COVID-19 diagnostic methods in developing countries

Md. Maniruzzaman1 · Md. Missile Islam7 · Md. Hazrat Ali3 · Nobendu Mukerjee4,5 · Swastika Maitra6 · Mohammad Amjad Kama7,8,9,10 · Arabinda Ghosh11 · Melvin A. Castrosanto12 · Athanasios Alexiou13,14 · Ghulam Md. Ashraf15,16 · Priti Tagde17 · Md. Habibur Rahman18

Received: 16 March 2022 / Accepted: 19 May 2022 / Published online: 27 May 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
COVID-19 has become one of the few leading causes of death and has evolved into a pandemic that disrupts everyone’s routine, and balanced way of life worldwide, and will continue to do so. To bring an end to this pandemic, scientists had put their all effort into discovering the vaccine for SARS-CoV-2 infection. For their dedication, now, we have a handful of COVID-19 vaccines. Worldwide, millions of people are at risk due to the current pandemic of coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2). Despite the lack of clinically authorized antiviral medications and vaccines for COVID-19, clinical trials of many recognized antiviral agents, their combination, and vaccine development in patients with confirmed COVID-19 are still ongoing. This discovery gave us a chance to get immune to this disease worldwide and end the pandemic. However, the unexpected capacity of mutation of the SARS-CoV-2 virus makes it difficult, like the recent SAS-CoV-2 Omicron variant. Therefore, there is a great necessity to spread the vaccination programs and prevent the spread of this dreadful epidemic by identifying and isolating afflicted patients. Furthermore, several COVID-19 tests are thought to be expensive, time-consuming, and require the use of adequately qualified persons to be carried out efficiently. In addition, we also conversed about how the various COVID-19 testing methods can be implemented for the first time in a developing country and their cost-effectiveness, accuracy, human resources requirements, and laboratory facilities.

Keywords SARS-CoV-2 · Viral invasion · Immunity · RT-PCR · ELIZA · SHERLOCK · Coronavirus · Antigen

Introduction
SARS-CoV-2 is an RNA virus, a new and highly aggressive coronavirus that causes the severe acute respiratory syndrome. Animals like birds and mammals, including humans, are susceptible to coronaviruses (Rabaan et al. 2020). As proven by the SARS-CoV, MERS-CoV, and SARS-CoV-2 outbreaks, Coronaviruses (CoV) can transfer from animals to people. COVID-19 is an upper respiratory tract infection caused by the SARS-CoV-2 virus in humans. An infection can induce various symptoms, ranging from a simple disease (such as a common cold) to a potentially fatal infection. Therefore, early infection diagnosis is critical for differentiating COVID-19 patients from other unwell people (Carter et al. 2020).

The SARS-CoV-2 virus is a betacoronavirus. Since the discovery of SARS-CoV in humans in 2003, thousands of coronaviruses have been sequenced. Alpha-CoV, Beta-CoV, Gamma-CoV, and Delta-CoV are the four genus of viruses (Letko et al. 2020). As a result, they were divided into three distinct groups, each with its genetic ancestry. SARS-CoV-2 is a member of the Clades-1 group, which...
also includes SARS-CoV, according to phylogenetic analysis of coronavirus genomes. RaTG13’s spike (S) gene shares around 93 percent sequence identity with other SARSr-CoVs discovered in humans and a wide range of animal taxa, making it the coronavirus with the most direct relation to SARS to date. A thorough examination of the SARS-CoV-2 and SARS-CoV sequences revealed less than 80% identity indicating distinct (Letko et al. 2020; Rahman et al. 2021). Millions of COVID-19 have been infected by the newest SARS COV2 coronavirus. Viruses can spread unchecked in infected tissues since there is no defense against them in humans and they can evade the body’s natural immune
The global public health care system has been devastated by COVID-19, which was not designed to deal with a health crisis of this magnitude, necessitating a significant upgrade of resources, infrastructure, COVID-19 detection systems (e.g., testing laboratories, kits), hospital capacity expansion, and the development of a new COVID-19 method. In addition, the WHO-recommended guidelines for testing laboratories and kits for COVID-19 detection are missing or inadequate in many developing countries (World Health Organization 2020a, b).

When flu-like symptoms first appeared in Wuhan, Hubei Province, China, on December 31, flu-like symptoms caused the virus was formally diagnosed as SARS-CoV-2 on January 7. The virus was confirmed to be SARS-CoV-2 the following day, and after multiple control incidents of COVID-19, Chinese authorities imposed a state of lockdown on the country. However, after successfully implementing a broad testing strategy early in the pandemic, several countries like China, South Korea, New Zealand, and Vietnam limited the spread (Carter et al. 2020; Akter et al. 2021). COVID-19 identification can currently be accomplished via CRISPR-based techniques, real-time polymerase chain reaction (RT-PCR), isothermal nucleic acid amplification technology, and serological assays, among other approaches.

The developing countries are struggling to give full coverage to the public of COVID-19 testing and track the infection because of limited testing laboratories and kits. On the other hand, COVID-19 cases and mortality increase each day. To increase testing capacity, they must invest in new infrastructure, laboratory facilities, and appropriately trained personnel. However, developing and implementing those facilities for COVID-19-affected countries will be costly, whereas this pandemic has severely harmed their economies (Kheirallah et al. 2020, Shah et al. 2020). However, the epidemiology and transmission of COVID-19 are unclear, and there is no specific vaccine and treatment yet. The purpose of this review is to elucidate the etiology, epidemiology, symptoms, and transmission according to the recent data. Furthermore, we also focus on the possible diagnostic approaches.

Fig. 2 The concern of the transmission mechanism of SARS-CoV-2 via droplets
Characteristics of SARS-CoV-2 and targeting SARS-CoV-2 viral entry mechanisms and the immune responses

Coronavirus is a type of virus that has an envelope that is either circular or oval and is sometimes pleomorphic. The coronavirus has a diameter of 50–200 nm and a genome that is approximately 30,000 nucleotides in length. SARS-CoV-2 is distinguished by the presence of club-shaped spike-like projections on its surface, which give the virus the appearance of a solar corona. This unique coronavirus carries the genetic information for four major structural proteins. Proteins implicated in the virus’s replication include the spike protein(S), membrane protein (M), an envelope protein (E), and nucleocapsid (N). The capsid is a protein shell that contains the nuclear capsid, or N-protein, which is attached to the virus’s single positive-strand RNA and allows the virus to hijack human cells and transform them into viral factories. The N protein encircles the viral RNA genome and is required for both replication and transcription to take place. The N-terminal of the N protein in MHV and IBV virions binds to genomic and sub-genomic RNAs and is involved in viral replication and transcription. The M-protein is the most abundant protein on the surface of the coronavirus, and it is thought to be the primary organizer of the virus’s assembly (Tagde et al. 2021a, b, Mukerjee et al. 2021). Coronavirus is a contagious virus that can be spread through inhalation or direct contact with the body. A virus droplet inhaled as a result of coughing or sneezing (a single sneeze can produce up to 10,000 droplets) or by contact with a contaminated surface is the major source of infection (see Fig. 1a).

