COVID-19; MOLECULAR DIAGNOSIS, PREVALENCE AND CONTROL IN SUDAN (REVIEW)

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ABSTRACT

The last day of 2019 delivered the first report to the World Health Organization (WHO) about a group of cases of pneumonia of unknown etiology in Wuhan, China. Subsequent investigations identified the new comer; a novel corona virus related to severe acute respiratory syndrome corona virus (SARS-CoV) and thus was termed as SARS-CoV-2. Being very contagious, the new virus led the era of "COVID-19" which is the acronym of "corona virus disease 2019,". Globally , as of 4:14pm CEST,14 October 2020 ,there have been 38,002,699 confirmed cases of COVID 19, including 1,083,234 deaths, where as in the Sudan ,from Jan 3 to 4:14 pm CEST, there have been 13,691 confirmed cases with 836 deaths reported to WHO. Validated and accurate laboratory testing for Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) is a crucial part of the timely management of COVID-19, supporting the clinical decision-making process for infection control at the healthcare level and detecting asymptomatic cases. Rapid and accurate molecular diagnostic technologies are crucial for the screening, isolation, treatment, prevention and control of COVID-19. Currently, nucleic acid detection-based techniques and rapid diagnostic tests that detect antigens or antibodies specific to 2019-nCoV infections are the primary diagnostic tools. In this article, we provide a molecular overview on the SARS-CoV-2 virus and summarize tremendous efforts that have been made to develop a rapid confirmatory diagnostic test for COVID-19, also in this article we aim to present a critical performance analysis of commercially available molecular diagnostics and reviews major factors influencing their diagnostic performance .

Keywords: Molecular diagnosis , COVID-19 Prevalence , COVID-19 Control, 2019-nCoV, RT-PCR.

INTRODUCTION

The last decade has witnessed the rise of epidemics and pandemics such as 2012s corona virus disease (MERS, middle-eastern respiratory syndrome) in the Middle East (Cotten M *et al*,.1993). 2014s Ebola virus disease (EVD, formerly known as Ebola hemorrhagic fever) in West Africa (Baize S, *et al*,.2014) 2015s zika virus disease in Latin America . Campos GS, *et al*,.2015) and 2019s corona virus disease (COVID-19) in Wuhan, China (Wu JT, *et al*,.2020). These emerging infectious diseases pose a grave threat to human health and the global economy. Lately, the novel corona virus (SARS-CoV-2, severe acute respiratory syndrome corona virus 2) aggressively spread throughout the world causing the COVID-19 pandemic (WHO, .2019). Several researchers globally and in Sudan were published good articles in this important topic (H. N. *et al*,.2020; A. Afzal, 2020; Marlin Touma, 2020; Wei Feng, *et al*,.2020; NING LI[1], *et al*,.2020; Robert ,and Arkadiusz, 2020)

Prevalence and Control of COVID 19 in Sudan

Sudan is the second largest country in Africa, with a total population of 43 849 260, located in the northeastern part of Africa, neighboured by countries with a high number of COVID-19 cases, such asEgypt and the Gulf Arab countries. Before the announcement of the first case of COVID-19, the Sudan Federal Ministry of Health had strengthened the measures at entry points and, on 13 April, the government announced a partial lockdown. However, because of the weak application of these preventive measures, and the open borders of Sudan with neighbouring countries, these measures were not effective; a large number of people refused and escaped quarantine(H. N. *et al* ,.2020) .

The Government of Sudan is taking a lot of measures against COVID-19 to guarantee and fulfill its responsibilities to its people. From the day on which the first case was reported in Khartoum State, on 13 March 2020, all services and measures have been used with the maximum capacity to guarantee the safety of people's lives in the country. In that time, all cases have had a history of travel, suggesting that transmissions are imported from elsewhere in the country. The Government of Sudan is implementing COVID-19 prevention methods with their interventions, such as early diagnosis and contact tracing, risk communication, social distancing, quarantine and isolation, to prevent the spread of COVID-19, closing the bridges linking the cities of Khartoum, suspension of prayers in mosques and churches, and partial lockdown. Some of the Darfur region states have closed their borders and imposed travel restrictions to reduce the movement of individuals. However, the implementation of these precautions is complicated by the weakness of Sudan's transition government and its fragmented health system (H. N. *et al* ,.2020).

Isolation centre and quarantine for COVID-19 in Sudan According to the World Bank, Sudan's hospital-bed capacity was 0.8 per 1000 people in 2013 . Up to 3 June 2020, Sudan had established 36 isolation centers (IC) in all its states with bed capacity around 985 beds and 198 intensive care unit beds ; for the total population of Sudan this capacity is very low by international standards . The government of Sudan equipped quarantine centers in all Sudanese states; these received 2374 individuals and had discharged 2189 of them up to 3 July 2020 (H. N. *et al* ,.2020) .

The government of Sudan established seven centers with PCR facilities for the diagnosis of SARS-CoV-2 in five of the 18 Sudanese states. Two centers were in Khartoum State (NPHL and Military Hospital laboratory), and there was one centre in each of the Red Sea (Port Sudan), Elgazera (Wadmadani), North Kordofan (Elobied) and South Darfur (Nyala). The current testing capacity of these diagnostic centers is 800 samples per day. However, overall capacity remains low. In addition to this low testing capacity, there are several other problems that limit its work, including sample collection and transportation (H. N. *et al* ,.2020) .

The Federal Ministry of Health identified the first case of COVID-19 on 12 March 2020. United Nations organizations and their partners created a Corona Virus Country Preparedness and Response Plan (CPRP) to support the Government. On 14 March 2020, the Government approved measures to prevent the spread of the virus which included reducing congestion in workplaces, closing schools and banning large public gatherings. From 8 July 2020, the Government started to ease the lock-down in Khartoum State. The nationwide curfew was changed from 6:00 pm to 5:00 am and bridges in the capital were re-opened. Travelling between Khartoum and other states is allowed and airports will gradually open pending further instructions from the Civil Aviation Authority. Now Schools and universities will

gradually open. Work at Government institutions started on 12 July, at 50 per cent capacity and literally be 100 per cent capacity with precautions. By 11 October, the number of confirmed cases had increased to 13,691. This increase is attributed mainly to local transmission of cases **(**OSHA,. 11oct 2020**).**

