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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:

- NucleoSpin RNA Virus, Mini kit for viral RNA from cell-free fluids (Macherey-Nagel; Cat. No 740956)
- Superscript™ III Platinum One-Step qRT-PCR Kit (Invitrogen™; Cat No. 11732020 or 11732088)

- Primers and Probe sequences

  WH-NIC N-F: 5'-CGTTTGGTGGACCCTCAGAT-3'

  WH-NIC N-R: 5'-CCCCACTGCGTTCTCCATT-3'

  WH-NIC N-P: 5'-FAM-CAACTGGCAGTAACCA-BQH1-3'

Protocol:

1. Viral RNA extraction, performed according to NucleoSpin RNA Virus manufacturer's instructions

2. Preparation of one-step monoplex RT-PCR master mix as below. The reactions should be set up on ice. Negative and positive controls should be performed.
   [Note: Primers and probe are used at different concentrations]

Reagent	Volume (µL) per reaction
H_2O (RNAse free)	5.5
2x PCR Reaction Mix	12.5
SuperScript-Taq Mix	0.5
Primer WH-NIC N-F (40µM)	0.5
Primer WH-NIC N-R (40µM)	0.5
Probe WH-NIC N-P (10µM)	0.5
Total reaction mix	20
Template RNA, add	5
Total volume	25

3. Set the follow RT-PCR conditions:

Temperature (°C)	Time	No of Cycles
50 95	30 min 2 min	1
95 55	15 sec 30 sec	45

Results Evaluation: Negative control should be undetected. A positive control should be detected with Ct ≤ 38.