SARS-CoV and MERS-CoV are human coronaviruses (MERS-CoV). The other human coronaviruses are 229E and NL63 from the family, and OC43 and HKU1 from the genus. The RNA of this new SARS-CoV-2 virus is 30 km long, as the RNA of the old SARS and MERS viruses. In comparison, a known bat coronavirus and SARS-CoV share 96% and 79.5% genome sequence similarities. According to another source, this virus’ genomic sequence resembles SARS-CoV by 45–90%, but MERS-CoV by just 20–60%. Shorter gene sequences in SARS-CoV-2. It has a longer sequence than MERS-CoV. Figure 1b shows two techniques for targeting the ACE2 receptor. In both cases, the S protein of SARS-CoV binds to the human zinc peptidase ACE2, which is expressed in a range of tissues such as the lung, heart, kidney, and intestinal cells. Both the N- and C-terminal S1 subunits contain the receptor-binding domain (RBD), while the C-terminal S2 subunit is required for spike trimerization and virus-host membrane fusion. The membrane-bound host protease TMPRSS2 is responsible for activating the S2 site for conformational changes and viral entry (Tagde et al. 2021a, b, Goyal et al. 2022).

An overview of COVID-19 symptoms

In some cases, those who have been infected with COVID-19 will experience symptoms between 2 and 14 days of infection, but in other cases, the disease may not reveal itself for up to 27 days following infection. Despite this, according to a group of Chinese scientists, the average incubation period is approximately 5.2 days in length. Over this period, there has been no significant change in the number of peripheral blood leukocytes (PBL) or lymphocytes in the blood. In the majority of cases, the viruses spread throughout the body, affecting the lungs, heart, gastrointestinal tract, and circulatory system, among other organs. About 7–14 days after beginning, primary lesions become considerably worse, and PBL, which comprises both T and B cells, begins to decline significantly. According to the findings of the COVID-19 study, the duration between the onset of symptoms and death might range between 6 and 41 days, with a median of 14 days. Even though this duration is reliant on two crucial factors, namely, the patient’s age and immune function, it is generally considered to be a reasonable amount of time. It is important to note that the number of instances among adults over the age of 70 is larger than the number of cases among people under the age of 70. As a result of the infection with COVID-19, a variety of symptoms such as high fever and dry coughing are experienced, as well as fatigue and muscle soreness. Other symptoms include sneezing, headaches, hemoptysis, dyspnea, sputum production, lymphopenia, sore throat, and respiratory issues. COVID-19 is a virus that may infect both humans and animals and spread throughout the world. There are three categories of symptoms: the most common symptoms (such as fever, dry cough, and tiredness), the less common symptoms (such as aches and pains, conjunctivitis, sore throat, diarrhea, and headache), the serious symptoms (such as loss of taste or smell, a rash on the skin, and discoloration of the fingers and toes), and the most serious symptoms of the disease (i.e., difficulty during breathing or shortness of breath, chest pain or pressure, and loss of speech or movement). In comparison to the general population, COVID-19 is more likely to infect children, the elderly, and those individuals with diabetes, cancer, heart disease, or lung disease (Hossain et al. 2020). Infection with COVID-19 resulted in gastrointestinal (GI) symptoms, including diarrhea, in some individuals; however, only a tiny fraction of patients with SARS-CoV or MERS-CoV experienced similar gastrointestinal symptoms. Because of this, it is necessary to check urine and fecal symptoms to rule out the likelihood of an effective transmission channel through.
doctors, nurses, or other healthcare professionals (as shown in Fig. 3).

Individuals suffering from respiratory distress syndrome have been identified, whereas critically ill patients require intense care due to respiratory failure, septic shock, or organ failure. Additionally, the likelihood of contracting COVID-19 increases if there is shortness of breath, coughing, or if a person has come into touch with a COVID-19-infected person or travels through a COVID-19-affected area (Ghosh et al. 2021). In this case, the clinical test should be administered to the affected individuals, and infected areas should be sealed off. Patients infected with COVID-19, on the other hand, may recover quickly because of their high immunity capacity and return to their pre-infection state, whilst others may require additional time due to health issues or age constraints (Hossain et al. 2020).

Currently instigated diagnostic approaches

Real-time polymerase chain reaction

RT-PCR, which combines reverse RNA transcription into complementary DNA (cDNA) with amplifying specific DNA targets, has been used as a quantitative laboratory technique (Fronhoffs et al. 2002). In the case of RNA virus’s presence detection through RT-PCR, RNA-dependent DNA polymerase is used for reverse transcriptase. Small DNA sequence primers were designed to target and bin specific complementary sequences of the particular virus genome in the inertial detection process. As a result, and using RNA-dependent DNA polymerase, cDNA copies were produced. In RT-PCR, this amplification is monitored using various fluorescence dyes or DNA probes labeled with fluorescence molecules that target a specific part of the genome (Tombaloglu et al. 2022).

RT-PCR can be used as a one-step process or as a two-step process. Generally, the one-step RT-PCR procedure is preferred for virus detection because whole reaction is run in a single tube with the primers and reagents in the one-step procedure. This makes it relatively less complicated, needs less time, and decreases the possibility of errors or contamination. At the same time, the two-step procedure is much more flexible and gives higher sensitivity.

Specimen

Nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage specimens, stool, and urine specimens are kept at 2–8 °C (≤ 4 days) or frozen in dry ice at – 70 °C or below (WHO Guidelines) (World Health Organization, 2020a, b).

Primers and probe designing

Coronaviruses are large, positive-stranded RNA viruses with the giant genomes of any RNA virus, spanning between 27 and 32 kb (Lu et al. 2020). The genomic RNA in this virus combines with the basic nucleocapsid (N) protein to produce a helical cap with a diameter of 80 to 120 nm and a length of 80 to 120 nm. The membranes of the virus are made up of three viral proteins. The spike (S), a type I glycoprotein, the membrane protein (M), and the envelope membrane protein (EMP) are all glycoproteins.

