Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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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)whichemerged in Wuhan city of Hubei province. It causes acute febrile illness with respiratory distress syndrome (ARDS). In 21 st century SARS-CoV-2 emerged as highly pathogenic corona virus for humans after SARS and MERS. World health organization declaredCOVID-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 clad 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 2million 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 ismost commonly used sampling technique (Zou et al., 2020).However bronchoalveolar lavage fluid, sputum endotracheal aspirates may have greater sensitivity than upper respiratory track 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 assayii) 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

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Table 1
Overview of RT-PCR-based in-vitro diagnostic assays for SARS-COV-2 approved for Emergency Use of Authorizations by FDA.

	Type of Sample	Target Gene	Sensitivity/limit of the Assay	Apparatus Used	Manufacturer
Single Target Gene Assay					
Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV	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	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 (QS7) instrument with software version 1.3	Laboratory Corporation of America (LabCorp)
ScienCell™ 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	Scien Cell Research Laboratories, Inc.
New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)- PCR	Nasopharyngeal and oropharyngeal swab and 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
CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)	Nasopharyngeal oropharyngeal swab, sputum, lower respiratory tract aspirates, BAL and nasopharyngeal wash/aspirate or nasal aspirate)	Nucleocapsid (N) gene	1000 copies/ mL.	Applied Biosystems 7500 Fast Dx Real-Time PCR System with SDS version 1.4 software.	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 orophanryngeal swab	Nucleocapsid (N) gene.	4 0 copies/mL	BD MAX System	BioGX (USA)
Lyra SARS-CoV-2 AssayTaqPath COVID-19 Combo Kit	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	Nasopharyngeal and oropharyngeal swab	RdRp gene	4290 copies/mL	BioMolecular system	Co-Diagnostics, Inc
Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay	Oropharyngeal swab	RdRp gene	330copies/ mL	Applied Biosystems 7500 Real-Time PCR System, or Roche Light Cycler 480 II, or Bio-Rad CFX 96	Primer design Ltd
Two target Gene assays					
Abbott RealTime SARS-CoV-2	Nasopharyngeal swab	N and RdRp genes	100 copies/ml	m2000 Real time System	Abbott Laboratories,USA
Xpert Xpress SARS-CoV-2	Nasopharyngeal, nasal, or mid-turbinate swab and/or nasal wash/ aspirate specimens	N2 and E genes	250 copies/ml	Gene Xpert Instrument	Cepheid
Simplexa™ COVID-19 Direct	Nasopharyngeal swab	ORF1ab and S genes	500 copies/ml	LIAISON MDX	DiaSorin Molecular
BioFire® COVID-19 Test	Nasopharyngeal swab	ORF1ab and ORF8 genes	330 copies/ml	FilmArray 2.0 and/or the FilmArray Torch Instrument Systems	BioFire diagnostic
PerkinElmer New Corona virus Nucleic Acid Detection Kit	Nasopharyngeal and oropharyngeal swab	ORF1ab and N genes	20 copies/ml	Pre-NAT II Automated Workstation and Applied Biosystems 7500 Real-Time PCR	PerkinElmer, Inc
cobas SARS-CoV-2	Nasopharyngeal and oropharyngeal swab	ORF1 and E genes	0.007 and 0.004 TCID50/ml	Cobas 6800/8800 Systems	Roche Diagnostics
ARIES SARS-CoV-2 Assay	Nasopharyngeal swab	ORF1ab and N genes	No info	MAGPIX System	Luminex Corporation

Table 1 (*Continued*)

	Type of Sample	Target Gene	Sensitivity/limit of the Assay	Apparatus Used	Manufacturer
Multiple target gene Assays					
QIAstat-Dx Respiratory 2019-nCoV Panel	Nasopharyngeal swab	ORF 1b poly gene, RdRp gene and E gene	500 copies/ml.	QIAstat-DX	QIAGEN GmbH
NxTAG CoV Extended Panel Assay	Nasopharyngeal swab	ORF1ab, N and E genes.	5000 copies /mL	MAGPIX® instrument using NxTAGCoV Extended Panel Assay File for SYNCT™ Software	Luminex Molecular Diagnostics, Inc.
TaqPath™ COVID-19 Combo Kit	Nasopharyngeal swab, nasopharyngeal aspirate, and BAL	ORF1ab, N gene, S gene, MS2	10GCE/reaction	Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument	Thermo Fisher Scientific, Inc Life Technologies Corporation

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, BALF= Bronchoalveolar lavage fluid

difference in detection limit of the assays is noted. The former has 100-150 copies/mL and later detects in 100 copies/mL. Similarly, nine assays have targeting nucleocapsid (N) gene. Sensitivity of these kits range from 40copies/mL on BD MAX System S to 10^5 copies/mL on m/s GenMark ePlex instrument.CDC has developed real-time PCR assays used for SARS-COV-2.This has primers and probes targeting two region N1& N2 of viral nucleocapsid gene and human RNAase P gene as an internal control that ensure successful RNA extraction. The assay has analytical sensitivity of500copies/mL (Zhen et al., 2020). Primer design COVID-19 Genesig Real-Time PCR assay targeting polypeptide RdRp gene has also been developed (Table 1 (a)).

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 ORFlab gene in combination with nucleocapsid (N), structural (S) and envelope (E) genes. The detection limit of these assays ranges from20 copies/mL to 500 copies/mL(Table 1 (b)).

Although, Realtime RT PCR is a predominant method for detection of all types of Coronavirus, including SARS CoV-2, the availableRealtime 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) (400copies/mL) by TaqPath™ COVID-19 Combo Kit from m/s Applied Biosystemsto 2500 GCE/reaction (5000 copies/mL) by NxTAGCoV Extended Panel Assay. QIAstat-Dx Respiratory 2019-nCoV Panel also gives a favorable sensitivity (500copies/mL) by multitarget detection of SARS CoV-2(Table 1(c).

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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Ethical approval

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