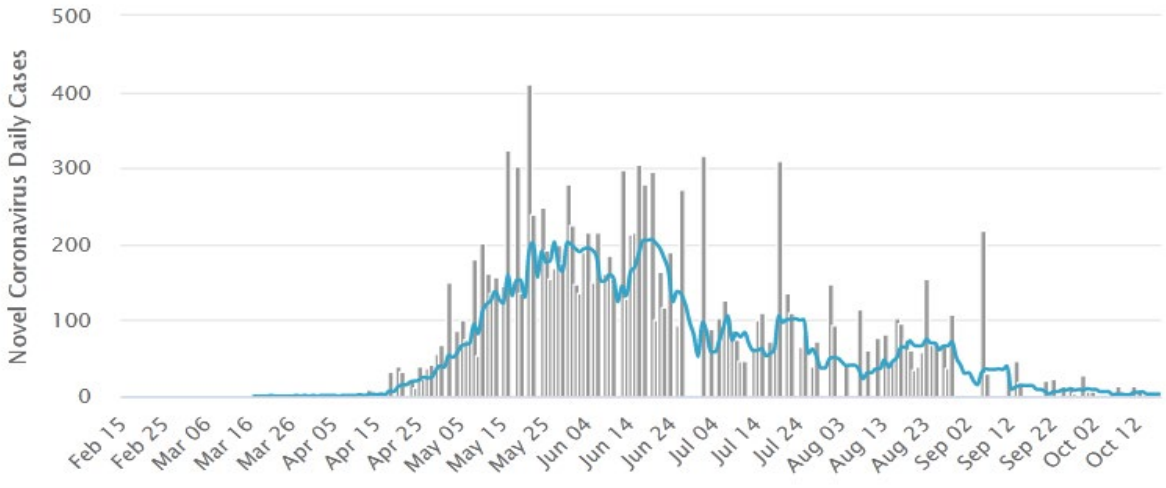
As of 29th July 2020, 185 of 197 UN countries are affected by this pandemic and despite all containment efforts, the number of COVID-19 infected people is rising above 16.5 million with over 655 thousand deaths accounting for the global fatality rate of ~ 3.96% . WHO Covid 19 (Dashboard ,.2020);TrackCorona ,.2020). Globally , as of 4:14pm CEST,14 October 2020 ,there have been 38,002,699 confirmed cases of COVID 19, including 1,083,234 deaths, where as in the Sudan ,from Jan 3 to 4:14 pm CEST, there have been 13,691 confirmed cases with 836 deaths reported to WHO. Table 1,2 and fig. 1,2,3 (World meter ,.2020)

WHO Region	New cases in last 7 days (%)	Change in new cases in last 7 days	Cumulative cases (%)	New deaths in last 7 days (%)	Change in new deaths in last 7 days*	Cumulative deaths (%)
Americas	804 735 (35%)	6%	17 794 771 (48%)	20 509 (52%)	-5%	588 867 (55%)
South-East Asia	575 763 (25%)	-6%	7 911 036 (21%)	7 750 (20%)	-8%	126 917 (12%)
Europe	694 275 (31%)	34%	6 918 265 (19%)	6 172 (16%)	16%	246 709 (23%)
Eastern Mediterranean	138 751 (6%)	10%	2 605 478 (7%)	3 173 (8%)	13%	66 329 (6%)
Africa	29 169 (1%)	11%	1 227 719 (3%)	991 (3%)	27%	27 255 (3%)
Western Pacific	26 199 (1%)	6%	651 841 (2%)	633 (2%)	26%	14 265 (1%)
[+] Other	-	-	741 (<1%)	-	-	13 (<1%)
Global	**2 268 892 (100%)**	**10%**	**37 109 851 (100%)**	**39 228 (100%)**	**<1%**	**1 070 355 (100%)**

Table.[1] Number of COVID-19 confirmed cases and deaths reported in the last seven days by countries, territories and areas, as of 11October202

Reporting Country/Territory/Area	New cases in last 7 days	Cumulative cases	Cumulative cases per 1 million population	New deaths in last 7 days	Cumulative deaths:	Cumulative deaths per 1 million population	Transmission classification
Eastern Mediterranean	138751	2605478	3565	3173	66329	91	
Iran (Islamic Republic of)	28 134	496 253	5 908	1 547	28 293	337	Community transmission
Iraq	24 193	400 124	9 948	443	9 790	243	Community transmission
Morocco	18 613	149 841	4 060	279	2 572	70	Clusters of cases
Tunisia	10 315	31 259	2 645	180	456	39	Clusters of cases
Jordan	9 249	23 998	2 352	93	181	18	Community transmission
Lebanon	9 078	52 558	7 700	57	455	67	Community transmission
United Arab Emirates	7 373	105 133	10 630	17	443	45	Community transmission
Libya	5 599	41 686	6 067	45	623	91	Community transmission
Oman	5 544	104 129	20 391	74	1 009	198	Community transmission
Pakistan	4 316	318 932	1 444	57	6 570	30	Clusters of cases
Kuwait	4 110	110 568	25 891	35	655	153	Community transmission
Bahrain	2 977	75 287	44 245	15	273	160	Clusters of cases
Saudi Arabia	2 947	338 944	9 736	168	5 018	144	Sporadic cases
Qatar	1 439	127 778	44 351	3	219	76	Community transmission
Egypt	812	104 387	1 020	70	6 040	59	Clusters of cases
Afghanistan	458	39 799	1 022	15	1 477	38	Clusters of cases
Syrian Arab Republic	344	4 673	267	17	221	13	Community transmission
Somalia	102	3 847	242	0	99	6	Sporadic cases
Sudan	12	13 670	312	0	836	19	Community transmission
Yemen	10	2 055	69	6	596	20	Community transmission
Djibouti	5	5 423	5 489	0	61	62	Sporadic cases
Territories[8]							

Table[2] Number of COVID-19 confirmed cases and deaths reported in the last seven days in Eastern Mediterranean , as of 11October202**0.**



Figure[1] shown Confermid Caseses in Sudan from march to october 2020.

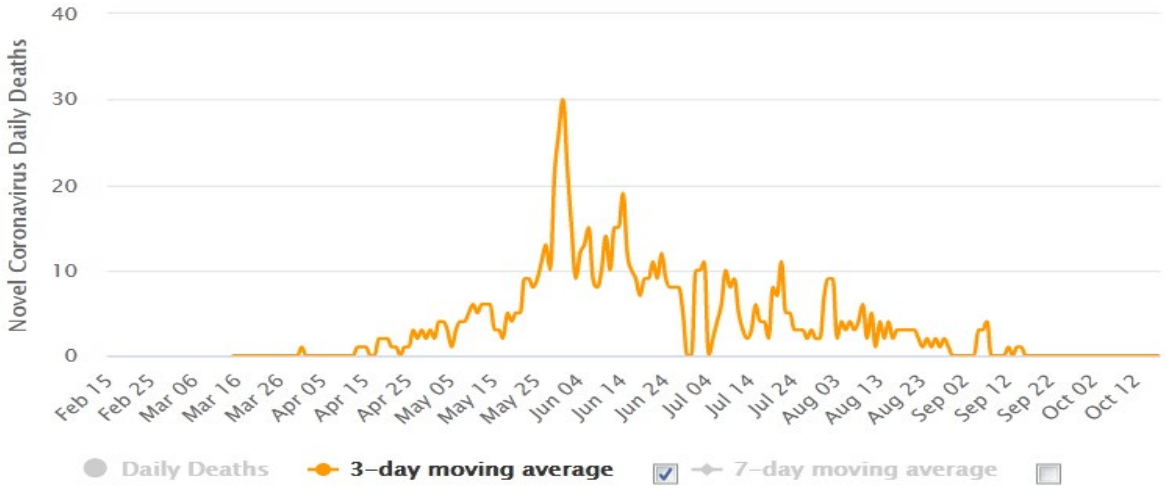


Figure [2] shown death Caseses in Sudan from march to october 2020.