For SARS-CoV-2 detection, the design process generally involves two main steps:

1. Sequence alignment and primer design.
2. Assay optimization and testing.

When the researchers examined the genomes of SARS-related viruses, they observed three conserved regions in which the RdRP (RNA-dependent RNA polymerase) gene and the E gene (envelope protein gene) exhibit high analytical sensitivity for identification and characterization. The N gene (nucleocapsid protein gene) has poorer analytical sensitivity than the other genes (Corman et al. 2020; Kakhki

![Fig. 3 Patients with COVID-19 infection suffered from systemic and respiratory problems](image-url)
et al. 2020). Scientists used sections of the virus’s genetic sequence specifically targeted by researchers while developing primers and probes for the COVID-19 testing kit, which was created to detect the SARS-CoV-2 virus. For RT-PCR tests, primers and probes for the nucleocapsid (N), spike (S), ORF1a, and ORF1b or ORF8 regions; RNA-dependent RNA polymerase (RdRP); and envelope (E) genes were constructed (Corman et al. 2020; Carter et al. 2020; Rabaan et al. 2020).

In many countries, scientists have sequenced SARS-CoV-2 variants and examined different genes of the virus genome to design primers and probes for RT-PCR. For example, the National Institute of Health (Thai NIH) located in Thailand worked on N genes, Institut Pasteur located in France worked on RdRP (2 targets) genes, and the Center for Disease Control and Prevention (CDC) located in China worked on ORF1ab, N genes (Table 1). The most used genes are N, ORF1a, ORF1b, and E, but most of those countries (Table 1) recommended gene “E” (Barreto et al. 2020).

Here are a few examples of successful primer and probes designed testing kits with high sensitivity: TaqPath COVID-19 Combo kit (ThermoFisherAppliedBiosystems), Allplex 2019-nCoV Assay (Seegene), Cobas SARS-CoV-2 (Roche) are some of the few earliest COVID-19 test kits (Carter et al. 2020; Saylor et al. 2020; Lai et al. 2021).

**RT-PCR for the SARS-CoV-2 detection methods**

In reaction to the launch of the COVID-19 testing kit, many laboratories worldwide produced their COVID-19 testing kits. Each COVID-19 testing kit has followed different targeted regions of the SARS-CoV-2 genomes, and each testing kit’s PCR protocol can vary depending on the manufacturer’s instruction (as shown in Fig. 4).

The Centers for Disease Control and Prevention (CDC) created their-PCR test method (COVID-19), which is known as the RT-PCR Diagnostic Panel (RT-PCRDP). According to the CDC protocol of the RT-PCRDP testing kit, the nucleic

---

**Table 1** There are a few examples of countries, institutes, and their respective target genes (Barreto et al. 2020)

| Country   | Institute                                      | Target genes                          |
|-----------|------------------------------------------------|---------------------------------------|
| China     | Center for Disease Control and Prevention (CDC) | ORF1ab, N                             |
| France    | Institut Pasteur                               | RdRP (2 targets)                      |
| Germany   | Charité—Universitätsmedizin Berlin             | RdRP, E, N                            |
| Hong Kong SAR | The University of Hong Kong (HKU)        | ORF1b-nsp14, N                        |
| Japan     | National Institute of Infectious Diseases (NIID) | Pancorona, multiple targets, spike protein |
| Thailand  | National Institute of Health (Thai NIH)       | N                                     |
| USA       | Centers for Disease Control and Prevention (CDC) | N (3 target regions)                  |

---

**Fig. 4** The process of RT–PCR assay
acid was extracted from the clinical samples and blended with the master mix before running. The mixture was through an RT-PCR thermocycler for 30 min at 50 °C. The incubation temperatures are then determined for to experiment (UNG). The following procedures are carried out after incubation at 25 °C for 2 min, reverse transcription at 50 °C for 15 min, enzyme activation at 95 °C for 2 min, and amplification at 95–55 °C for 3–30 s; (the probe for quenching fluorophores has been cleaved). When utilized in conjunction with the thermocycler, the thermocycler monitors the fluorescence signal and records the amplification progress in real-time (Tahamtan and Ardebili 2020). It is recommended that studies be carried out independently for each target, with the analyst manually changing the threshold setting. To verify specimen quality, all clinical samples should be tested for the presence of the human RNase P (RNP) gene. If not sooner, positive results should be achieved within 35 rounds (Kevadiya et al. 2021).

As a consequence, the presence of enough nucleic acid from the human RNase P gene, as well as the Specimen’s acceptable quality, is both indicated. The thresholds should be changed as needed to stay inside the exponential phase of the fluorescence curves while remaining above any background signal. There should be no fluorescence growth curves that cross the threshold line by negative template Control. If the reaction is successful, the positive template control reaction should produce positive results for each target. The human specimen control (HSC) is a second negative control that looks at the nucleic extraction technique and the reagent integrity. For primer/probe sets unique for 2019-nCoV (Tahamtan and Ardebili 2020), the HSC should be negative, and the HSC should be negative for all primer/probe sets.

RT-PCR has a high sensitivity to early infection detection and is a more flexible, well-established, and proven method. For COVID-19 diagnosis, many testing kits are available based on RT-PCR.

However, there are also a few significant limitations of the RT-PCR test. One of its risks is false-negative results like other methods and the lengthy or time-consuming process requiring expensive laboratory equipment and highly skilled laboratory personnel. In addition, it is a highly sensitive and complex process (Tahamtan and Ardebili 2020). For example, due to poor collection, transit, or handling, insufficient levels of the virus may be present in the samples, resulting in a false-negative result. Furthermore, if analysts are not adequately trained and knowledgeable about testing techniques, generating a false result is easy. Also, mismatches between primers and probes and target sequences can result in decreased assay performance and possible false-negative results. But despite those possible errors, RT-PCR is still very reliable and has proven its effectiveness. According to a study, the viral load in a sample of two COVID-19 people decreased with time. But considering developing countries’ economic conditions, RT-PCR’s most significant disadvantage is costly and time-consuming.

