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Short Communication

Recent Advances in Molecular diagnosis curbing the COVID-19

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WHO assigned name SARS-CoV-2 to virus causing Corona Virus Disease (COVID-19) which emerged in Wuhan city of Hubei province. It causes acute febrile illness with respiratory distress syndrome (ARDS). In 21st century SARS-CoV-2 emerged as highly pathogenic corona virus for humans after SARS and MERS. World health organization declared COVID-19 outbreak a public health emergency of international concern on 30 of January 2020 (WHO, 2020; Tang et al., 2020; Wu and McGoogan, 2020). The genome of coronavirus and its phylogenetic analysis indicate that it placed in distinct clade from other human β corona virus which caused SARS and MERS. On 28th April 2020 SARS-CoV-2 has spread to 213 countries. It infected more than 2 million people and resulted in 193825 deaths globally. The exact number of infected people with SARS-CoV-2 is not known, as many asymptomatic cases go undetected (Kobayashi et al., 2020). From the study of Diamond Princes cruise ship cases, the estimate reported 17.9% asymptomatic cases (Mizumoto et al., 2020). Therefore asymptomatic individuals are infectious like symptomatic individuals and transmit the disease further. In the absence of vaccine and proper treatment, currently available efficient lever to reduce the transmission of SARS-COV-2 is to identify and isolate persons who are contagious (Wu et al., 2020).

The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of affected cases, assessment of the extent of the outbreak, monitoring of intervention strategies and surveillance studies. FDA approved a number of molecular tests for emergency use to address the pandemic confronting the World (FDA, 2020). Broad testing will help identify the infected, allowing proper quarantining, treatment and control of its spread. This study gives a brief of FDA Emergency Use Only recommended nucleic acid diagnostic modalities along with the limit of detection, target gene, type of sample and name of kits and developer details (Table 1).

Nucleic acid detection technologies available for the detection of SARS-CoV-2 are RT-PCR and sequencing. The use of high throughput sequencing techniques is limited due to equipment dependency and cost. RT-PCR routinely used for the detection of SARS-CoV-2, acts as a gold standard platform because of its high sensitivity (Corman et al., 2020). Different types of sampling techniques are used for detection include throat swab, nasopharyngeal swab, bronchoalveolar lavage fluid, sputum and endotracheal aspirates. Nasopharyngeal sample most commonly used sampling technique (Zou et al., 2020). However bronchoalveolar lavage fluid, sputum endotracheal aspirates may have greater sensitivity than upper respiratory tract samples (Wang et al., 2020). Improper sampling technique may lead to false negative results. False negativity may be minimized by using flocked swab as it enhance the collection and release of cellular material and preferred those swab who have plastic or aluminium shaft. Sample transportation is another risk factor that contributes in false negativity of infectious sample. Collected samples undergo RNA extraction followed by RT-PCR for target detection. Three types of strategies have been described for target detection) single gene target assay(i) double gene target assay iii) and multiplex assay.