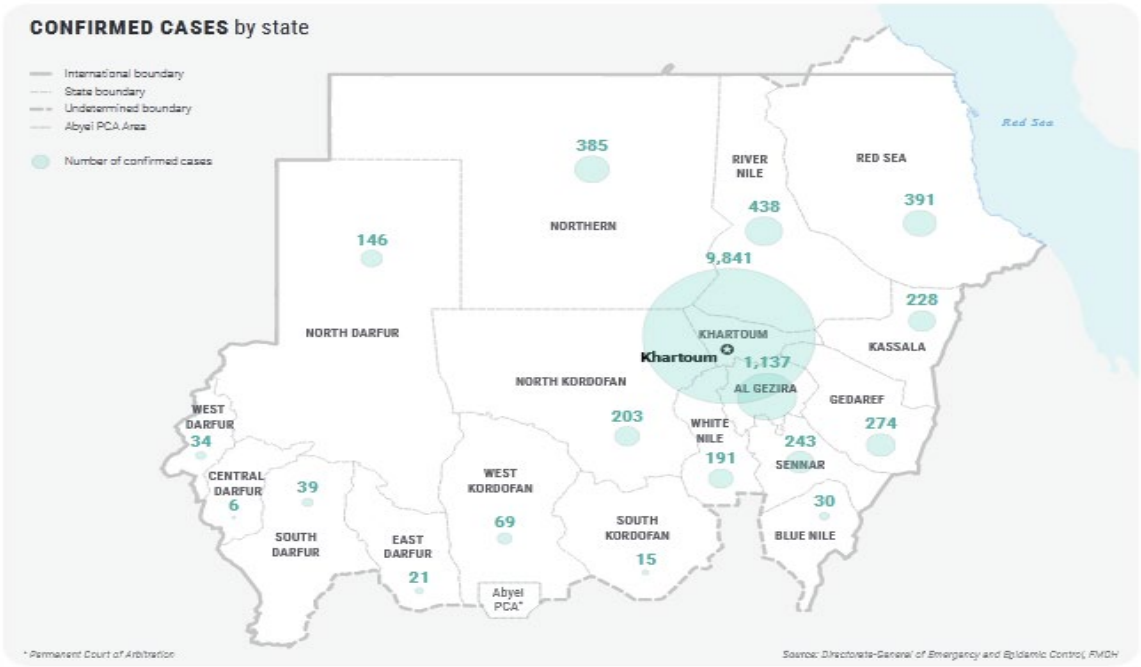


Figure [3] shown Confermid Caseses in Sudan by state from march to october 2020.

SARS-CoV-2 Structure and Life cycle

Structure and infection of SARS-CoV-2 as shown in figure [4], is mediated by binding of the receptor binding domain of the S1 region of spike protein to angiotensin-converting enzyme 2 (ACE2) receptors on the surface of host cells(Letko, M.,*et al*,.2020; Hoffmann, M,. 2020). The spike protein is subsequently primed by cleavage at the S1/S2 site by the transmembrane protease serine 2 (TMPRSS2) (Hoffmann, M,. 2020), which exposes a fusion peptide that merges viral and cell plasma membranes. This membrane fusion at the cell surface deposits the genome into the cytoplasm, leading to translation of ORF1a and ORF1b and production of the polyprotein 1a (pp1a) and pp1ab, respectively. Pp1a and pp1ab are self-cleaved into 16 nonstructural proteins (Nsps) by the viral proteases Nsp3 and Nsp5. Nsps 1 to 16 coalesce to form a replicase/transcriptase complex (RTC) containing multiple enzymes, such as the Nsp7-Nsp8 primase, the Nsp12 RNA dependent RNA polymerase (RdRp), the Nsp13 helicase/triphosphatase, the Nsp14 exoribonuclease, the Nsp15 endonuclease, and the Nsp10-Nsp16 N7- and 2′O-methyltransferases(Lu, R.;*et al* ,.2020), Within this RTC, the RdRp polymerizes full length and partial length RNA complementary to the viral genome (negative sense RNA) which serve as templates for nascent synthesis of positive sense RNA genomes as well as subgenomic RNA species. The subgenomic RNAs encode the aforementioned structural proteins (E, M, S, N) as well as putative accessory proteins (Du, L *et al* ,.2009; Kim, D,. *et al* ,.2020). The E, M, and S proteins enter the endoplasmic reticulum (ER), and the N proteins bind positive sense RNA genomes, and these virion components are subsequently combined in the ER-Golgi apparatus compartment (ERGIC). These newly formed SARS-CoV-2 viruses are then released from cells through vesicle transport (exocytosis).

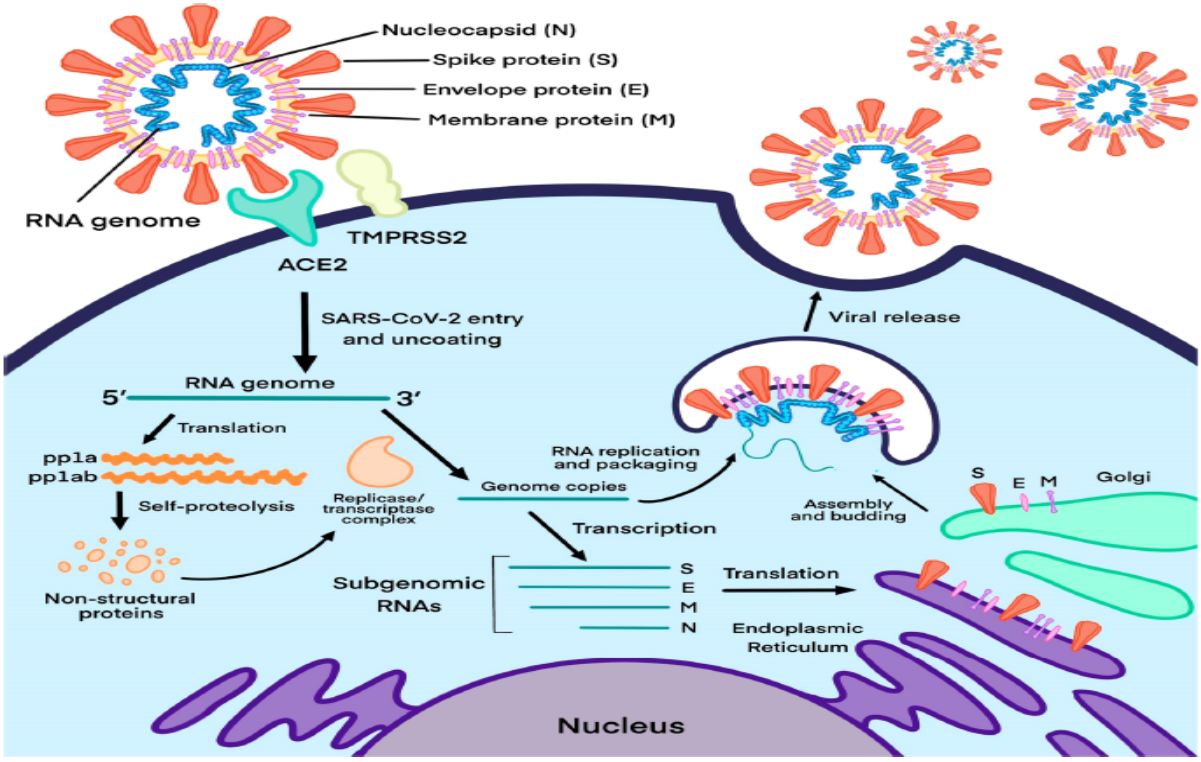


Figure [4] Schematic presentation of SARS-CoV-2 structures and its life cycle (Hoffmann, M,. 2020;Kim, D,. *et al* ,.2020).