**Isothermal nucleic acid amplification technologies**

Nucleic acid testing promises rapid, sensitive, and specific diagnosis of infection. Still, the complex biochemical nature of clinical samples and the low abundance of nucleic acid targets in most clinical samples to solve this problem, nucleic acid amplification will be required (Craw and Balachandran 2012). For nucleic acid amplification, PCR, RT-PCR, and isothermal amplification technologies, including reverse transcription loop-mediated isothermal amplification (RT-LAMP), and CRISPR-based assays, were used. However, PCR or RT-PCR requires multiple temperature changes for each cycle, but isothermal nucleic acid amplification needs constant temperature and a single temperature cycle (Carter et al. 2020; Karthika et al. 2021). Therefore, for COVID-19 detection, several methods have been developed.

**Reverse transcription loop-mediated isothermal amplification**

In one step, a single heat cycle methodology is used to combine a reverse transcription step (which takes place at a temperature between 60 and 65 °C) with a nucleic acid amplification method. It is a less time-consuming alternative to reverse transcription-polymerase chain reaction. The use of RT-LAMP for the early diagnosis of COVID-19 has recently gained popularity in clinical laboratories (Mori and Notomi 2009; Bokelmann et al. 2021). Diagnostic approaches based on RT-LAMP technology can detect SARS-CoV-2 viruses from swabs or saliva without the need for RNA. To execute the RT-LAMP assay, it is essential to employ primers that have been mainly designed to be compatible with the target region and primers produced from virus genomic studies. It is crucial to use a set of four primers that are particular for the target gene/region to maximize the sensitivity of RT-LAMP (Carter et al. 2020). Colorimetry, spectrophotometry, and real-time fluorescence (Srivastava et al. 2020), among other techniques, can be used to detect amplification results with the naked eye. According to Abbott Diagnostics, the COVID-19 diagnostic test is based on RT-LAMP technology and targets the RdRP gene. Clinical samples are acquired in 5 min using nasal, nasopharyngeal, and throat swabs, while negative findings take 13 min. Positive results take 5 min to obtain, while negative results take 13 min. The test has been approved for usage outside a laboratory setting (Carter et al. 2020). A DNA-dependent DNA polymerase is used to amplify cDNA, allowing for quick detection of cDNA using a DNA-binding dye (SYTO-9, ThermoFisher
Clustered regularly interspaced short palindromic repeats–based assays

CRISPR-associated enzymes, such as Cas9, Cas12, and Cas13, are located in the genomes of prokaryotes such as bacteria or archaea and play a vital role in the antiviral defense mechanism of prokaryotes. Cas9, Cas12, and Cas13 can be programmed to target and cut viral RNA sequences (Zhang 2019). There are three different types of CRISPR-Cas-12a-based detection approaches available: SHERLOCK (Specific High-sensitivity Enzymatic Reporter UnLOCKing), DETECTOR (DNA Endonuclease-Targeted CRISPR Trans Reporter), and AIOD-CRISPR assays (Kellner et al. 2019; Ding et al. 2020a).

The SHERLOCK technique developed by Sherlock Biosciences uses Cas13. This reporter RNA sequence can be activated by SARS-CoV-2-specific guide RNA, and it has shown substantial promise for the development of next-generation POC molecular isotherms. Mammoth Biosciences and Sherlock Biosciences, two companies, founded by CRISPR pioneers, are exploring utilizing CRISPR to identify SARS-CoV-2. A 316-bp pUCIDT-AMP plasmid and AIOD-CRISPR technology were used to build the COVID-19 detection method at the University of Connecticut Health Center. The AIOD-CRISPR experiment was carried out using SARS-CoV-2 N gene cDNA (N plasmid). In terms of specificity, the AIOD-CRISPR assay has a positive signal in real-time and visual detections without any cross-reactions with non-SARS-CoV-2 targets. Using a single, one-pot reaction system, this method eliminates the need for separate pre-amplifying and post-amplifying steps and the necessity to move the amplified product between systems. Using a 5-FAM (Fluorescein) fluorophore and a 3-Iowa Black® FQ quencher, we were able to identify ssDNA-FQ. As part of the TwistAmp® Liquid Basic package, one will receive one RPA reaction buffer, one Basic E-mix, one Core Reaction Buffer, 14 mg of magnesium acetate, 320 ng of primers, and 1.2 mg of deoxynucleoside triphosphate. This enzyme cleaves ssDNA-FQ reporters nearby when activated, which causes fluorescence. SARS-CoV-2 RNA and DNA were detected in 40 min using the AIOD-CRISPR test (Ding et al. 2020b). Dual crRNAs were added to AIOD-CRISPR throughout the design phase to increase sensitivity. It is an isothermal nucleic acid detection technology that uses a single reaction system that is precise, quick, reliable, and practically single-molecule sensitive.

Serological and immunological assay

Serological assay, also known as serology test or antibody test, was used for the detection presence of antibody-like immunoglobulin M (IgM) and immunoglobulin G (IgG) against particular diseases by analyzing patient serum, plasma, or any other biological fluids samples (Bonelli et al. 2020; Carter et al. 2020). A few days after developing some infectious disease, our body produces an antibody to counter the cause of infection. As a first response, an IgM antibody was produced and switched to IgG after a few weeks (IgG also indicates post-infection immunity) (Carter et al. 2020). Few variants of serological tests are available: for example, hemagglutinin-inhibition tests, neutralization tests, enzyme-linked immunosorbent assays (ELISA), flocculation tests (Bonelli et al. 2020; Carter et al. 2020). qSARS-CoV-2 IgG/IgM Rapid Test, manufactured by Cellex Inc, is the first serology test granted by FDA on April 1, 2020 (Machado et al. 2020, Huergo et al. 2021) (Fig. 5).

Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) is a microwell, plate-based assay technique designed for quantitative/qualitative assay commonly used to measure antibodies, antigens, proteins, and glycoproteins in biological samples. ELISA is used in medical laboratories around the world. Examples include diagnosis of HIV infection, pregnancy tests, and measurement of cytokines or soluble receptors in cell supernatant or serum (Lequin 2005; Eguasi Inkabi et al. 2017). ELISA is based on specific antigen–antibody reaction and high specificity and sensitivity, high efficiency, simple procedure, and cost-effective (Sakamoto et al. 2018). The ELISA technique is easy to perform for many samples (Vidal and Catapani 2005). ELISA assay measures the amount (qualitative and quantitative) of specific antibodies or proteins present in the sample after the infection, like COVID-19 (as shown in Fig. 6). This assay does not require handling infectious virus, can be adjusted to detect different antibody types in serum or plasma, and help to define previous exposure to SARS-CoV-2 in the population (Amanat et al. 2020).

