REVIEW

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CRISPR AS A NOVEL TECHNIQUE FOR COVID-19 DIAGNOSIS: A REVIEW

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To this moment, the human coronavirus disease COVID-19 that occurs as a result of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection is still a critical case that provokes concern around the world. In January 2022, there were over 300 million infections and over 5 million fatalities from COVID-19. As a countermeasure against this rapid spread, there is a vital need for effective and low-cost diagnosis methods in order to control the danger of this pandemic. CRISPR technology has proved its efficiency in detecting COVID-19 due to its simplicity, specificity and high sensitivity. This paper reviews the state-of-the-art of developing the CRISPR platforms for the purpose of COVID-19 diagnosis and treatment. Limitations and challenges of CRISPR in terms of nucleic acid analytical methods for viral infection diagnosis are discussed.

K e y w o r d s: COVID-19, CRISPR, SARS-CoV-2, Cas protein, RT-PCR.

The new strain of coronavirus is SARS-CoV-2, which has caused the COVID-19 outbreak first in Wuhan, China, and has caused a real worldwide health issue [1, 2]. Accordingly, the World Health Organization (WHO) stated on March 11, 2020, that COVID-19 has become a pandemic. As of January 2022, over 300 million individuals were infected and over 5 million individuals died from this virus [3]. Comparatively, this virus is more contagious and transmittable compared to SARS-CoV-1 and MERS-CoV [4]. SARS-CoV-2 had a 95% similarity to bat-CoV and a 70% homology with SARS-CoV-1 [5]. Additionally, SARS-CoV-2 is mostly related to lung illness and may infect other organs in the body, such as the liver and kidney [1].

Early detection of a viral infection could enable us to reduce the chance of the disease spreading to others. Accordingly, there are many technologies for the detection of viral infection. Presently, the most widely used technology is PCR, and COVID-19 detection kits largely depend on real-time PCR [6]. On the other hand, modern technology, such as CRISPR, provides a glimmer of hope not just for tackling COVID but also as a weapon against unknown future pandemics [7].

The discovery and development of the CRISPR system in recent years has provided a new path of research into diagnostic techniques [8]. CRISPR was discovered in 1987, when a group of researchers found a unique repeating DNA sequence in *Escherichia coli* [9].

CRISPR is considered the third generation of gene editing tools, and it has been developed from a natural defense mechanism that helps bacteria fight viral invasion. Further, the CRISPR system is divided into two classes and six types, with more than 19 subtypes and many associated proteins [10]. There are many CRISPR platforms that have been developed in order to combat COVID-19, such as SHERLOCK and DETECTR [7].

