Laboratory testing procedure of 2019-nCoV by real-time RT-PCR

developed by the National Institute of Viral Disease Control and Prevention, China CDC

I. Specimen collection

Respiratory specimens must be collected for each suspected 2019 novel coronavirus (2019-nCoV) infection. In severe cases, specimens of the lower respiratory tract (e.g. bronchi or alveolar lavage) are preferred.

Specimen type:

1. Upper respiratory tract specimens: includes swabs for pharynx, nasal swabs, nasopharyngeal swabs, and deep cough sputum.
2. Lower respiratory tract specimens: including respiratory tract extracts, bronchial lavage fluid, alveolar lavage fluid, and lung biopsy specimens.

II. Specimen detection

The conventional method for detecting 2019-nCoV infection is real-time reverse transcription polymerase chain reaction (rRT-PCR). Primers and probes targeting open reading frame 1ab (ORF1ab) and nucleocapsid protein (N) genes in the 2019-nCoV genome are recommended.

1. Primer and probe sequence:

Target 1 (ORF1ab) :

Forward primer (F) : CCCTGTGGGTTTTACACTTAA

Reverse primer (R) : ACGATTGTGCATCAGCTGA

Fluorescent probe (P) : 5 '- the FAM - CCGTCTGCGGTATGTGGAAAGGTTATGG - BHQ1-3'

Target 2 (N) :

Forward primer (F) : GGGGAACTTCTCCTGCTAGAAT

Reverse primer (R) : CAGACATTTTGCTCTCAAGCTG

Fluorescent probe (P) : 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'

Nucleic acid extraction and fluorescence quantitative RT-PCR reaction system, please refer to the usage instructions by manufacturer.

2. Judgment of results

Negative: no Ct value or Ct 40.

Positive: Ct value <37 can be reported as positive.

Suspicious: the Ct value is between 37-40, it is recommended to repeat the experiment. If the Ct value is less than 40 and the amplification curve has obvious peaks, the sample should be judged as positive, otherwise negative.

3. To confirm a positive case in the laboratory, the following conditions should be met:

The specific RT-PCR results of two targets (ORF1ab and N) of 2019-nCoV in the same sample were positive.

Negative results also cannot exclude 2019-nCoV infection, and factors that may produce false negatives need to be excluded, including: poor sample quality, such as respiratory tract samples from oropharynx and other sites; Early or late collection of samples; Failure to properly store, transport and process samples; Reasons for the existence of technology itself, such as; Virus mutation, PCR inhibition, etc.

III. Verification of the sensitivity and specificity of the detection method.

1. Sensitivity: the nucleic acid test results were still positive after 105-fold dilution of the clinical samples;

2. Specificity: 2019-nCoV-specific ORF1ab and N primers, probe detection system do not cross-react with other 6 coronaviruses and influenza viruses that can infect humans.