ELISA is a qualitative detection method of the presence of IgM at early-stage infection for diagnosing the COVID-19 or IgG test for post-infection immunity in human plasma (Bundschuh et al. 2020; Alhalabi et al. 2020)).

This method is a microwell technique, so first, the viral protein was coated on the plate wells. In the second step, collected human specimens were introduced with the coat. Suppose antiviral antibodies are present in the patient
specimens. In that case, it will produce an antibody-protein complex that can be visually detected using additional tracer antibodies in the third step causing colorimetric or fluorescent-based readout. This method will take about 10 to 30 min to give the result (Ding et al. 2020a). KT-1033 EDI Novel Coronavirus COVID-19 ELISA kit manufactured by Epitope Diagnostics can detect the presence of IgG/IgM of SARS-CoV-2 from serum samples (Kaltenbach et al. 2020). V ITROS-Immunodiagnostics Products, anti-SARS-CoV-2 total reagent pack manufacturer by Ortho-Clinical Diagnostics targets SARS-CoV-2 IgG/IgM from blood serum/plasma it cannot distinguish IgG/IgM and (Goss et al. 2021).

ELISA has a few limitations that make it inferior to other methods. One of the limitations is the disability to detect efficiently COVID-19 during the very early stage of infection, because the early-stage body might not be responsive enough to produce antibodies or in the process of building antibodies and create a gap. ELISA assay gives high sensitivity, but in the case of COVID-19 diagnosis, it is not very competitive till now comparatively to other methods (Statement from Commissioner of Food and Drugs—Food and Drug Administration, USA, April 7, 2020).

**Lateral flow immunoassay**

Lateral flow immunoassay is also known as lateral flow immunochromatographic assays. These tests are widely used in medical diagnostics for home testing, point of care testing, or laboratory use, for example, home pregnancy tests (Magiati et al. 2019). It has good sensitivity, specificity, good stability, relatively low cost, and a short timeline for development (Wong and Tse 2009; Wang et al. 2020).

Lateral flow immunoassay is a qualitative chromatographic assay, and it is a very rapid diagnostic test. It can give a result within 10–15 min (Carter et al. 2020). Lateral flow tests operate on the same principles as the enzyme-linked immunosorbent assays (Wong and Tse 2009). Following the method currently, many SARS-CoV-2 testing kits are available. It is mainly designed to detect the presence of SARS CoV-2 antibody using serum/plasma/whole blood. In this assay, serum/plasma/blood sample runs along the surface of a testing device pad via capillary flow and device testing zone coated with a labeled antigen that can bind with the SARS-CoV-2 antibody. When anti-SARS-CoV-2 antibodies are present, they bind to the labeled antigen and turn into captured antibody–antigen complex, visualizing it as a colored test band (Wu et al. 2020). For example, COVID-19 IgG/IgM rapid test cassette device manufactured by Hangzhou Biotest Biotech Co. L can detect SARS-CoV-2 IgG/IgM antibodies within 5–20 min using serum/plasma/blood samples (Dellière et al. 2020). Similarly, the COVID-19 IgG/IgM Point of Care Rapid test device manufactured by Aytu Biosciences/Orient Gene Biotech can detect SARS-CoV-2 IgG/IgM antibodies within 2–10 min (Yang et al. 2020; Boiko et al 2022).
Discussion

COVID-19 was met with a state of lockdown and massive diagnostic testing of the virus by developed country governments, which quickly expanded testing capacity and produced or stored necessary equipment to trace, isolate, and treat those who were infected as well as contain the virus’ spread, as they had done in the aftermath of the SARS-CoV-2 outbreak, which crossed international borders and had no known effective treatment. Similar to what is happening in developed nations, an alarming number of developing country governments are adopting similar policies without regard for their country’s unique circumstances or other challenges (Chowdhury and Jomo 2020). As a result, they made only modest progress in detecting, isolating, and treating sick people and restricting the virus’s transmission. Developing countries were forced to adopt already established testing processes that were exceedingly sensitive and highly specific. However, those approaches were not always cost-effective. For example, at the beginning of the COVID-19 emergency in Bangladesh, there was only one dedicated RT-PCR laboratory for COVID-19 at the Institute of Epidemiology Disease Control and Research (IEDCR). But within seven months, Bangladesh had opened 103 more
COVID-19 testing RT-PCR laboratories (Average cost per test $19.83 and $26.84), and 15 are GeneXpert method (Cost more than $ 24). And for rural area coverage, the government establishes an overall 40 Ag. RDT (Antigen-based Rapid Diagnostic Test) testing centers with the WHO assistance (Till March 29, 2021, only over 16 033 tests have been conducted) (Cost between $2.92 and $3.50) (according to WHO report, March 30, 2021).

Similarly, in Malaysia, the Ministry of Health (MOH) took RT-PCR as a gold standard for COVID-19 testing with an accuracy of 99.9% (in Malaysia, the price per test is now anywhere between $43 and $60) and also recognized Antigen Rapid Test Kit (RTK-Ag) with an accuracy 90% (Each kit costs $35.92). On the other hand, in India, according to the Indian EXPRESS December 31, 2021, report, the Health Ministry has introduced several other types of tests to ramp up the COVID-19 testing rate to 2.15 lakh per day. The tests are RT-PCR tests (in India, the price per test is now anywhere between $29.53 and $40.27), Rapid antibody tests (Cost between $6.71 and $8.05), Rapid antigen tests (Each kit costs $4.50), and TruNat tests (Cost $17.45).