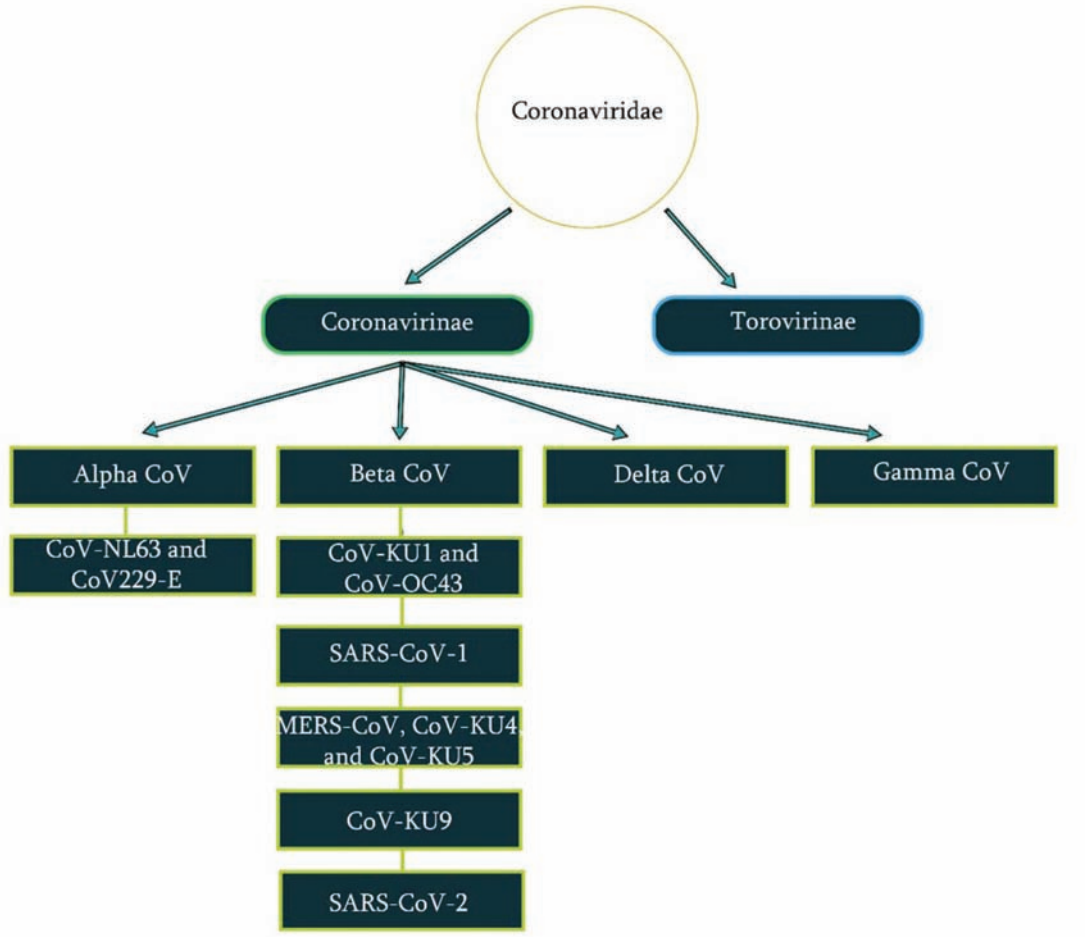

Fig. 1. The Coronaviridae family's typology

Background of Coronaviruses

The Coronaviridae family includes single-stranded RNA, envelop, and large viruses. They are classified as the biggest RNA viruses recognized, with a genome size of 25-32 kb and a 118-136 nm diameter of the virion. The Coronavirinae and Torovirinae are two subfamilies of the Coronaviridae family [11]. The Coronavirinae subfamily is divided into four coronavirus genera based on genetic and immunological characteristics (Alpha, Beta, Delta, and Gamma). While Alpha and Beta-coronaviruses infect mammals, Delta and Gamma-coronaviruses mainly infect birds [12, 1].

The coronaviruses (CoVs) are positive sense, enveloped viruses with a spherical body covered in spikes (Fig. 1). The name "corona" refers to the shape of the virion under electron microscopy, in which spike extensions from the virus envelope provide the impression of a crown, or corona in Latin [13].

The early two human CoVs, CoV-OC43 and CoV-229E, have been recognized since the mid-

Abbreviations: COVID-19 – coronavirus disease 2019; SARS-CoV-1-CoV-2 – Severe acute respiratory syndrome coronavirus 2; S – spike; N – nucleocapsid; E – envelope; M – membrane; ORF1ab – open reading frame 1ab; RT-qPCR – reverse transcription quantitative polymerase chain reaction; CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats; Cas – CRISPR-associated protein; crRNA – CRISPR-derived RNA; gRNA – guide RNA; dCas9 – dead CRISPR-associated protein 9; RT–LAMP – reverse transcription loop-mediated isothermal amplification; dsDNA – double-stranded DNA; ssDNA – single-stranded DNA; RdRp – RNA dependent RNA polymerase; RPA – recombinase polymerase amplification; hACE2 – human angiotensin-converting enzyme II; PAM – protospacer adjacent motif; RT-RAA – reverse transcript recombinase aided amplification; PFS – protospacer flanking site; pre-crRNA – Precursor CRISPR-RNA; DSB – double-strand break.

