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Technique: Real Time RT-PCR

Methodology: The protocol is designed in order to target the *N* gene to detection of 2019-nCoV infection.

Material:

- QIAamp Viral RNA Mini Kit (Qiagen)
- QuantiTect Probe RT-PCR Kit (Qiagen)
- LyghtCycler96 (Roche)
- Primers and Probes sequences

  NIID_2019-nCOV_N_F2: 5'-AAATTTTGGGGACCAGGAAC-3'

  NIID_2019-nCOV_N_R2: 5'-TGGCAGCTGTGTAGGTCAAC-3'

  NIID_2019-nCOV_N_P2: 5'-FAM-ATGTCGCGCATTGGCATGGA-BHQ-3'

Protocol:

1. Viral RNA extraction, performed according to QIAamp viral RNA mini kit manufacturer's instructions

2. Preparation of Primer FW, Primer RV and Probe Mix. Concentration should be 500 nM, 700 nM and 200 NM, respectively.

3. Preparation of one-step monoplex RT-PCR master mix as below. Negative and positive controls should be performed.

Reagent	Volume (µL) per reaction
DDW	3.8
2x Master Mix	10
RT Mix	0.2
Primer NIID_2019-nCOV_N_F2	1 (Mix of the components)
Primer NIID_2019-nCOV_N_R2	
Probe NIID_2019-nCOV_N_P2	
Total reaction mix	20
Template RNA, add	5
Total volume	25

4. Set the follow RT-PCR conditions:

Temperature (°C)	Time	No of Cycles
50 95	30 min 15 min	1
95 60	15 sec 1 min	40

Results Evaluation: In the present work, negative control was undetected, positive control (500 copies) had an average Cq value of 35.0, and the specimen had 36.7.