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Biosensors-based approaches for other viral infection detection

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21.1 Introduction

Contamination and infection due to viruses are one of the major reasons for diseases amounting to hundreds of thousands of deaths every year (Alvarez et al., 2017). In recent years, several novel and reemerging viral diseases have seen an outstanding rise in occurrence like coronavirus disease 2019 (COVID-19) which has arguably altered the socio-economic strata and also pointed out the shortcomings in medical biology and diagnostics at a global scale (Blumenthal et al., 2020). The overwhelming burden of global infection numbers exceeded expectations from federal healthcare programs and medical facilities (Hamid et al., 2020). The current situation pointed out that the cost of reliable tests is disproportionately high in case of frequent repetition of tests on one person. Also, the speed of distribution, implementation, and evaluation of tests are unsatisfactory in the current situation, both technically and logistically. Viruses are nonliving obligate parasites and require living host cells to replicate and propagate (Tong & Revill 2016). Due to their error-prone replication mechanism and fast mutations, the protective layers and mechanisms change quickly, thus helping them to evade immune reactions in the host cell (Smith, 2017). This has led to reemergence and also establishing new virus strains. Medicinal biology and conventional diagnostic practices are now facing a challenging task to upkeep with the pace of these occurrences. Therefore to improve the quality of detection and to help in the reduction of cost, major advancements are required in the detection techniques to better prepare the healthcare system for a similar novel virus infection in the future.

One of the crucial elements in the point-of-care diagnosis and recovery from pathogenic diseases is early detection. Conventional diagnostic approaches for virus detection include virus isolation and screening from clinical samples, polymerase chain reaction (PCR)-based assays (Mackay et al., 2002) and enzyme-linked immunosorbent assays (ELISA) (Torane & Shastri 2008), and immunofluorescence assays (Kiptoo et al., 2004), etc. On the other hand, several techniques are useful for the quantification of viruses measuring viral infectivity (such as plaque assay, TCID₅₀), quantifying viral genetic material or protein (qPCR, western blotting) (Gueudin et al., 2012; He et al., 2004) or directly counting viral load using flow cytometry (Zamora & Aguilar 2018). Despite the good accuracy, these methods are time-consuming, labor-intensive, and arguably less-
economical in terms of storage and handling of the required chemical. Also, it is imperative to mention that the conventional diagnostics practices are unfeasible to perform on-site due to the requirement of specialized machines and suitable laboratory environments. To reduce the challenges, biosensor-based approaches have been studied extensively for fast detection and provide an excellent opportunity for on-site diagnosis with less labor and time.

Advancement in interdisciplinary biological research combined with the knowledge of nanomaterial chemistry contributed critically to the development of biosensors. A biosensor is an analytical device with physiochemical detecting properties that enable it to detect analytes of interest which usually possess biological importance (Dincer et al., 2019). Biosensors can convert the biological response into an optical or electrochemical measurable signal through which the presence or the concentration of a specific analyte can be detected and calculated (Castillo et al., 2004). Presently, biosensors are used in a wide range of clinically significant biomedical diagnostics due to fast, reliable, point-of-care detection abilities with real-time monitoring of treatment and disease progression, environmental monitoring, food control, forensic and biomedical research (Hsu et al., 2008; Lee et al., 2012; Luo et al., 2009). For the development and fabrication of biosensors, transducer materials are incorporated into the sensing platform which can detect the analyte using optical, or electrochemical techniques by measuring the generated current, light, or frequent signal. The biosensor functions through the interaction of a specific analyte with the immobilized bioreceptor including enzymes, peptides, oligonucleotides, DNA to generate a measurable signal. The strategy to modify biosensors is a suitable diagnostic approach not only for known viruses but also can be used for a new or highly divergent family of viruses.