1960s [14]. At present, there are seven known coronaviruses that can infect humans [1]. Two alpha-CoVs (CoV-NL63 and CoV-229E) and five beta-CoVs (CoV-KU1, CoV-OC43, SARS-CoV-1, MERS-CoV, and SARS-CoV-2) belong to the CoV family [15]. However, people with low-immunity are more susceptible to the common cold signs caused by four viruses: CoV-229E, CoV-OC43, CoV-NL63, and CoV-KU1 [16]. Other strains (SARS-CoV-1 and MERS-CoV) are of animal origin and have been associated with occasional deadly diseases in animals [17]. Fig. 1 shows the Coronaviridae family's typology.

The genomic structure of Coronaviruses

Coronaviruses have the biggest genomes of any identified RNA viruses (26.4-31.7 kb), with a G/C ratio ranging from 32 to 43 percent. Thus, due to its huge genome, this virus family has more flexibility in terms of accepting and editing genes [18]. However, tree genetic analysis of SARS-CoV-2 showed that SARS-CoV-2 is a member of the Beta-coronavirus genus that comprises Bat SARS-like-CoV, SARS-CoV-1, and MERS-CoV [19]. Additionally, there are about 380 amino acid replacements among SARS-CoV-2 and SARS-like-CoVs, essentially centered on non-structural protein genes, and 27 mutations in genes encoding the viral S protein [20]. The genome of SARS-CoV-2 has 14 open reading frames (ORFs) in its genetic material that code for 27 proteins [21]. At the 5' end of the genome, ORF1a and ORF1ab encode accordingly the PP1a and PP1ab polypeptides. Subsequently, PP1a and PP1b break down into 11 and 16 proteins, cascadingly. On the other hand, the 3' end of the genome encodes four proteins (Nucleocapsid, Membrane, Envelope, and Spike) as well as eight accessory proteins [22]. Furthermore, these proteins play an important role in genome stability and virus reproduction [23]. Fig. 2 shows the structure of the SARS-CoV-2 virus.

Origin of SARS-CoV-2

There are many theories about the origin of SARS-CoV-2. First, biological evolution drove the virus to its present virulent form in a non-human carrier before jumping into humans. As a result of