Although COVID-19 testing kit cost-related information is minimal and not organized, but according to obtained data from open source, RT-PCR is the world delayered standard method of testing COVID-19 infection. Among the all-available molecular assays for diagnosis of COVID-19, 90% use RT-PCR technologies, 6% isothermal amplification, and 2% CRISPR-based technologies for the detection of SARS-CoV-2 as serological and immunological detection methods like ELISA and lateral flow immunoassay (Carter et al. 2020). Even though reverse transcription-polymerase chain reaction (RT-PCR) has been the most extensively used method for detecting SARS-CoV-2, real-time PCR test kits have several drawbacks, including high false-negative rates due to various factors. This highly complex operation will necessitate a highly qualified operator and a costly laboratory. Isothermal amplification, for example, is exceptionally sensitive, specific, and cost-effective, as well as rapid and straightforward to conduct because it does not require a highly trained operator or expensive laboratory equipment. Serosurveys will be tremendously helpful in determining the actual attack rate and infection fatality rate in various human groups and charting the kinetics of the antibody response. Two serological and immunological assays that will be highly useful in this regard are ELISA and lateral flow immunoassay. Serological tests continue to raise several questions, including the lag period that occurs during the early stages of infection when the body’s immune response is still developing. The possibility is that serological and immunological assays may not detect the presence of the SARS-CoV-2 virus. Also, many concerns have regarding the sensitivity and specificity of several types of serological and immunological assays.

| Name of kit | Type of test kit | Time | Cost per test (USA) | Sensitivity |
|-------------|------------------|------|---------------------|-------------|
| Cobas RT-PCR Kit, 480 T/kit | RT-PCR Kit | Results in ~3.5 h | $10.90 | 95.0% |
| DETECTOR BOOST™ CRISPR-based | Results in 30 – 40 min | $3.50 | Among the COVID-19 positive (PPA) 95.0% were detected and among negative (NPA) 100% |
| DETECTOR BOOST™ CRISPR-based | Positive results within 5 min and negative results within 13 min | $5 | 97.1% |
| ID NOW COVID-19 | Isothermal nucleic acid amplification technology | Between 10 and 30 min | – | 41.2% (0–7 days), 91.7% (7–14 days), 98.6% (15 + days) |
| SGti-flex COVID-19 IgG | Serological test | Between 10 and 30 min | – | (PPA)/sensitivity of 90.48% and (NPA)/specificity of 98.95% |
| The Rapid COVID-19 IgM/IgG Combo Test Kit | Lateral flow immunoassay | Results in 15 min | – | |

Nowadays, many laboratory techniques and test kits are available, and among them, the RT-PCR test was the world delayered standard method of testing COVID-19 infection. Among the all-available molecular assays for diagnosis of COVID-19, 90% use RT-PCR technologies, 6% isothermal amplification, and 2% CRISPR-based technologies for the detection of SARS-CoV-2 as serological and immunological detection methods like ELISA and lateral flow immunoassay (Carter et al. 2020). Even though reverse transcription-polymerase chain reaction (RT-PCR) has been the most extensively used method for detecting SARS-CoV-2, real-time PCR test kits have several drawbacks, including high false-negative rates due to various factors. This highly complex operation will necessitate a highly qualified operator and a costly laboratory. Isothermal amplification, for example, is exceptionally sensitive, specific, and cost-effective, as well as rapid and straightforward to conduct because it does not require a highly trained operator or expensive laboratory equipment. Serosurveys will be tremendously helpful in determining the actual attack rate and infection fatality rate in various human groups and charting the kinetics of the antibody response. Two serological and immunological assays that will be highly useful in this regard are ELISA and lateral flow immunoassay. Serological tests continue to raise several questions, including the lag period that occurs during the early stages of infection when the body’s immune response is still developing. The possibility is that serological and immunological assays may not detect the presence of the SARS-CoV-2 virus. Also, many concerns have regarding the sensitivity and specificity of several types of serological and immunological assays.

Table 2 A visual compression among a few available test kits (Carter et al. 2020; Lee et al. 2021)
Conclusion

Rapid, dependable, and concrete point-of-care testing (POCT) is required to expand diagnostic coverage and rapidly identify infected persons, isolate them, and begin treatment. At the same time, impoverished countries must afford to participate. For example, isothermal amplification methods such as those used in the CRISPR-Cas approach are less expensive than RT-PCR, easier to perform (only one temperature cycle is required), and have accuracy comparable to RT-PCR is significantly more accurate than serological testing. According to recent findings, isothermal amplification using the CRISPR-Cas technique may be the most cost-effective and best way for point-of-care testing in low-income nations.

Author contribution Conceptualization, methodology: Md. Maniruzzam, Nobendu Mukejee; writing the original draft and methodology and collecting data, Md. Maniruzzam, Md. Missile Islam, Md. Hazrat Ali, Nobendu Mukejee, Swastika Maitra, Mohammad Amjad Kamal, Asmita Ghosh, Arabinda Ghosh, Melvin A. Castrosanto, Athanasios Alexiou, Gulam Md Ashraf, Priti Tagde, and Md. Habibur Rahman; supervision: Athanasios Alexiou and Md. Habibur Rahman. All authors approved for submitting the final manuscript.

Data availability Not applicable.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests Not Applicable.

References

Akter R, Chowdhury MA, Rahman MH (2021) Flavonoids and polyphenolic compounds as potential talented agents for the treatment of Alzheimer’s disease and their antioxidant activities. Curr Pharm Des 27(3):345–356. https://doi.org/10.2174/1381612826666201102102810

Amanat F, Stadlbauer D, Strohmeier S, et al (2020) A serological assay to detect SARS-CoV-2 seroconversion in humans. medRxiv: the preprint server for health sciences. https://doi.org/10.1101/2020.03.17.20037713

Alhalabi O, Iyer S, Subbiah V (2020) Testing for COVID-19 in patients with cancer. EClinicalMedicine 23:100374

Barreto HG, de Pádua Milagres FA, de Araújo GC et al (2020) Diagnosing the novel SARS-CoV-2 by quantitative RT-PCR: variations and opportunities. J Mol Med 98:1727–1736. https://doi.org/10.1007/s00109-020-01992-x

Bokelmann L, Nickel O, Maricic T et al (2021) Point-of-care bulk testing for SARS-CoV-2 by combining hybridization capture with improved colorimetric LAMP. Nat Commun 12:1467. https://doi.org/10.1038/s41467-021-21627-0

Bonelli F, Sarasin A, Zierold C, et al (2020) Clinical and analytical performance of an automated serological test that identifies S1/S2-neutralizing IgG in COVID-19 patients semiquantitatively. J Clin Microbiol 58. https://doi.org/10.1128/JCM.01224-20

Boiko DL, Skrypnikov AM, Shkodina AD, Hasan MM, Ashraf GM, Rahman M (2022) Circadian rhythm disorder and anxiety as mental health complications in post-COVID-19. Environ Sci Pollut Res 5:1–8. https://doi.org/10.1007/s11356-021-18384-4

Bundschuh C, Egger M, Wiesinger K, et al (2020) Evaluation of the Edil enzyme linked immunosorbent assays for the detection of SARS-CoV-2 IgM and IgG antibodies in human plasma. Clinica Chimica Acta; International Journal of Clinical Chemistry 509:79–82. https://doi.org/10.1016/j.cca.2020.05.047

