SARS-Cov2 N1, or SARS-Cov2 N2 probe is used in each reaction.

7 Transfer 11 µL of cDNA to each PCR tube containing master mix.

7.1 In addition cDNA made from human samples, a comercial cDNA control for SARS-Cov2 is also used with each probe as a control.

8 Mix samples and centrifuge to collect at the bottom of the tube.

9 Obtain a DG8 droplet generator cartridge (Bio-Rad 1864008) and transfer 20 µL of sample to the sample well.

10 Transfer 70 µL of droplet generation oil (Bio-Rad 1863005) to the oil well.

11 Secure the gasket (Bio-Rad 1863009) over the cartridge, and used the QX droplet generator (Bio-Rad 186-4002) to generate droplets.

12 Transfer 40 µL droplets to a 96 well plate (Bio-Rad 12001925).

13 When all droplets for the experiment have been transferred, cover plate with pierceable foil (Bio-Rad 181-4040) with the RED LINE SIDE UP AT THE TOP,

14 Heat seal with a plate sealer (Bio-Rad 181-4000).

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15 Transfer plate to a Real-Time thermocycler.
•10 min. at 95ºC
•40 cycles (30 s 94ºC, 1 min. 60ºC)
•10 min. at 98ºC.
•Hold at 4ºC.

16 Transfer plate to droplet reader and run.

Analysis

17 Analysis is performed with the Quantasoft software associated with the droplet reader.

18 Lower limit of quantitation is 10 copies/ul.

19 Lower limit of detection is 1 copy/ul.

20 A single present copy/ul in both N1 and N2 probes is classified as a suspected positive test.

21 A human RNaseP probe is used to confirm a quality sample of RNA was obtained from each subject.

22 A 500 copy/ul cDNA standard is run with the N1 and N2 probes on each plate as a positive control.

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