



Laboratory Detection of Novel Corona Virus 2019 using Polymerase Chain Reaction

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Since mid-December 2019, several cases of a pneumonia like disease (with symptoms including fever, difficulty in breathing, cough and invasive lesions on both lungs) of unknown cause have emerged in the central Chinese city of Wuhan. Chinese authorities made a preliminary determination that the causative agent is a novel coronavirus(2019-nCoV). (1) Coronaviruses are enveloped RNA viruses belonging to Coronaviridae family and the order Nidovirales. This subfamily consists of four genera alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus on the basis of their phylogenetic relationships and genomic structures. These subfamilies are broadly distributed for causing infections in humans and other mammals. (2) The alphacoronaviruses and betacoronaviruses infect only mammals. The gammacoronaviruses and deltacoronaviruses infect birds, but some of them can also infect mammals. The source of betacoronavirus 2019-nCoV is still unknown, although initial cases have been linked with south Huanan seafood market. (3) Viral infections already known to produce similar symptoms are influenza, parainfluenza, Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV). (4) Laboratory investigations reported raised plasma levels of L2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNF α in patients. (1)

WHO reports day by day telling about spread of infection over entire globe. At time of writing this report, 2019-nCoV infection in humans has been reported in Australia, France Japan, Malaysia, Nepal, Singapore, South Korea, Taiwan, Thailand and United States. (5) Pakistan, being a partner of China Silk Route, has two-way movement of citizens, is at major risk of epidemic in the country. It is, thus, important to have designed and developed diagnostic assay for confirmation of 2019-nCoV infection, if any. Authors surveyed for recent reports of 2019-nCoV and gathered significant genomic and molecular information about the virus. Data sources utilized for this purpose were GenBank, Global Initiative on Sharing All Influenza Data (GISAID) and virlogical.org.

Corman et al. reported diagnostic assay using real-time RT-PCR. Method is good to differentiate between 2019-nCoV and SARS-CoV. (6) But we used information given in by Huang et al. to develop 2019-nCoV RNA detection technique. This laboratory procedure should be performed in Biosafety level III settings. Details of assay are as following:

RNA Extraction: Laboratories can extract virus RNA using any readymade RNA miniprep kit for downstream analysis.

PCR Recipe:

Forward primer: 5'-TCAGAATGCCAATCTCCCCAAC-3'

Reverse Primer: 5'-AAAGGTCCACCCGATACATTGA-3'

Mastermix: SuperScript III Platinum Sybr Green One-Step qRT-PCR Kit

Amplification conditions of PCR reaction are given in Figure 1.

Pathogen detection: Following real-time RT-PCR, presence of 2019-nCoV can be detected through real-time PCR results.

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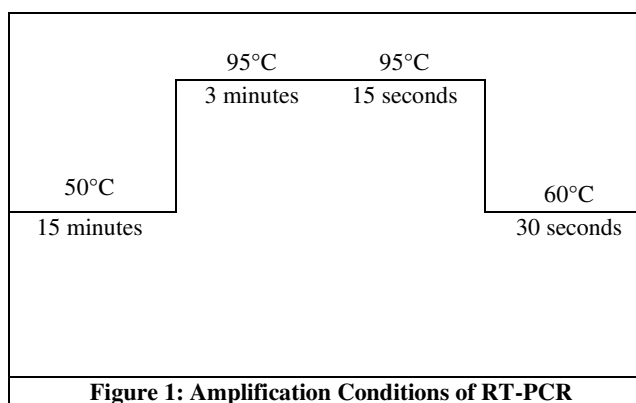


Figure 1: Amplification Conditions of RT-PCR

6. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance*. 2020;25(3):2000045.