Carter LJ, Garner LV, Smoot JW et al (2020) Assay techniques and test development for COVID-19 diagnosis. ACS Cent Sci 6:591–605. https://doi.org/10.1021/acscentsci.0c00501

Chowdhury AZ, Jomo KS (2020) Responding to the COVID-19 pandemic in developing countries: lessons from selected countries of the Global South. Development (Society for International Development) 1–10 https://doi.org/10.1057/s41301-020-00256-y

Corman VM, Landt O, Kaiser M, et al (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 25https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

Craw P, Balachandran W (2012) Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review. Lab Chip 12:2469. https://doi.org/10.1039/c2lc40100b

Dao Thi VL, Herbst K, Boerner K, Meurer M, Kremer LP, Kirrmaier D, Freistaedter A, Papagiannidis D, Galmozzzi C, Stanifer ML, Bouland S (2020) A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. Sci Transl Med 12(556):eabc7075

Dellière S, Salmona M, Minier M, et al (2020) Evaluation of the COVID-19 IgG/IgM rapid test from Orient Gene Biotech. J Clin Microbiol 58.https://doi.org/10.1128/JCM.01233-20

Ding X, Yin K, Li Z et al (2020a) Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. Nat Commun 11:4711. https://doi.org/10.1038/s41467-020-18575-6

Ding X, Yin K, Li Z, Liu C (2020b) All-in-One Dual CRISPR-Cas12a (AIOD-CRISPR) Assay: a case for rapid, ultrasensitive and visual detection of novel coronavirus SARS-CoV-2 and HIV virus. bioRxiv: the preprint server for biology. https://doi.org/10.1101/2020.03.19.998724

Eguasi Inkabi S, Pushpamithran G, Richter P, Attakora K (2017) Exercising and education management among patients with cancer. EClinicalMedicine 23:100374

Huergo LF, Selim KA, Conzentiino MS et al (2021) Magnetic bead-based immunoassay allows rapid, inexpensive, and quantitative

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

 Springer
Kevadiya BD, Machhi JK, Herskovitz J, Oleynikov MD, Blomberg WR, Kellner MJ, Koob JG, Gootenberg JS et al. (2019) SHERLOCK: nucleic acid detection with CRISPRR nucleases. Nat Protoc 14:2986–3012. https://doi.org/10.1038/s41596-019-0210-2

Kellner MJ, Ross JJ, Schnabl J, et al. (2020) A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing. bioRxiv. https://doi.org/10.1101/2020.06.23.166397

Kheirallah KA, Alsinglawi B, Alzoubi A et al. (2020) The effect of strict state measures on the epidemiologic curve of COVID-19 infection in the context of a developing country: a simulation from Jordan. Int J Environ Res Public Health 17:6530. https://doi.org/10.3390/ijerph17106530

Kevadiya BD, Machhi JK, Herskovitz J, Oleynikov MD, Blomberg WR, Bajwa N, Soni D, Das S, Hasan M, Patel M, Senan AM (2021) Diagnostics for SARS-CoV-2 infections. Nat Mater 20(5):593–605. https://doi.org/10.1038/s41563-020-00906-z

Karthika C, Swathy Krishna R, Rahman M, Akter R, Kaushik D (2021) COVID-19, the firestorm in 21st century: a review on coronavirus disease and its clinical perspectives. Environ Sci Pollut Res 28(46):64951–64966. https://doi.org/10.1007/s11356-021-16654-9

Lai C-C, Wang C-Y, Ko W-C, Hsueh P-R (2021) In vitro diagnostics of coronavirus disease 2019: Technologies and application. J Microbiol Immunol Infect 54:164–174. https://doi.org/10.1016/j.jmii.2020.05.016

Lalil MA, Langmade JS, Chen X et al. (2021) Rapid and extraction-free detection of SARS-CoV-2 from saliva by colorimetric reverse-transcription loop-mediated isothermal amplification. Clin Chem 67:415–424. https://doi.org/10.1093/clinchem/bvaa267

Lee CK, Tham JWM, Png S et al. (2021) Clinical performance of Roche cobas 6800, Lumixen ARIES, MiRXES Fortitude Kit 2.1, Altona RealStar, and Applied Biosystems TaqPath for SARS-CoV-2 detection in nasopharyngeal swabs. J Med Virol 93:4603–4607. https://doi.org/10.1002/jmv.29640

Lequin RM (2005) Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clin Chem 51:2415–2418. https://doi.org/10.1373/clinchem.2005.051532

Letko M, Marzi A, Munster V (2020) Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol 5:562–569. https://doi.org/10.1038/s41556-020-0668-y

Lu R, Zhao X, Li J et al. (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet 395:565–574. https://doi.org/10.1016/S0140-6736(20)30251-8

Magiati M, Myridaki VM, Christopoulos TK, Kalogiani DP (2019) Lateral flow test for meat authentication with visual detection. Food Chem 274:803–807. https://doi.org/10.1016/j.foodchem.2018.09.063

Machado BA, Hodel KV, Barbosa-Junior VG, Soares MB, Badaro R (2020) The main molecular and serological methods for diagnosing COVID-19: an overview based on the literature. Viruses 13(1):40. https://doi.org/10.3390/v13010040

Mori Y, Notomi T (2009) Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother 15:62–69. https://doi.org/10.1007/s10156-009-0669-9

Muñoz-Planas N, Ghosh AK, Dolai M (2021) Treatments discovery so far on SARS-COV-19: a brief report

Rabanal AA, Al-Ahmed SH, Sah R et al. (2020) Genomic epidemiology and recent update on nucleic acid–based diagnostics for COVID-19. Curr Trop Med Rep 7:113–119. https://doi.org/10.1007/s40750-020-00212-3

Rahman MH, Akter R, Behl T, Chowdhury MA, Mohammed M, Bulbuli JI, Elshenawy SE, Kamal MA (2020) COVID-19 outbreak and emerging management through pharmaceutical therapeutic strategy. Curr Pharm Des 26(41):5224–5240. https://doi.org/10.2174/18162826666200713174140

Rahman MH, Akter R, Kamal MA (2021) Prospective function of different antioxidant containing natural products in the treatment of neurodegenerative diseases. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders). 20(8):694–703. https://doi.org/10.2174/1871257319666200721253611

Sakamoto S, Putulan W, Vimolmangkang S et al. (2018) Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. J Nat Med 72:32–42. https://doi.org/10.1007/s11356-017-1144-z