The virus titer in the early phases of a productive infection is usually low or challenging to be efficiently detected by microscopy-based detections. PCR-based detection techniques are aimed at the detection of viral genetic material that requires additional steps of sample preparation. The phenotypic traits exhibited by the biological macromolecules on the surface of pathogens are excellent markers for the detection by a biosensor using an appropriate bioreceptor. By targeting the structural surface proteins or the internally expressed functional proteins, biosensors have been developed for fast and easier diagnosis. Using appropriate recognition molecules or bioreceptor, miniaturized biosensors are capable of detecting both viral proteins and genetic material at very low concentrations (femtomolar or nanomolar), which gives an edge over conventional detection techniques. In the last decades, tremendous achievements have been recorded in terms of associating novel nanoparticles to design biosensors for several medically important viruses (Bai & Spivak 2014; Birnbaumer et al., 2009; Feng et al., 2018; Inan et al., 2017; Jin et al., 2018; Prabowo et al., 2017; Riedel et al., 2014). In this chapter, various strategies of development and advancements in the field of biosensors to detect a few viruses are discussed based on the latest research findings. Finally, the scopes and future perspectives of the biosensor research are assessed to understand the current situation and its shortcomings.

21.2 Appropriate target sites for the detection of viruses

21.2.1 Surface protein

Viruses are nonliving biological entities with a protective membrane called capsid or nucleocapsid (Fig. 21.1). It helps to protect the viral core with genetic material from physical stress. Some
viruses develop an outer layer called an envelope composed of specialized envelope proteins. Both capsid and envelope exhibit a range of glycoproteins in form of receptors or ligands which are excellent molecular signatures for the specificity of the individual virus. The structural and physical characteristics of the viral capsid are extremely important and relevant to establishing infection and propagation. Conventionally the viral infection of a target cell is dependent on the interaction between viral spike protein and the specific host cell receptor. A peplomer or spike glycoprotein protrudes from the viral envelope or capsid has been studied extensively. Spike proteins are crucial for viral infectivity and host cell specificity. For example, the influenza virus has two types of spike proteins, (1) hemagglutinin, triangular and spike-shaped, and (2) neuraminidase, mushroom-shaped. The interacting domain of the spike protein makes it an excellent analyte for the detection of a specific virus. Along with spike proteins, several other transmembrane surface antigens have been also used as an analyte, such as hepatitis B virus surface antigen (Walters et al., 2020), glycoprotein 41 (gp41), and glycoprotein 120 (gp120) in HIV (Lifson et al., 2016), etc.
21.2.2 Viral protein

Viruses are nonliving entities and thus all kinds of viruses require a host for replication. After the introduction of viral genetic material into the host cell, they either use their proteins/enzymes or also use host cell machinery to synthesize viral protein and to complete packaging new viral particles. Along with several structural proteins, both transmembrane and surface, many regulatory proteins are also used as target analytes to detect a virus in biological samples, one of the most important such viral proteins is RNA-dependent RNA polymerase (RdRp). This protein can be a potential target to develop a biosensor to detect HIV and DENV and also be used for the diagnosis of SARS-CoV-2 under laboratory conditions (Chan et al., 2020; Qiu et al., 2020; Won et al., 2020). Targeting the viral proteins can add another challenge as oftentimes viral proteins from viruses of the same family are evolutionary conserved. As a result, the structural domains of these conserved proteins are similar as seen in crystallographic studies. Attempt to identify conserved protein to conclude the virus type can result in false positives due to cross-react.

21.2.3 Genetic material

Viral DNA or RNA consists of various sequences of evolutionarily conserved domains that have been used in sequencing to distinguish a specific strain. Single-stranded artificial nucleotides in the form of DNA or RNA possess outstanding ability to detect and bind to specific targets. These short genetic materials are about 10–100 nucleotides long and the targets include wide ranges of amino acids (Ellington & Szostak 1992), proteins, pathogens (Torres-Chavolla & Alocilja 2009), and mammalian cells. Aptamer-based biosensors have been generated in which the hybridization takes place between the receptor material and a specific domain in the viral genome. Some of the gene targeting aptamer-based approaches have been discussed in the following chapters in detail.

21.3 Importance of biosensor in the diagnostics

Advancement in the current understanding of nanotechnology has pushed the innovation of new nanomaterials with various special properties such as magnetic, electrical, etc. Fabrication of nanostructures and surface modifications have helped to develop small-sized biosensors with efficiency to detect the significantly low amount of analytes. The requirement of advanced diagnostics such as fast detection and enhancement in specificity, selectivity, and being profitable has opened opportunities for biosensors to be studied and used for the detection of biological analytes. Integration of new recognition materials and nanoparticles with various physicochemical properties has increased the feasibility and analytical figures of the merits of the biosensors. Improvement in analytical performances has made biosensors approachable and appropriate for the point of care devices for the fast detection of viruses using a specific recognition module. Different types of biosensors for medical uses exist today. Based on the mechanism and outcome they are classified as optical, electrochemical, piezoelectric, magnetic, thermal, etc. Advantages of biosensors are fast detection ability, the requirement of analyte in a lesser amount, and less labor such that they can be used by the end-user. Questions also remain at large regarding reproducibility and sensitivity as the research and integration of biosensors in real-time diagnostics are still in the early stages.
21.4 Detection of mammalian viruses by a biosensor