Genome of SARS-CoV-2

Genome (RNA) is ~ 30,000 nucleotides long comprising a structural gene unit that encodes S, E, M, and N proteins and two large, open reading frame genes (ORF1a and ORF1b) that encrypt sixteen nonstructural proteins (NSP) including RNA-dependent RNA polymerase (RdRp) .fig. [5] (Kim D *et al* .,2020; Alanagreh L *et al* .,2020).

The findings of high-throughput genomic sequencing of SARSCoV-2 are regularly deposited on the global initiative on sharing all influenza data (GISAID) (GISAID,.2020). The development of oligonucleotides (primers and probes) for molecular diagnosis of COVID-19 was initiated as soon as these findings were made public on 10th Jan 2020 (Sheridan C,2020;GISAID,. 2020). CDC (USA), China CDC, Charité Germany, Institute Pasteur (France), NIID Japan, Hong Kong University, and NIH Thailand researchers developed forward/reverse primers and probes for real-time RT-PCR-based molecular diagnostics.. Thanks to these research and development efforts, the first test kits were available for clinical diagnosis of the disease by 4th Feb 2020 (FDA ,.2020).
Since then, several molecular diagnostic kits have been commercialized for the detection of the SARS-CoV-2 genome. These diagnostics are approved for emergency use in the current pandemic situation by major healthcare authorities worldwide.

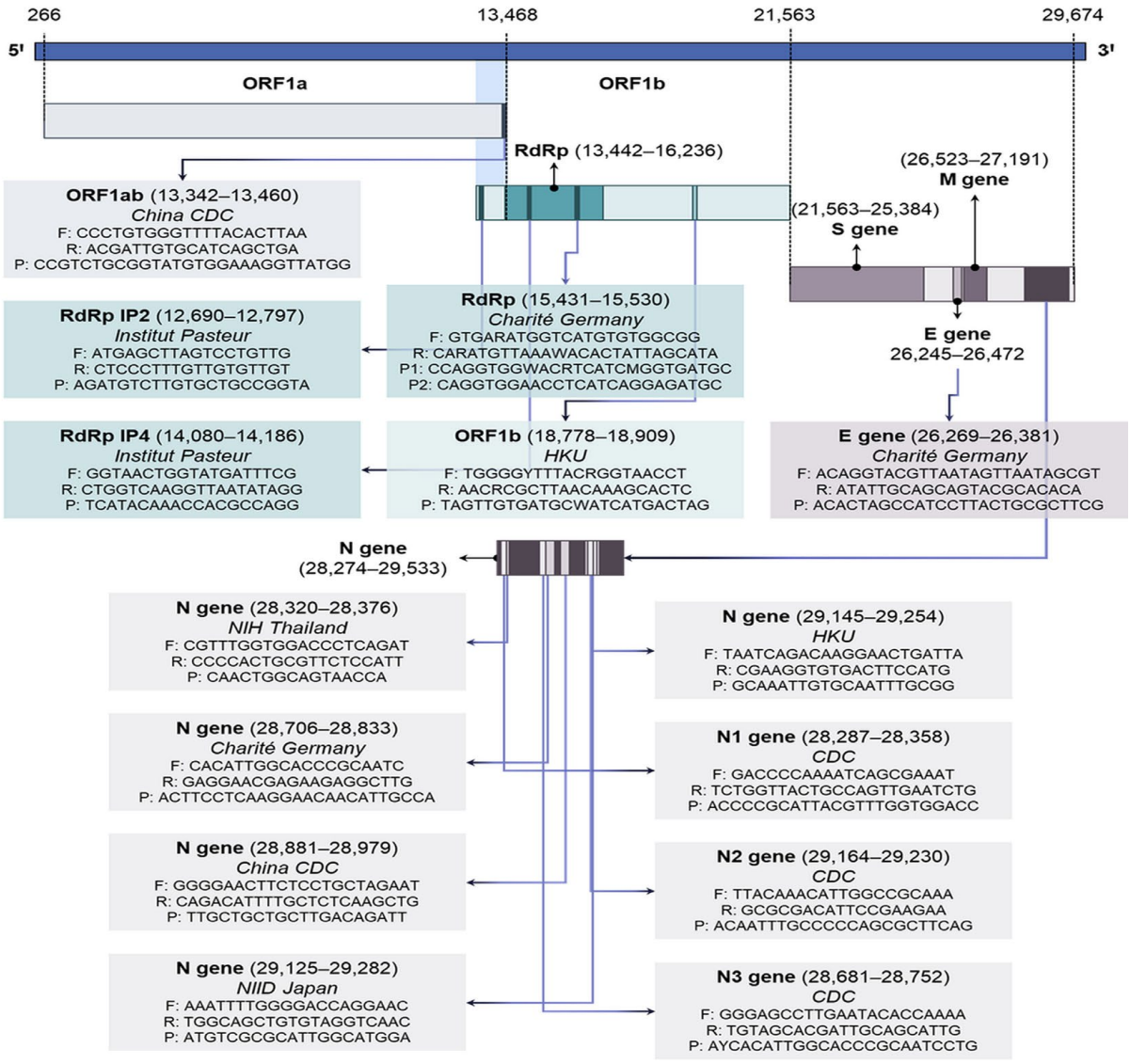


Figure [5] shown the Genome of SARS-CoV-2

COVID-19 symptoms and incubation period

The symptoms of COVID-19 are similar to common illnesses such as a cold or influenza. You may have one or more of the following:

- a new or worsening cough
- fever (at least 38˚C)
- shortness of breath
- a sore throat
- sneezing and runny nose
- temporary loss of smell.

Shortness of breath is a sign of possible pneumonia and requires immediate medical attention. Some people may present with less typical symptoms such as only: fever, diarrhoea, headache, myalgia (muscle pain), nausea/vomiting, or confusion/irritability. Symptoms can take up to 14 days to show after a person has been infected. The virus can be passed onto others before they know they have it – from up to two days before symptoms develop(WHO,.2019).

For COVID-19, the mean incubation period and the mean serial interval are 5.2 days (95% CI: 4.1–7.0 days) and 7.5 days (95% CI: 5.3–19.0 days), respectively; while the basic

reproduction number (R0) is reported to be 2.2 (95% CI: 1.4–3.9), i.e. a SARS-CoV-2 carrier can spread it to ~ 2.2 persons on average, the virus life cycle. Therefore, to limit the spread of SARS-CoV-2 and overcome the COVID-19 crises it is important to identify the suspected individuals and isolate them, which requires indefatigable diagnostic testing(Li Q *et al*., 2020).