The sensitivity of RT-PCR varies greatly, depending upon the target region of the virus used for amplification. Variation in the detection rate of some kits was observed but none of the assays showed cross-reactivity with other respiratory (corona) viruses (van Kasteren et al., 2020). Commercially available assays no longer reported result in copies of viral RNA per milliliter (Table 1) To compare their reported sensitivity/limit of the assay results have been equalized into copies /mL RT-PCR Kit for Detecting SARS-2019 of m/s BGI Genomics and Panther Fusion SARS-CoV-2 of m/s Hologic, both targets open reading frame ORF 1ab gene but
| Type of Sample | Target Gene | Sensitivity/limit of the Assay | Apparatus Used | Manufacturer |
|----------------|-------------|-------------------------------|----------------|--------------|
| Single Target Gene Assay | Nasopharyngeal swab, throat swabs and BALF | ORF1ab gene | 100-150 copies/mL | Applied Biosystems 7500 Real-Time PCR System | BGI Genomics Co. Ltd |
| Panther Fusion SARS-CoV-2 Kit for Detecting SARS-2019-nCoV | Nasopharyngeal and oropharyngeal swab | ORF1ab gene | 100 copies/mL | Panther Fusion system | Hologic, Inc. |
| ePlex SARS-CoV-2 Test | Nasopharyngeal swab | Nucleocapsid (N) gene | 100000 copies/mL | GenMark ePlex instrument and Software | GenMark Diagnostics, Inc. |
| Ipsum Diagnostics Coronavirus Test | Nasopharyngeal swab | nucleocapsid (N) gene | 8500 copies/mL | Thermofisher Applied Biosystems QuantStudio 12 K Flex instrument. | Ipsum Diagnostics Atlanta GA |
| COVID-19 RT-PCR Test | Nasopharyngeal, oropharyngeal swab, sputum, lower respiratory tract aspirates, BAL and nasopharyngeal wash/aspirate or nasal aspirate | Nucleocapsid (N) gene | 6250 copies/mL | Applied Biosystems QuantStudio7 Flex (Q5T) instrument with software version 1.3 | Laboratory Corporation of America (LabCorp) |
| ScientCell™ SARS-CoV-2 Coronavirus Real-time RT-PCR | Nasopharyngeal, oropharyngeal swab | Nucleocapsid (N) gene. | 3162 copies/mL | LightCycler® 96 Real-Time PCR System with LightCycle | Scient Cell Research Laboratories, Inc. |
| New York SARS-CoV-2 Real-time Reverse Transcriptase (RT-) PCR CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC) | Nasopharyngeal, oropharyngeal swab, sputum | Nucleocapsid (N) gene. | 1000 copies/mL | Applied Biosystems 7500 Fast Dx Real-Time PCR System with SDS version 1.4 software | Wadsworth Center, New York State Department of Public Health’s Centers for Disease Control and Prevention’s (CDC) |
| NeuMoDx SARS-CoV-2 Assay | Nasopharyngeal and oropharyngeal swab | Nucleocapsid (N) gene. | 150 copies/mL | NeuMoDx Molecular Systems | NeuMoDx Molecular, Inc |
| Quest SARS-CoV-2 rRT-PCR | Nasopharyngeal, oropharyngeal swab, sputum, tracheal aspirates and BALF | Nucleocapsid (N) gene. | 51 copies/mL | Applied Biosystems 7500 Real Time PCR System | Quest diagnostic |
| BioGX SARS-CoV-2 | Nasopharyngeal and oropharyngeal swab | Nucleocapsid (N) gene. | 40 copies/mL | BD MAX System | BioGX (USA) |
| Lyra SARS-CoV-2 Assay | Nasopharyngeal swab, nasopharyngeal aspirate and BAL | Non-structural polyprotein (pp1ab) | 800 copies/mL | Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Roche 93 LightCycler 480, or Qiagen Rotor-Gene Q | Quidel Corporation |
| Logix Smart™ Coronavirus Disease 2019 (COVID-19) Kit Primerdesign Ltd COVID-19 genesig Real-Time PCR assay | Nasopharyngeal and oropharyngeal swab | RdRp gene | 4290 copies/mL | Applied Biosystems 7500 Real-Time PCR System, or Roche Light Cycler 480 II, or Bio-Rad FX 96 | Co-Diagnostics, Inc |
| Two target Gene assays | Nasopharyngeal swab, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens | N and RdRp genes | 100 copies/mL | m2000 Real time System | Abbott Laboratories, USA |
| ABBOTT RealTime SARS-CoV-2 Xpert Xpress SARS-CoV-2 | Nasopharyngeal swab | N2 and E genes | 250 copies/mL | Gene Xpert Instrument | Cepheid |
| Simplexa™ COVID-19 Direct BioFire® COVID-19 Test | Nasopharyngeal swab | ORF1ab and 5 genes | 500 copies/mL | Liaison MDX FilmArray 2.0 and/or the FilmArray Torch Instrument Systems | DiaSorin Molecular BioFire diagnostic |
| PerkinElmer New Corona virus Nucleic Acid Detection Kit | Nasopharyngeal swab | ORF1ab and ORF8 genes | 330 copies/mL | Pre-NAT II Automated Workstation and Applied Biosystems 7500 Real-Time PCR | PerkinElmer, Inc |
| cobas SARS-CoV-2 | Nasopharyngeal and oropharyngeal swab | ORF1ab and N genes | 20 copies/mL | FilmArray 2.0 and/or the FilmArray Torch Instrument Systems | Roche Diagnostics |
| ARIES SARS-CoV-2 Assay | Nasopharyngeal swab | ORF1 and E genes | 0.007 and 0.004 TCID50/mL | MACPDX System | Luminex Corporation |
Table 1 (Continued)