21.4.1 Dengue

Dengue fever is a mosquito-borne viral infection caused by the dengue virus (DENV) consisting of a positive sense single-strand RNA. High-temperature fever, rash, vomiting, and nausea are some of the major symptoms of dengue infection, which start to appear approximately 3–12 days after the infection. Clinically, there are four serotypes of DENV (1, 2, 3, and 4). While DENV infection generally causes mild asymptomatic illness, severe dengue can lead to shock syndrome and death. There is a lack of specific treatment for DENV infection, but early diagnosis and access to proper medical attention reduce the risk and fatality rate to below 1% in case of severe dengue.

Conventional methods for the detection of DENV in a biological sample are virus isolation, qPCR, ELISA test using DENV-specific antibodies. While these tests produce reliable results, major limitations are also present involving logistics and several steps for sample preparation that are not feasible to be done on-site rapidly. Several biosensors have been recently developed for the rapid detection of DENV. Targeting the DENV genetic material as the analyte, a genosensor was developed using palladium and platinum with zinc nanoparticles as the matrix for having higher catalytic efficiency and a large surface area (Singhal et al., 2017). A complementary sequence to a conserved region in the genome of all four serotypes of DENV was used as a receptor to detect the virus. Another aspect of developing a reliable biosensor is to target nonstructural protein 1 (NS1) of DENV. Detection of NS1 is particularly beneficial as to report the infection at early stages in both mosquito and the host. Detection of the virus from the vector involves several time- and labor-consuming steps requiring specific expertise and expensive instruments. To facilitate this challenge, developing a biosensor to detect DENV NS1 is appropriate. NS1 is a well-studied viral protein present in patient sera and is an excellent biomarker for the selection of analytes for biosensor development. To demonstrate a surveillance method an immunosensor was developed to measure the amount of NS1 protein present in the adult mosquito homogenate (Sylvestre et al., 2014). Anti-NS1 monoclonal antibodies were functionalized on the single-wall carbon nanotube and immobilized on the gold microelectrode. Detection of NS1 protein in the sample by the antibody produced an electrochemical signal to confirm detection. The design of this biosensor is beneficial in terms of cost-effectiveness and flexibility to be adjusted for other similar analytes. Affinity peptides have also been designed and chosen to detect NS1 in the clinical sample (Kim et al., 2019). This peptide was covalently modified on the gold electrode surface and showed good recognition capability with free NS1 on the sample (Fig. 21.2).

21.4.2 Human immunodeficiency virus

Human immunodeficiency virus (HIV) is a retrovirus and belongs to a subset of the family called lentiviruses. It has a viral RNA that gets retro-transcribed into viral DNA using host cell machinery. Lentiviruses are conventionally slow in establishing the infection and the final stage of it is known as acquired immunodeficiency syndrome (AIDS) which is still one of the outstanding challenges remaining for biology and medicine. HIV primarily infects CD4+ T cells to start replication, after which it can stay in a dormant phase for many years. Early detection of HIV can be life-saving and thus there is a requirement for rapid and specific biosensors. HIV-1 serotype is the most common
form to establish infection. Hybridization techniques have been used to prepare an effective biosensor for virus detection. Using aptamer selective to HIV gene a molecularly imprinted polymer (MIP) electrochemiluminescence biosensor was developed for the specific detection (Babamiri et al., 2018). Europium sulfide nanocrystals are strong agents for chemiluminescence. The gene-specific aptamer was used as a template to detect HIV genes. This strategy demonstrated the ability
to target HIV genetic material as an analyte, which can be detected rapidly with similar biosensing platforms.