Types of diagnostic tests for COVID-19

The dependable diagnostic solutions have been immediately developed and marketed to help early diagnosis of COVID-19 (Sheridan C, 2020 ;Satyanarayana M,. 2020). Thus far, two types of diagnostic tests have been commercialized:

[1]Molecular diagnostics:

That detect part of the viral genome (i.e. RNA) in the respiratory tract specimens; and second, is

[2] Serological or antibody tests:

That detect SARS-CoV-2 specific antibodies in serum specimens (Sheridan C. 2020). Molecular diagnostics are used to identify symptomatic or asymptomatic SARS-CoV-2 carriers, and the basic performance criterion for these tests is high clinical sensitivity to avoid false-negative results. On the other hand, the objective of serological tests is to identify individuals with an active immune response to the SARS-CoV-2 antigen, and the basic performance criterion for these tests is high clinical specificity to rule out false-positive results (Sethuraman *et al.,*2020) published a timeline for the detection of viral RNA (molecular diagnosis) and immune-response antibodies (serological diagnosis), which revealed that molecular diagnostics could identify infected individuals a week before the onset of symptoms while antibodies could only be detected _ 8 days after the symptoms appear. Therefore, molecular diagnostics are essentially the only tests for early diagnosis of COVID-19 (Abbasi J. 2020).

In this article we aimed to present a critical performance analysis of commercially available molecular diagnostics and reviews major factors influencing their diagnostic performance. The criteria for the selection of molecular diagnostic tests is two-fold: (a) only those tests are selected that have been approved by major healthcare authorities around the world, and (b) most importantly, those molecular diagnostic kits are shortlisted that have been independently tested by WHO or relevant healthcare authorities for their clinical sensitivity and specificity.

Molecular diagnostics for COVID-19

The following are the current molecular diagnostic methods of COVID-19

RT-PCR Technology

At present, molecular techniques based on the real-time (quantitative) reverse transcriptase-polymerase chain reaction (RT-PCR) are considered the gold standard for COVID-19 diagnosis(Park G-S, *et al* ,.2020;Shen M, *et al* ,2020). Real-time RT-PCR detects amplified SARS-CoV-2 genome in sputum, nasopharyngeal or oropharyngeal swabs, bronchoalveolar lavage fluid, nasal or nasopharyngeal aspirate, and lower respiratory tract aspirates. A typical RT-PCR test can take 4–6 h from sample to result (Sheridan,.2020) . However, RT-PCR has proven to be extremely handy in clinical settings to consistently perform a large number of tests. As of 29th July 2020, >260 million COVID-19 tests have been performed in the most impacted countries worldwide (Statista GmbH,.2020).

Since the development of the 2019-nCoV epidemic, China has recommended RT-PCR technology as a guideline for the COVID-19 diagnosis and treatment program (NHC ,2020).

The Chinese Center for Disease Control and Prevention recommends the use of primers and fluorescent probes (FAM, BHQ1 and TAMRA) targeting 2019-nCoV ORF1ab and nucleocapsid protein (N) gene regions(NIVDCP,.2020) .

The Department of Clinical Laboratory of the Third Hospital of Chongqing Municipal People's Hospital compared the detection performance of 2019-nCoV for six of the kits (Shengxiang Biotechnology 2019-nCOV rapid nucleic detect kit, Beijing Ka You Di 2019-nCoV ORF1ab/N gene RNA detection kit for nucleic acid-free extraction, Shuoshi Biotechnology 2019-nCOV detection kit, Zhongyuan 2019-nCOV nucleic detect kit, Zhong Shan An Da 2019-nCoV ORF1ab/N nucleic detection kit and Beijing Zhuo Cheng Hui Xin 2019-nCoV ORF1ab/N gene double fluorescent RT-PCR kit) and reported that the detection capabilities of each kit for weakly positive samples were different (Guo YY,. *et al* 2020). Furthermore, certain kits were able to double-positively detect Orf1ab and N, while other kits could only detect one of them.

At present, RT-PCR nucleic acid detection serves an irreplaceable role in the diagnosis of 2019-nCoV and is the most important molecular diagnostic method in the early stage of the epidemic(NHC,.2020). However, there are limitations due to tedious, time-consuming operation, required biosafety laboratories ranked Class II or above centralized inspection and shortage of personnel and qualified biosafety sites in the epidemic area(Espy MJ,.2006) . Furthermore, there are shortcomings in responding to the rapidly increasing demand for the diagnosis of patients with suspected 2019-nCoV pneumonia and asymptomatic infections (Espy MJ,.2006; Kaul KL,.2020). Additionally, recent research on patients infected with 2019-nCoV demonstrated that the positive rates of early stage nucleic acid detections of oropharyngeal swabs, anal swabs and blood were 53.3, 26.7 and 40%, respectively, while the positive rate of anal swabs was even higher than oral swabs in the late stage of infection. Notably, the actual positive rate is only 30-50% when collecting suspected patient samples through routine throat swabs at the outpatient fever clinic, with many of the samples producing false-negative results , despite the considerable pressure to prevent and control the 2019-nCoV(Zhang W,. *et al*,.2020) .

Nucleic Acid Detection Technology

Nucleic acid detection is an important diagnostic tool for the clinical diagnosis, segregation, rehabilitation and discharge of patients, and was also the ‘gold standard’ for the detection of 2019-nCoV infection in the early stage of the epidemic(NHC ,.2020). Current nucleic acid detection methods include RT-PCR, isothermal amplification and high-throughput sequencing. At present, specimens tested by commercial nucleic acid kits mainly comprise throat swabs, or pharyngeal swabs, nasopharyngeal swabs, sputum and alveolar lavage fluid (Shen M, *et al* ,2020 ; China NMPA ,.2020).

High-throughput Sequencing Technology

Gene sequencing is the most accurate and reliable technology for the detection of viruses and other pathogenic ‘emergency’ infectious diseases. Additionally, it is the only method to dynamically track genome variation in pathogens. In the early stage of the epidemic, the Chinese Center for Disease Control and Prevention identified and analyzed the genome of 2019-nCoV based on second-generation sequencing metagenomics technology (mNGS) within five days and reported that the similarity between the nucleotide sequence of 2019-nCoV and SARS or bat-derived strains were 79 and 96%, respectively (Armstrong GL,.*et al* 2019).

The China National Medical Products Administration has approved a gene sequencing system (ultra-high-throughput sequencer DNBSEQ-T7), supporting analysis software and nucleic acid detection kits, which can identify and diagnose coronaviruses, including 2019-nCoV and other infectious respiratory pathogens and enable rapid detection of viral sequences (China NMPA ,. 2020). The DNBSEQ-T7 sequencer can complete the entire 2019-nCoV detection process (from sample extraction to result reporting) in 20 h. The sample detection throughput is 50-200 per cycle and each sample can obtain an average data output of >100 M, ensuring highly accurate results for 2019-nCoV detection. However, mNGS has the limitations of high equipment and testing costs, long detection cycles, complicated procedures and a lack of standardization. Furthermore, the sequencing depth of certain samples is not always appropriate (MGI ,. 2020).

Nanopore sequencing is a third-generation genome sequencing technology that provides real-time analysis and rapid insights. It is a physical sequencing technology based on alterations in electrical signals (Bowden R,.2019) . Nanopore sequencing does not require enzymes to amplify samples and directly performs full-length sequencing of 2019-nCoV (30). This method has the advantages of long sequencing length, low cost, high throughput and non-labeling (Bowden R,.2019; van Dijk EL *et al*,.2018). However, nanopore sequencing has not yet been approved by the China National Medical Products Administration. Hangzhou Center for Disease Control and Prevention completed the first 2019-nCoV genome assembly using only nanopore data on February 12, 2020 . Final assembly results were 100% consistent with the reference genome without the correction of other sequencing technologies. The development of a real-time and rapid viral genome sequencing solution through nanopore sequencing is expected to become a powerful technology and resource support for combating viral epidemics worldwide(CPC HMC,.2020).