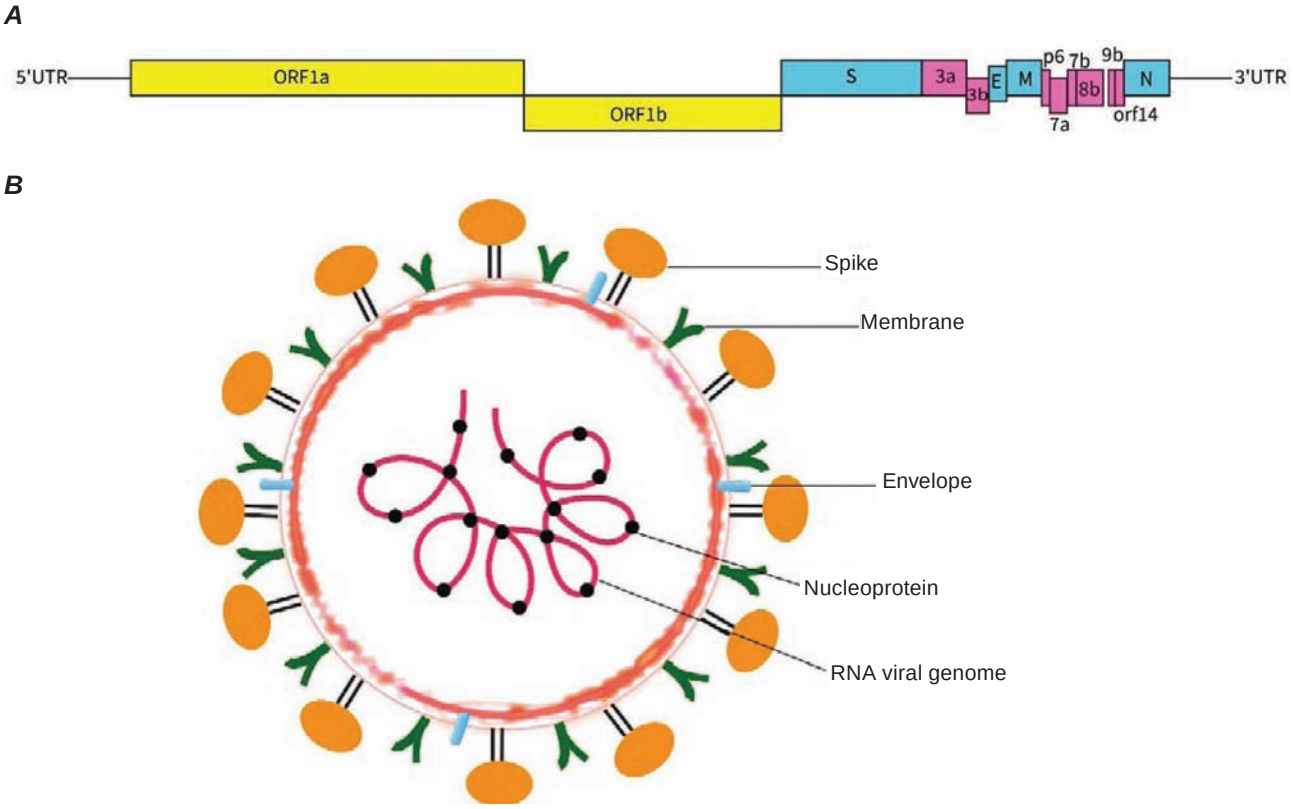

Fig. 2. ***A*** *– The structure of SARS-CoV-2. Yellow represents ORF1a and ORF1b. Blue represents structure proteins (S, E, M, and N). Pink represents accessory proteins (3a, 3b, p6, 7a, 7b, 8b,9b, and orf14).* ***B*** *– SARS-CoV-2 RNA genome and essential structural proteins*

the similarity between SARS-CoV-2 and bat-COV, bats are considered the most possible hosts, and it is possible that bats serve as intermediary hosts between humans and bats. Second, a non-pathogenic strain of the virus was transmitted from animals to people, where it eventually developed into its present infectious condition. Additionally, the pangolin coronavirus might infect a person, either immediately or via an intermediate vector such as ferrets or civets [1, 24]. Third, the virus was modified in a lab and then freed or escaped inadvertently into the outside world. However, the genetic characteristics of SARS-CoV-2 are much incompatible with lab-based hypothesises of transmission [25].

Pathogenesis of SARS-CoV-2

Similarly, to SARS-CoV-1, the ACE2 receptor is also used by SARS-CoV-2 for the entrance of human cells [26]. The spike glycopeptide in SARS-CoV-2 is made up of two subunits: S1 (which binds to the ACE2 receptor and allows the virus to adhere to the target cells' surfaces) and S2 (for fusion with the cell membrane) [27, 28]. Similar to other CoVs, the receptor-binding domain (RBD) is a singular area of spike protein that facilitates contact with the cell target [29]. while the internal protease TMPRSS2 controls the division and activity of S protein, resulting in freed, fusion-catalyzed patterns on the host cell membrane [26]. However, at the nuclear level, the ACE2/SARS-S structure has been described, and the competence of ACE2 use has been discovered to be considered as the main indicator of SARS disease transmission [30]. As a result, the S proteins play a major role in determining the cell specificity and contagious character of CoVs [29]. SARS-CoV-2 might affect all the cells with strong ACE2 expression [31]. Thus, a high level of ACE2 expression was linked with more organ damage in COVID-19 patients [32]. In addition to ACE2, the presence of furin protein in many human tissues might play an essential role in causing damage to these tissues [26, 33].