The HIV genomic transcript is 9.2 kb long in the unspliced form and codes for nine major proteins including structural polyproteins and regulatory proteins. Benefiting from the excessive study and data available for HIV proteins, targeting the interacting domains is a feasible idea for developing a biosensor for the detection of the virus. Gp41 is a well-studied transmembrane envelope protein in HIV-I and is crucial for the fusion of the virus into the host cell (Soumajit et al., 2021; Weiss, 2003). Quantification of gp41 signal has been previously correlated to the infection progression level and to assess the efficacy of treatment. Using a structural epitope or immunodominant region of the gp41 as an analyte, a biosensor exhibited efficient detection of the protein from the sample (Lu et al., 2012). The imprinting of the gp41 epitope on the quartz crystal microbalance chip showed great affinity toward binding the protein specifically. Using a label-free optical sensing platform employed with nanostructured photonic crystals to capture intact virus, detection and quantification have been possible from biological samples (Shafiee et al., 2014). The adsorption and binding of the virus on the biosensor surface induced a shift in the resonant peak wavelength value which was further measured to quantify the virus (Fig. 21.3).

21.4.3 Zika

Zika is a mosquito-borne virus (ZIKV) from the Flavivirus family that was initially identified in monkeys and later in humans in 1952. ZIKV infections have been majorly identified in Africa, Asia, and the Americas in past years. The infection generally causes mild disease and also severe conditions if not treated properly leading to hospitalization. Long-term ZIKV infection and also during pregnancy cause serious birth defects such as microcephaly and improper brain formation in the fetus. ZIKV infection has also been associated with Guillain-Barre syndrome in adults where the immune system attacks and damages nerve cells. Many people with ZIKV infection exhibit no or only mild symptoms. Conventionally existence of ZIKV in blood or urine is tested by detecting the presence of viral RNA or by testing with IgM antibody, which is needless to mention time-consuming and not cost-effective. There is no specific treatment still available for the ZIKV infection. Therefore, it is imperative to say that the need for rapid and highly sensitive detection of ZIKV on-site by advanced biosensing platforms is high. Several biosensing platforms have been prepared to target the ZIKV proteins as analytes. Targeting nonstructural protein 1 (ns1) and domain III of the envelope protein (EDIII) a biosensor was demonstrated to simultaneously detect antigens of ZIKV (Cabral-Miranda et al., 2018). As a receptor, a chimeric recombinant protein was used composed of ns1 and EDIII to avoid cross-reactivity to DENV. Antibodies placed on the conductive carbon surface facilitated the detection of antigens to finally quantify the viral load. Regular and controlled surveillance of ZIKV circulation in mosquitoes in infected areas is a critical strategy to keep the risks of infection spread at bay. Due to the presence of a characteristic electrostatic surface at the C-terminal, ns1 of the ZIKV can be distinguished among other flaviviruses (Song et al., 2016). This specific characteristic has helped to produce antibodies specific to ZIKV ns1 to avoid false positives arising from cross-reactivity. A graphene-based biosensor was prepared with this anti-ns1 antibody functionalized on the surface to specifically detect the ZIKV. Recombinant ZIKV ns1 was used as the target to generate measurable response and this setup successfully demonstrated fast and specific detection of the virus. When tested against other
flaviviruses, the biosensor failed to generate a signal as expected. Biosensing platforms like this can be very useful to execute controlled surveillance for the virus circulation in vectors as shown in other viruses like the Japanese encephalitis virus.

CRISPR/Cas9 system was discovered as a bacterial defense mechanism to identify and degrade foreign genetic material. An optical geno-biosensor was developed using the CRISPR/Cas9 system to specifically bind ZIKV RNA (Pardee et al., 2016). Being a paper-based cell-free system, the use of this biosensor in real-time on-site diagnostics is beneficial. The specificity of this platform is very high as the specific hybridization between the biosensor recognition material with viral RNA
based on CISPR/Cas9 system can detect a mismatch of nucleotide sequence from other viruses, as reported against DENV RNA. The mechanism of action of this platform depends on the hybridization of the ZIKV RNA which in turn regulates the synthesis of chromogenic substances as a measurable signal. When tested with DENV RNA, the amplification of RNA was not enough to generate a satisfactory signal demonstrating the specificity of the biosensor towards the detection of ZIKV RNA.