2019-nCov will continue to mutate during the transmission process. An analysis of 103 2019-nCov genomic data collected from a public database (Global Initiative on Sharing All Influenza Data; https://www.gisaid.org/) from December 24, 2019 to February 5, 2020 demonstrated that these virus strains underwent a total of 149 point mutations and that most mutations occurred recently(Tang X,.2020). If a mutation is located in the primer or probe binding site, the sensitivity and accuracy of existing RT-PCR detection kits will be affected. High-throughput sequencing technology can compensate for the limitations of RT-PCR, effectively increase positive rates and monitor possible mutations (van Dijk EL,. *et al*,.2018). Furthermore, sequencing could simultaneously providing a more comprehensive pathogen genome analysis of critical illness and patients with complex infections, provide more information about infectious pathogens and identify drug resistance genes to guide clinical medication. However, due to the high cost, long procedure times and complex testing processes, it has not become a routine clinical batch testing technology (Quainoo S,.2017) .

Isothermal Temperature Nucleic Acid Amplification Technology and loop-mediated Isothermal Amplification (LAMP) Technology

Developed in 2000, LAMP is a fast and highly specific technology for gene amplification under constant temperature conditions(Notomi T *et a*l,. 2000). RT Loop-Mediated Isothermal Amplification (RT-LAMP) combines RT with LAMP, can be used directly for RNA detection and has previously been used in the identification of various respiratory RNA viruses, including SARS-CoV and MERS-CoV (Shirato K, *et al*.,2014; Hong TC,. *et al*.,2014) . Based on this, by adding a fluorescence quenching probe (QProbe), fluorescence RT-LAMP technology be used for the detection of MERS-CoV(Shirato K, *et al*., 2018) . In order to make the detection of LAMP amplification products more accurate, the combination

of nucleic acid detection and immunogold labeling technology has resulted in an improved RT-LAMP-combined nucleic acid strip detection technology (RT-LAMP-NAD), which has been used for the detection of Ebola virus (Xu C, *et al*., 2016) .

On February 25, 2020, the team of Dr Xiushan Yin, the director of the Institute of Applied Biology of Shenyang University of Chemical Technology and the team of Michael B Chancellor at Royal Oak Beaumont published an article about RT-LAMP on MedRxi. The article optimized a specific and accurate detection method for 2019-nCoV and provided multiple primer sequences directed at Orf1ab region (Yu L, *et al*., 2020; Lamb LE,2020). The entire reaction process takes approximately 15-45 min. This simple analytical method can be used on biological samples outside of central laboratories to monitor isolated populations or to assist in screening at entrance areas . Numerous domestic institutions have announced that they have developed a 2019-nCoV isothermal amplification kit, which requires only 'one-time opening and one-step operation', and can complete amplification reactions as fast as 15 min. Test results are fast and easy, and can be seen macroscopically . Relevant products have entered the review process of the China National Medical Products Administration and certain products have obtained registration certificates (China NMPA ,.2020).

Although LAMP technology has the advantages of simplicity, sensitivity, specificity, speed and is inexpensive and has low hardware requirements, the development of a kit using this technology is more complicated than an RT-PCR kit and involves multiple pairs of primers. Therefore, the development and clinical application of LAMP in 2019-nCoV pneumonia epidemic is slower than RT-PCR(Li Y,.2017).

Isothermal Temperature Nucleic Acid Amplification Technology and Recombinase Aided Amplification (RAA)

RAA technology utilizes recombinases, single-stranded binding proteins and DNA polymerases to perform nucleic acid amplification under isothermal (37˚C) conditions(Piepenburg O, *et al* ,. 2006) .

Using RAA technology, the Institute of Viral Disease Control and Prevention of the Chinese Center for Disease Control and Jiangsu Qitian Gene Biotechnology Co., Ltd. jointly developed a new coronavirus (2019-nCoV) nucleic acid isothermal amplification rapid detection kit. After nucleic acid is extracted, it only takes 8-15 min to detect 2019-nCoV nucleic acid. Following parallel comparison with commercial quantitative PCR (qPCR) kits approved by the China National Medical Product Administration (NMPA), the kits have a 100% positive compliance rate, a 100% negative compliance rate and a total compliance rate of 100%, which are equivalent . The kit has been evaluated by the First Affiliated Hospital of Zhejiang University School of Medicine (92 clinical samples), Zhejiang Center for Disease Control and Prevention (104 clinical samples) and Jiangsu Province's Center for Disease Control and Prevention (100 samples) . The kit is recommended for qualitative detection of clinical 2019-nCoV to identify patients with suspected infection. The kit is considered suitable for use in prefecture-level laboratories and is currently applying for a China NMPA approval number(NIVDCP,.2002) .

RAA technology is relatively new in the current nucleic acid detection technology field. The advantage of rapidity, sensitivity and specificity of RAA technology may aid in the detection, screens, isolation for suspected 2019-nCoV infections(Piepenburg O, *et al* ,. 2006) .

Nucleic Acid Mass Spectrometry

Nucleic acid mass spectrometry is a novel type of soft ionized biological mass spectrometry technology that has been developed recently based on atrix Assisted Laser Desorption Ionization-Time of Flight technology and is very simple and efficient (Karas M, *et al* 2000). This procedure integrates the high-throughput of chip technology and the high sensitivity of mass spectrometry technology without the requirement for complex biological information analysis and is mainly used for the detection of known mutations . A single reaction of nucleic acid mass spectrometry can perform 20-50 PCR amplifications simultaneously and can detect dozens of pathogens at once (Gao X, *et al* ,. 2013). Nucleic acid mass spectrometry is a very useful tool for the differential diagnosis of respiratory infections(Jang KS and Kim YH .,2018).

It was previously announced that the successful development of a nucleic acid mass spectrometry kit that can simultaneously detect 2019-nCoV and 20 other common respiratory infection pathogens . The detection limit is as low as 100 copies/ml and 96 pieces of single-chip with a manual operation time of 30 min. Furthermore, 1,504 tests can be completed in 24 h . Additionally, the kit can detect other RNA viruses that cause respiratory diseases, including influenza A and B (Li M, Jiang ., *et al* 2020).

Nucleic acid mass spectrometry has high throughput analysis, is simple to operate and is inexpensive, nucleic acids are difficult to ionize, are unstable and easily generate fragments. This makes it difficult to parse spectrum data. It is necessary to continuously improve the resolution of the detector to promote its use(Gao X, *et al* 2013).