| Apparatus Used | Manufacturer                        | Target Gene                  | Sensitivity/Limit of the Assay | Type of Sample       |
|----------------|-------------------------------------|------------------------------|-------------------------------|---------------------|
| QIAstat-Dx Respiratory 2019-nCoV Panel | Qiagen GmbH | nCoV Panel and E gene | 500 copies/mL | Nasopharyngeal swab |
| TaqPath™ COVID-19 Combo Kit | Applied Biosystems | ORF1ab, N gene, S gene, MS2 | 10 GCE/reaction | Nasopharyngeal swab |
| CDC= Centre for disease control and prevention; EUA = Emergency Use Authorization; FDA = U.S. Food and Drug Administration. N=Nucleocapsid, E= envelop, ORF= open reading frame, RdRp= RNA-dependent RNA polymerase. | | | | 

The detection limit of these assays ranges from 20 copies/mL to 500 copies/mL.

Among PCR assays that target two gene, Abbott Realtime SARS-CoV-2 m2000 RT System uses a combination of N and RDRP gene while four other assays target ORF1ab gene in combination with nucleocapsid (N), structural (S) and envelope (E) genes.

Although, Realtime RT PCR is a predominant method for detection of all types of Coronavirus, including SARS-CoV-2, the available Realtime RT PCR kits have failed to detect the virus at early stages and give false negative results (Rothe et al., 2020). The rapidly mutating nature of coronaviruses also demands a more accurate method for detection. Thus, multiplex Realtime RT-PCR systems using multiple genes (combinations of ORF lab gene, N gene, S gene and MS2 (Coat protein), RDRP gene) simultaneously amplified and tested has been developed. This may play important role in avoiding false negative results. The sensitivity of these assays ranges from 10 GCE/reaction (Genomic Copy Equivalents) (4000 copies/mL) by TaqPath™ COVID-19 Combo Kit from m/s Applied BioSystenm 2500 GCE/reaction (5000 copies/mL) by NxTACoV Extended Panel Assay. QIAstat-Dx Respiratory 2019-nCoV Panel also gives a favorable sensitivity (5000 copies/mL) by multitarget detection of SARS-CoV-2 (Table 1c).

Variation in the detection rate of some kits was observed but none of the assays showed cross-reactivity with other respiratory (corona) viruses.

The intensive testing for SARS-CoV-2 infection will help to identify infected and quarantining at appropriate time curb the spread of infection. The information on all the parameters provides an insight to both laboratories and clinical teams to identify the correct suitable platform. This will help them make informed decisions on use of kit, based on their need for accurate diagnosis of patients suffering from novel human corona virus. Amidst the pandemic situation, it is now imperative to develop assays, which can be deployed easily in developing and underdeveloped countries, remote locations, and decentralized laboratory systems as well.

Conflict Of Interest

All authors do not have any conflict of interest including any financial, personal or other relationships with other people or organizations of submitted work.

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