21.4.4 Hepatitis

Hepatitis is an inflammation of the liver caused by a family of viruses. Predominantly five types of hepatitis viruses have been studied and are in circulations, namely A, B, C, D, and E. Among them, hepatitis B infection is the most notorious and causes approximately 800,000 deaths worldwide due to liver cirrhosis and malignancy. Hepatitis B virus surface antigen (HBsAg) is a biomarker for acute hepatitis B infection found in serological tests. Using commercially available anti-HBsAg functionalized on microelectrode and recombinant HBsAg generated in Pichia pastoris as recognition element, a surface plasmon resonance-based biosensing platform demonstrated efficiency for the rapid detection of hepatitis B virus (Tam et al., 2017). Gold nanoparticles have also been used in the biosensor for the sequence-specific hybridization of viral DNA (Hassen et al., 2008). Using magnetic nanoparticle in an impedimetric biosensor simultaneous detection of hepatitis B virus and HIV genomes were achieved. Genome-specific DNA probes were modified with biotin and further functionalized on to streptavidin-coated magnetic nanoparticles for the specific and sensitive detection of viral presence. Using specific antibodies modified on to microelectrode surface an impedimetric biosensing platform demonstrated efficiency to detect the hepatitis E virus from the sample. This pulse-triggered electrochemical biosensor was fabricated with gold-embedded polyaniline nanowires to increase the catalytic and electrical ability. The attachment of the virus to the matrix introduced an external electrical pulse which was then measured to confirm the specific detection of hepatitis E virus from the samples.

21.4.5 Influenza virus

Influenza is a viral disease that causes a pandemic every year resulting in tremendous medical and financial burden. Based on the antigenic variants among Matrix (M) protein and Nucleocapsid (N) protein, the virus is categorized into three groups. Conventional detection methods lack sensitivity and are time-consuming, while rapid antigen detection test is used regularly. Real-time polymerase chain reaction (RT-PCR) is oftentimes used for detection, but like other regular techniques, it is expensive and time-consuming. These shortcomings led to the fabrication and development of biosensors for the specific detection of the influenza virus.

In a notable work, matrix protein 2 was targeted for the selective detection of the influenza A virus H9N2 subtype (Bai & Spivak 2014; Birnbaumer et al., 2009; Feng et al., 2018; Inan et al., 2017; Jin et al., 2018; Prabowo et al., 2017; Riedel et al., 2014). Antimatrix protein 2 antibodies were used along with magnetic nanoparticles to isolate the virus from the sample. In another effort, a nanosensor was developed with a DNA probe functionalized on the biosensor to bind to the genetic material of the influenza virus (Tam et al., 2017). The resulting hybridization of the DNA probe and viral DNA changed the conductance of the electrode surface which was correlated with
the amount of the virus in the sample. An optical fluorescence biosensor was prepared to detect recombinant hemagglutinin protein of the H5N1 influenza virus in human serum (Pang et al., 2015). This immunosensor was tested for the detection of the protein in aqueous buffer and human serum where the detection limit was 2 and 3.5 ng/mL, respectively. A surface plasmon resonance-based biosensor was developed to detect avian influenza virus H5N1 in poultry swab samples (Bai et al., 2012). The fabricated biosensor used an aptamer for virus detection in the sample solution (Fig. 21.4) (Table 21.1).

![Principle of SPR biosensor for detection of AIV H5N1. (A) Streptavidin immobilization; (B) Biotinylated aptamer immobilization; (C) Virus detection.](https://doi.org/10.3390/s120912506)

**FIGURE 21.4**

Principle of SPR biosensor for detection of AIV H5N1. (A) Streptavidin immobilization; (B) Biotinylated aptamer immobilization; (C) Virus detection.

*From Bai, H., Wang, R., Hargis, B., Lu, H., & Li, Y. (2012). A SPR aptasensor for detection of avian influenza virus H5N1. Sensors (Switzerland), 12(9), 12506–12518. https://doi.org/10.3390/s120912506.*

| Type of biosensor | Virus species                      | Recognition element                      | Reference               |
|-------------------|-----------------------------------|------------------------------------------|-------------------------|
| Optical           | Human adenovirus                  | Primer DNA                               | Jin et al. (2018)       |
| Optical           | Enterovirus 71                    | Major capsid protein VP1                 | Prabowo et al. (2017)   |
| Optical           | Epstein-Barr virus                | Oligonucleotide antigen                  | Riedel et al. (2014)    |
| Electrochemical   | Human rhinovirus serotype 2       | Molecularly imprinted specific polymer   | Birnbaum et al. (2009)  |
| Optical           | Japanese encephalitis virus       | Screen printed polymer                   | Feng et al. (2018)      |
| Optical           | Human papilloma virus 16 E7       | Antihuman papilloma virus 16 E7          | Inan et al. (2017)      |
| Optical           | Apple-stem pitting virus          | Aptamer                                  | Bai and Spivak (2014)   |
21.5 Importance and significance of biological analytes