Protein Detection Technology

Protein detection technology is mainly divided into pathogen antigen detection and host anti-body detection(Gao Y, *et al*,. 2018). Commonly used methodologies include colloidal gold(Huang C, *et al*,. 2018), immunofluorescence chromatography(Nuccetelli M, *et al* 2020), chemiluminescence(Lippi G, *et al*,. 2020; Padoan A, *et al*,. 2020) and ELISA (Aydin S: 2006). The colloidal gold method is easy to operate and can be directly visually interpreted (Kong D, *et al*,. 2016). The test can be completed in 15 min and, therefore, can be used for on-site material acquisition and on-site detection . Immunofluorescence chromatography is as easy to operate as colloidal gold and detection is fast; however, it requires instrument interpretation(Nuccetelli M, *et al*,. 2020); Wu HS, *et al*,. 2004) . The chemiluminescence method generally has high sensitivity and uses a full-automatic immunoanalyzer, which can complete detection without excessive manual operation (Lippi G, *et al*,. 2020; Padoan A, *et al*,. 2020). The detection time is generally ~30 min. ELISA can be interpreted using a conventional microplate reader(Gan SD and Patel KR:2013) . Generally, ELISA exhibits high sensitivity; however, the detection time is longer (≥1.5 h) and there are numerous operating steps (Aydin S: 2006) .

Value of the combined application of nucleic acid-protein detection technology

Results from a study from the Wuhan Clinical Frontier demonstrated that the titers of virus-specific IgM and IgG in serum were often low or lower than the detection limit . On admission, samples were collected from patients and by day 5 almost all patients had positive or elevated antibody levels. Among them, IgM positive rates increased from 50% (8/16) to 81% (13/16) and IgG-positive rates increased from 81% (13/16) to 100% (16/16) . Those results are the exact opposite of the relatively low positive rates of detection from nucleic acid molecule testing (Zhang W,. *et al*,.2020) . This indicated that, in the context of epidemiological history or clinical manifestations that meet diagnostic criteria, addition of

immunoassays based on specific antigen-antibody responses effectively compensate for the limitations of high false-negative rated of nucleic acid detection and reduce false diagnoses. Epidemic prevention and control have a great auxiliary role and immunoassays may therefore be useful(Lee CY, *et al*,.2020; Okba NMA, *et al*,.2020; Cutts FT and Hanson M: 2016).

A recently published study reported the latest clinical practice results at Ren Min Hospital of Wuhan University in the first-line diagnosis and treatment of 2019-nCoV (Xu WZ, *et al*,.2020). In that study, 19 patients with negative nucleic acid tests but with clinical symptoms and CT imaging features of COVID-19 were serologically tested. The results reported that 16 patients (84.21%) were positive for 2019-nCoV IgM antibodies and 18 patients (94.74%) were positive for 2019-nCoV IgG antibodies. The majority of the 2019-nCoV IgM and IgG antibody detection assays have high clinical specificity and clinical detection rates, and they should be used to confirm the status of nucleic acid detection negative samples. Furthermore, in terms of treatment monitoring and disease progression, the decline and disappearance of 2019-nCoV IgM concentration and the rise of 2019-nCoV IgG concentration indicated that patients gradually recovered and produced immunity to pathogenic 2019-nCoV.

The advantage of 2019-nCoV antibody tests is that the sample source is flexible and serum, plasma and whole blood can be obtained, avoiding the limitation of the current nucleic acid tests which collect upper respiratory tract samples and exposes medical personnel to a high exposure risk(Zhang W,. *et al*,.2020) . Compared with PCR and sequencing, the colloidal gold test kit is easier to operate without any instruments and equipment and the assay can be performed anywhere with minimal training (Kong D, *et al*,. 2016). However, China's State Drug Administration previously stated that, given the characteristics and current status of antigen/antibody detection reagents, its sensitivity and specificity are currently limited and cannot be used as the sole basis for diagnosis or exclusion of 2019-nCoV. Furthermore, it is not suitable for general population screening and can only be used as a supplement to existing viral nucleic acid detection assays(Shen M, *et al* ,.2020; NHC ,2020). In the first six editions of the 'Guidelines for the Diagnosis and Treatment of New Coronavirus Pneumonia' issued by the National Health Commission of China, the use of protein testing products is recommended to be limited to supplementary detection indicators for suspected cases of negative nucleic acid testing or used in conjunction with nucleic acid testing for the diagnosis of suspected cases. However, in the 7th version of the guidelines, which were released on March 3, 2020, positive antibody tests have been included as one of the diagnostic indicators alongside positive nucleic acid tests Briefly, the future application of multiple molecular diagnostic methods of nucleic acids, antigens and antibodies will shorten detection windows and increase positive detection rates. Furthermore, it will serve a crucial role in the molecular diagnosis of 2019-nCoV in laboratories(Shen M, *et al* ,. 2020) ..

Future trends

In the future, molecular diagnostic research of 2019-nCoV infections will speed up sample preparation, increase detection throughput and accuracy, improve detection automation level and develop novel technologies with low requirements and low costs for equipment and testing personnel(Chan JF, *et al* ,2020; Tahmasebi S,. *et al* ,2020). Due to antibody preparation requiring additional time, faster breakthroughs are expected in pathogen nucleic acid detection technology (Yan Y, *et al* ,.2020).

Efficient and safe pre-processing

For RT-PCR technology, nucleic acid extraction and processing affect the yield of viral nucleic acid for analysis (Hata A, *et al* ,.2011). This can be more difficult when certain sample types are used, as throat swabs and sputum often contain only trace amounts of virus . Efficient and fully automatic nucleic acid extraction equipment will provide better protection in terms of detection sensitivity and personnel safety (Tahamtan A and Ardebili A: 2020). The Covaris™ high-performance nucleic acid release system developed by Gene Company Ltd. is processed by Adaptive Focused can completely inactivate the virus in a very short time without affecting the quality of RNA extraction . Furthermore, the full release of the viral nucleic acid has the advantages of fast processing, efficient recovery, stability and reliability, which enables subsequent nucleic acid purification to obtain sufficient and high-quality viral RNA. It is the basis for improving detection sensitivity under existing conditions and can reduce inconclusive or false-negative results (Nauwelaers D,*et al* ,.2011).

Accurate quantification of viruses

According to previous reports, Apexbio-designed primer probes for Orf1ab and N sequence conserved regions of 2019-nCoV and developed a highly sensitive digital PCR new crown virus detection kit(Toms D, *et al* ,2020; Yan C, *et al* ,.2020) . Compared to qPCR, digital PCR is used for the absolute quantification of nucleic acid molecules. It can directly detect the copy number of the target sequence and the detection limit can reach a single copy . Furthermore, sensitivity, specificity, accuracy, resolution and tolerance is higher (Vogelstein B and Kinzler KW: 1999; Sykes PJ,. *et al* ,.1992). However, due to the high cost of equipment and tremendous workload, it is challenging to apply digital PCR to the initial stage of epidemic prevention and control, particularly in under-developed areas, on a large scale . However, digital PCR will still be extremely useful as it allows for absolute quantification and the detection of complex background samples, it can track the progress of disease and analyzes the viral load. Additionally, digital PCR will enable the evaluation of drug efficacy(Li H, *et al*,. 2018).

