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Technique: Real Time RT-PCR

Methodology: The assays design in the protocol are reactive with coronavirus under the subgenus *Sarbecovirus* that includes 2019-nCoV, SARS-CoV and bat SARS-like coronavirus. As SARS was eliminated in humans, positives cases in the performed assays should be considered as infection by 2019-nCoV.

The protocol intends the targeting of two genes: *N* and *Orf1b*. The first is recommended as a screening assay and the second as a confirmatory one. Sequence analyses is denoted as helpful in the confirmation of the results and in the distinguish between SARS-CoV and 2019-nCoV.

Material:

- QIAamp Viral RNA Mini Kit (QIAGEN, Cat# 52906) or equivalent
- TaqMan Fast Virus Master mix (ThermoFisher, Cat# 4444432)
- Ethanol (96–100%)
- MicroAmp Fast Optical 96-well reaction plate (ThermoFisher, Cat# 4346907)
- MicroAmp optical adhesive film (ThermoFisher, Cat# 4311971)
- Microcentrifuge (adjustable, up to 13 000 rpm)
- Adjustable pipettes (10, 20, 100, 200 μ L)
- Sterile, RNase-free pipette tips with aerosol barrier
- Vortex
- Microcentrifuge tubes (0.5mL and 1.5 mL)
- Thermocycler (ThermoFisher, ViiA™ 7 Real-Time PCR)
- Positive control (Available from HKU, e-mail: llmpoon@hkucc.hku.hk)
- Primer sets

- Primers and Probes sequences

Assay 1 (Target: *ORF1b-nsp14*; 132 bp)

HKU-ORF1b-nsp14F: 5'-TGGGGYTTTACRGGTAACCT-3'

HKU-ORF1b-nsp14R: 5'-AACRCGCTTAACAAAGCACTC-3'

HKU-ORF1b-nsp141P: 5'-FAM-TAGTTGTGATGCWATCATGACTAG-TAMRA-3'

Assay 2 (Target: *N*; 110 bp)

HKU-NF: 5'-TAATCAGACAAGGAACTGATTA-3'

HKU-NR: 5'-CGAAGGTGTGACTTCCATG-3'

HKU-NP: 5'-FAM-GCAAATTGTGCAATTTGCGG-TAMRA-3'

Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR

Protocol:

1. Viral RNA extraction, performed according to QIAamp viral RNA mini kit manufacturer's instructions
2. Preparation of one-step monoplex RT-PCR master mix as below:

Volumes (μ L) per reaction for each assay		
Reagent	Assay 1 (<i>ORF1b-nsp14</i>) Confirmatory	Assay 2 (<i>N</i>) Screening
H ₂ O (RNase free)	8.5	8.5
4x Reaction Mix*	5	5
Primer HKU-ORF1b-nsp14F (10 μ M)	1	-
Primer HKU-ORF1b-nsp14R (10 μ M)	1	-
Probe HKU-ORF1b-nsp141P (10 μ M)	0.5	-
Primer HKU-NF (10 μ M)	-	1
Primer HKU-NR (10 μ M)	-	1
Probe HKU-NP (10 μ M)	-	0.5
Total reaction mix	16	16
Template RNA, add	4	4
Total volume	20	20

* Reaction mix from TaqMan Fast Virus Master mix

3. Set the follow RT-PCR conditions (Both monoplex assays can be conducted under the same conditions):

Temperature ($^{\circ}$ C)	Time	No of Cycles
50	5 min	1
95	20 sec	
95	5 sec	40
60	30 sec	