Immunosensor or genosensors are remarkable devices that bring so much potential to the platform of advanced diagnostics. Viral surface and internal proteins generally exhibit evolutionarily conserved domains, which makes it easier to distinguish between families or species of viruses. Domain-specific antibody or aptamer makes the detection by biosensors advantageous in increasing the specificity and distinguishing between different viruses. Although the biosensor platform offers rapid detection with the requirement of a low amount of analyte, also it has some limitations. One difficulty involving aptamer technologies is that the aptamers are developed and screened under certain in silico parameters which do not always exactly replicate the conditions of complex clinical samples, so the structure, function, binding affinity, and specificity of aptamer could be changed in clinical samples. The fabricated biosensor faces interferences from nonspecific proteins and other metabolites, ions, amino acids present during the detection of analyte from clinical samples, which is a critical challenge for the specificity of the biomarker. Because of the overwhelming complexity of the biological samples, pretreatment is often required to concentrate the analyte and to reduce interferences thus minimizing false positives. Although the integration of electrochemically active nanoparticles into the sensing platform has greatly increased the sensitivity of the sensor, often times the detection of a specific virus is challenging due to the low titer of virus present in the sample. In this scenario, detection of a virus using a viral surface protein as an analyte is quite challenging. On the other hand, biosensors based on the recognition of viral internal protein or genetic material require an extra step of pretreatment of the sample to open up viral envelope or capsid by chemical or physical methods to provide access. When designing specific probes, researchers also have to count the possibility of nonspecific bindings of protein or RNA. Another major disadvantage of analytes can be strain-specificity. Oftentimes the structural domains of a particular protein can be different in various strains of a single virus, the domains in the genetic material as well. During the analysis and choosing the right probe, it is critical to study evolutionarily conserved domains to minimize the false-positive detection signals by the biosensor platform. To overcome the critical challenges and to be integrated into the real diagnostics, the new age biosensors must exhibit, (1) high sensitivity and selectivity with faster result production, (2) easy modification and excellent long life, (3) requirement of minimal sample and less labor, (4) excellent reproducibility, (5) simple interface for usage at hospitals and clinics, (6) miniaturization and easy portability, and (7) minimal costing.

21.6 Future scopes

The most effective and reliable way to detect viral particles in a clinical sample is quantitative real-time polymerase chain reaction (qRT-PCR. But it is also expensive, time-consuming, and requires a special machine and expertise for the detection. With the advancement in nanotechnology, new exciting options are now open to the use of fast and specific detection of microbes by miniaturized biosensors in real-time diagnostics. Compared to conventional detection techniques, the reported biosensors showed excellent ability to detect the microbe in the clinical sample efficiently in laboratory testing. But the real challenge remains. The development of biosensors is a critical process...
and hence the commercial production of them will require bulk nanomaterials. While the sensitivity of the platform relies upon the quality of the nanomaterials used, it is challenging to regulate and keep the same quality in different batches. The reported biosensors for different viruses are in the right direction of using viral components efficiently as an analyte. By solving the remaining challenges, it is not a far-fetched reality anymore to have biosensors in the real use of diagnostics.

**Abbreviations**

| Acronym | Description |
|---------|-------------|
| AIDS    | Acquired immuno deficiency syndrome |
| CRISPR  | Clustered Regularly Interspaced Short Palindromic Repeats |
| DENV    | Dengue virus |
| DNA     | Deoxyribonucleic acid |
| ELISA   | Enzyme-linked immunosorbent assay |
| HIV     | Human immunodeficiency virus |
| PCR     | Polymerase chain reaction |
| qPCR    | Quantitative polymerase chain reaction |
| qRT-PCR | Quantitative real-time polymerase chain reaction |
| RdRp    | RNA-dependent RNA polymerase |
| RNA     | Ribonucleic acid |
| RT-PCR  | Real-time polymerase chain reaction |
| TCID    | Tissue culture infectious dose |

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