Point-of-care testing (POCT)

The current technology platform used by the majority of POCT integrates nucleic acid extraction, amplification and detection on a micro fluidic chip that reduces detection complexity(Magro L, *et al*,. 2018). Systems presently used include GeneXpert from Danaher Corporation, as well as FilmArray from BioMérieux and Liat from Hoffmann-La Roche. Numerous domestic companies have developed nucleic acid POCT detection instruments and supporting detection reagents . The 2019-nCoV molecular cassette fluorescent PCR detection method launched by Transview Life can complete the detection of 4 samples in 1-1.5 h. Additionally, Orion BioScience, Inc. has completed the development of a new 2019-nCoV nucleic acid detection kit, which can detect 12 samples at a time(Sheridan C: 2020**)**.

POCT has the advantages of rapid results, unrestricted test sites and low professional skill requirements for operators(Khan AH, *et al*,. 2018). Therefore, the research and development of POCT nucleic acid detection technology is likely to be the general direction of future development of testing. Operators only need to add samples, such as swabs or blood, into the slot on ‘sample in-result out’ requirement, which will significantly simplify the detection process(Park S, *et al*,. 2018; Basile K, *et al*,. 2018) . POCT automatically completes nucleic acid amplification, signal collection and result analysis in a short time(Park S, *et al*,. 2018). However, POCT requires improvement due to lack of authoritative control experiments and lack of uniform national standards for manufactured products(Basile K, *et al*,. 2018).

Development of innovative technologies

The diagnosis of infectious diseases usually requires professional knowledge, sophisticated equipment and sufficient power sources; however, these are difficult to achieve in areas with poor economic foundations(Broadhurst MJ, *et al*,. 2016; Shorten RJ, *et al*,. 2016). The new generation of CRISPR-based molecular diagnostic technology [such as Specific High-sensitivity Enzymatic Reporter (SHERLOCK)], do not rely on electricity as much as PCR, has lower cost, faster times and simple operation(Gootenberg JS**,** *et al*,. 2017; Kellner MJ, *et al*,. 2019). The advantages of matching the efficiency and accuracy of qPCR technology have made significant contributions to the fight against Ebola outbreaks in Nigeria, where power is often lost(Broadhurst MJ, *et al*,. 2016; Shorten RJ, *et al*,. 2016) .

In response to the 2019-nCoV epidemic, the McGovern Institute for Brain Research of the Massachusetts Institute of Technology announced on February 14, 2020, that the team of Professor Feng Zhang, the inventor of SHERLOCK technology, used synthetic COVID-19 RNA to design two crRNA recognition specific sequences for S and Orf1ab. If the acid sample for 2-3 min, the presence of 2019-nCoV nucleic acid is determined by the appearance of black lines on the test paper .However, since CRISR technology has always had patent disputes, the implementation of CRISPR-based molecular diagnostic technology can be challenging from an economic perspective**(**Zhang F,. *et al* 2020).

Sample collection, Isolation and Testing

For sampling, the WHO recommends collecting specimens from both the upper respiratory tracts, nasopharyngeal (NP) and oropharyngeal (OP) swabs, in the spontaneously breathing patients, and the lower respiratory tract in the mechanically ventilated patients. Researchers have proven that NP swab specimen is superior to the OP swab specimen for the examination of SARS-CoV-2 (Zou L, *et al***,**.2020 ; Wang W, *et al*,.2020). As higher viral loads were detected in the nasal area than in the throat(Kim C, *et al*,.2011). Therefore, NP swab is the preferred sampling method for COVID-19 diagnosis.

Upon collection, the swabs should be placed immediately into a sterile transport tube containing 2–3 mL of either viral transport medium (VTM), Amies transport medium, or sterile saline (CDC,. 2020) and stored at 4 °C. Potential risks of NP swabs include (1) the production of aerosol during the sampling, which can impose infection risks to healthcare workers; (2) the inconsistent quality of NP swabs between collections, which may lead to false-negative results; and (3) the patient may experience discomfort during the sampling procedure. Aiming to address these potential risks, further studies demonstrated the efficacy of less invasive routes for sampling such as throat wash and sputum collection . Saliva collection was shown to yield greater detection sensitivity and consistency throughout the course of the infection when compared with patient-matched NP samples. Furthermore, saliva could enable self-administered sample collection for accurate large-scale SARS-CoV-2 testing(Wyllie AL, 2020) . Some authors suggested a potential value in testing both fecal and respiratory specimens to improve the test sensitivity (Tian Y, *et al***,**.2020) . However, this issue remains under debate, as the detection of viral RNA in stool may not reflect actual viral replication or infection(Lo IL, . *et al*,.2020). Although the assay is highly specific, the sensitivity is relatively low secondary to several factors including the viral load, virus replication, RNA isolation method, and the source and timing of swab collection in relation to the disease stage (Wölfel R,. *et al*,.2020) .

After collection, the clinical samples are subjected to RNA isolation. This is a time-consuming step that is also extremely important to avoid the false-negative results. The

optimal protocol for the isolation of RNA would ideally provide pure RNase-free nucleic acid and recover RNA quantitatively across a range of dilutions. Several protocols for RNA isolation have been used by different laboratory. The efficiency of RNA isolation protocols for SARS-CoV-2 from stool samples was discussed in a recent multicenter study (Petrich A, *et al*,.2006) .

CONCLUSIONS

The top priority for controlling the rapidly evolving SARSCoV-2-associated-COVID-19 is developing a diagnostic test of high performance. The use of viral culture is not feasible option for rapid diagnosis as it takes 3–5 days for SARS-CoV-2 to cause obvious cytopathic changes in vitro. In addition, virus isolation requires biosafety level-3 (BCL-3) facilities of limited availability in many medical centers. Serology tests have not yet been validated. Moreover, the issue of cross reactivity with SARS-CoV remains to be solved. Therefore, at present, a positive result in nucleic acid testing (NAT) using reverse transcription-polymerase chain reaction (RT-PCR) is the gold standard for diagnosing COVID-19 .The optimization of laboratory diagnostics is the most dynamically developing field in the time of the COVID-19 pandemic, supporting contemporary medicine, government decisions and healthcare strategies. The efforts of scientist–clinician teams focus, first of all, upon implementing the most reliable diagnostic tools; however, because COVID-19 is a new nosological entity, there are not enough data as of yet that would enable the determination of standards for the interpretation of serological POCTs.

As with any other infectious diseases, the diagnostic value of a test is not only about the method of collecting the material, the quality of the sample and the equipment applied. Equally essential pre-analytical considerations are also the time point when a sample is collected, as well as a suitable procedure (storage and handling) prior to analysis, from the moment of collecting the biological material to the assaying stage.

The clinical performance data reported by the manufacturers of commercially available tests are compared with the clinical evaluations performed by independent research labs and healthcare organizations, and the results are reviewed to define upcoming research and development challenges. Since the understanding of molecular diagnostics for COVID-19 is still evolving, their limitations in the current pandemic scenario are deliberated to ask forthcoming research questions that would improve the diagnostic technologies for COVID-19 and future emerging infectious diseases.

RECOMEDATIONS

- To limit the spread of SARS-CoV-2 and overcome the COVID-19 crises it is important to identify the suspected individuals and isolate them.
- In addition to excluding all other sources of respiratory infection, the decision on who to test should be based on the CDC guidance and local epidemiological data.
- The priority category includes persons with symptoms of suspected COVID-19 infection and persons without symptoms who are prioritized by healthcare providers, for any reason, including public health monitoring or screening according to state and local plans.

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