Saylor J, Mehta V, Choc LH et al. (2020) Rapid development and validation of a novel laboratory-derived test for the detection of SARS-CoV-2. Delaware J Public Health 6:10–15. https://doi.org/10.32481/djph.2020.07.004

Sharma S, Batra S, Gupta S, Sharma V, Rahman M, Kamal M (2022) Persons with co-existing neurological disorders: risk consideration, analysis and management in COVID-19 pandemic. https://doi.org/10.2174/1871257320666210308113457

Srivastava S, Jain V, Nag VL et al. (2020) Current avenues for COVID-19 serology. Ann Natl Acad Med Sci (India) 56:087–090. https://doi.org/10.1055/s-0040-1713709

Shah SA, Bungau S, Si Y, Xu H, Rahman M, Behl T, Gitea D, Pavel FM, Corb Aron RA, Pasca B, Nemet S (2020) Chemically diverse and biologically active secondary metabolites from marine phylum chlorophyta. Mar Drugs 18(10):493

Tablat A, Ardebelli A (2020) Real-time RT-PCR in COVID-19 detection: issues affecting the results. Expert Rev Mol Diagn 20:453–454. https://doi.org/10.1080/14737559.2020.1757437

Tagde P, Tagde S, Tagde P, Bhattacharyya T, Monzur SR, Rahman M, Otrisal P, Behl T, Abdel-Daim MM, Aleya L, Bungau S (2021a) Nutraceuticals and herbs in reducing the risk and improving the treatment of COVID-19 by targeting SARS-CoV-2. Biomedicines 9(9):1266

Tagde P, Tagde S, Bhattacharyya T, Tagde P, Chopra H, Akter R, Kausik D, Rahman M (2021b) Blockchain and artificial intelligence technology in e-Health. Environ Sci Pollut Res 28(38):52810–52831. https://doi.org/10.1007/s11356-021-16223-0

Tombuloglu H, Sabit H, Al-Khallaf H, Kabanja JH, Alsaeeed M, Al-Saleh N, Al-Suhaime E (2022) Multiplex real-time RT-PCR method for the diagnosis of SARS-CoV-2 by targeting viral N, RdRp and human RP genes. Sci Rep 12(1):1. https://doi.org/10.1038/s41598-022-06977-z

Vidal AMB, Catapani WR (2005) Enzyme-linked immunosorbent assay (ELISA) immunoassaying versus microscopy: advantages and drawbacks for diagnosing giardiasis. Sao Paulo Med J 123:282–285. https://doi.org/10.1590/S1516-31802005000600006

Wang D, He S, Wang X, Yan Y, Liu J, Wu S, Liu S, Lei Y, Chen M, Li L, Zhang J (2020) Rapid lateral flow immunoassay for the detection of human SARS-CoV-2 antibodies. ACS Sensors 6:703–708. https://doi.org/10.1021/acssensors.0c02544

Hossain M, Hasan A, Mumau AA, Uddin M, Wahed MI, Sarker S, Behl T, Ullah I, Begum Y, Bulbuli JI, Amran M (2020) COVID-19 outbreak: pathogenesis, current therapies, and potentials for future management. Front Pharmacol, 1590. https://doi.org/10.3389/fphar.2020.563478
fluorescence detection of SARS-CoV-2 RNA. Nat Biomed Eng 4(12):1150–1158
Wong R, Tse H (eds) (2009) Lateral flow immunoassay. Humana Press, Totowa, NJ
World Health Organization (2020a) Advice on the use of point-of-care immunodiagnostic tests for COVID-19. In: World Health Organization
World Health Organization (2020b) Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance. In: World Health Organization
Wu J-L, Tseng W-P, Lin C-H et al (2020) Four point-of-care lateral flow immunoassays for diagnosis of COVID-19 and for assessing dynamics of antibody responses to SARS-CoV-2. J Infect 81:435–442. https://doi.org/10.1016/j.jinf.2020.06.023
Yang T, Gentile M, Shen C-F, Cheng C-M (2020) Combining point-of-care diagnostics and Internet of Medical Things (IoMT) to combat the COVID-19 pandemic. Diagnostics 10:224. https://doi.org/10.3390/diagnostics10040224
Zhang F (2019) Development of CRISPR-Cas systems for genome editing and beyond. Q Rev Biophys 52:e6. https://doi.org/10.1017/S0033583519000052

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Md. Maniruzzaman 1 · Md. Missile Islam 2 · Md. Hazrat Ali 3 · Nobendu Mukerjee 4,5 · Swastika Maitra 6 · Mohammad Amjad Kamal 7,8,9,10 · Arabinda Ghosh 11 · Melvin A. Castrosanto 12 · Athanasios Alexiou 13,14 · Ghulam Md. Ashraf 15,16 · Priti Tagde 17 · Md. Habibur Rahman 18

1 Department of Pharmacy, Faculty of Science & Engineering, Varendra University, Rajshahi 6204, Bangladesh
2 Varendra University, Rajshahi 6204, Bangladesh
3 Department of Pharmacy, Faculty of Science & Engineering, International Islamic University Chittagong, Sitakundu, Chittagong 4318, Bangladesh
4 Department of Microbiology, Ramakrishna Mission Vivekananda Centenary College, West Bengal, Kolkata 700118, India
5 Department of Health Sciences, Novel Global Community Educational Foundation, Hebersham, NSW, Australia
6 Department of Microbiology, Adamas University, Kolkata, India
7 West China School of Nursing/Institutes for Systems Genetics, Frontiers Science Center for Disease-Related Molecular Network, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China
8 King Fahd Medical Research Center, King Abdulaziz University, P. O. Box 80216, Jeddah 21589, Saudi Arabia
9 Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka, Bangladesh
10 Enzymoics, Novel Global Community Educational Foundation, Hebersham, NSW, Australia
11 Microbiology Division, Department of Botany, Gauhati University, Guwahati 781014, Assam, India
12 Institute of Chemistry, University of the Philippines, 4030 Los Banos, Philippines
13 Department of Science and Engineering, Novel Global Community Educational Foundation, Hebersham, NSW, Australia
14 AFNP Med, Vienna, Austria
15 Pre-Clinical Research Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Saudi Arabia
16 Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia
17 Amity Institute of Pharmacy, Amity University, Noida 201301, India
18 Department of Global Medical Science, Wonju College of Medicine, Yonsei University, Gangwon-do, Wonju 26426, Korea