Besides S protein, there are three structural proteins that can contribute in some way to getting infected with SARS-CoV-2. Above all, the M protein is among the most essential proteins in the viral particle structure. Compared to the E protein, the M protein is more abundant in the viral molecule. Therefore, it plays a role in shaping and coordinating the virus structure as well as maturing the virus envelope, jointly with the E protein. However, the other role of the E protein includes releasing the viral from the infected cells [34-36]. At virus formation, the N protein ropes to the RNA of the virus, and the N protein is required to cover the virus RNA in the viral particles [37].

Diagnosis of COVID-19

COVID-19 can be detected by many techniques, such as genetic and serological testing. Each of these techniques has its own advantages and disadvantages [38]. Nowadays, the genetic test RT-qPCR is commonly used to diagnose COVID-19. In this case, the RT-PCR method use respiratory specimens such as throat and nasal swabs [39]. To do this, viral RNA is first collected from specimens and separated. Second, RT-qPCR is performed, which transforms viral RNA into DNA and magnifies it in order to create lots of copies. Third, the detection probe can be used to check if the person has virus RNA in his body [40]. However, the RT-PCR method has drawbacks in terms of the possibility of false-negative results, variations in diagnostic precision with illness progression, and a lack of readily available materials [41]. In contrast, sometimes serological assays are used instead of or in addition to RT-PCR in the diagnosis of patients' infections since it is inexpensive and easiest to perform at the point-of-care. Also, these assays have a distinct benefit on RT-PCR in that they can recognize people who have been infected with SARS-CoV-2 before, even if they have never been tested when sick [41]. Additionally, a new technology, such as CRISPR, has proven to be useful in combating viral outbreaks [7].

Background of the CRISPR/Cas system

Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) were initially identified in 1987 in *Escherichia coli* and were subsequently discovered in various bacterial species [9]. The CRISPR system includes a library of foreign genetic sequences as well as a method for detecting and eliminating any foreign RNA or DNA, such as viruses and plasmids. The CRISPR systems are found in 70% of bacteria and 90% of Archaea, and some have more than one CRISPR area on their chromosomes. Furthermore, the CRISPR array is made up of foreign DNA fragments called "spacers" or "memories" with the same repeated sequences that may or may not be palindromic, depending on the type of system [10].

On the other hand, the CRISPR proteins are encoded by genes located upstream of the repeat and spacer sequences (CRISPR-associated proteins, or Cas proteins). However, there are two functions for the Cas proteins: First, certain Cas proteins acquire and store portions of the foreign sequence via a process termed adaptation or spacer acquisition; second, other Cas proteins utilize the stored genomic sequences through expression and interference processes to detect and destroy invading nucleic acids. The Cas proteins are very variable in structure and composition among bacterial species [10, 42].

The CRISPR system contains many associated proteins with different types of CRISPR. The CRISPR/Cas class 1 system employs a mixture of many Cas proteins, while the class 2 system only employs one Cas protein with several domains [43]. In addition, Cas proteins have nuclease activity and need base pairing between guide RNA (gRNA) or CRISPR RNA (crRNA) with a target region on RNA or DNA and a protospacer adjacent motif (PAM) to find a specific target [44]. However, following the discovery of the CRISPR system as a natural defensive mechanism in bacteria, the researchers tried to modify it to make it useful for therapeutic and diagnostic purposes [45].

CRISPR as a novel diagnostic technique

CRISPR technology is a new technique for nucleic acid identification that has emerged in recent years. In fact, CRISPR was designed as an RNA-guided DNA endonuclease for genetic modifications, but it has grown into an efficient tool in many fields. As a result of the CRISPR protease capability for RNA identification, several platforms were created to detect COVID-19 infection in humans, such as SHERLOCK, DETECTR, and CARMEN [46]. Furthermore, many proteins associated with CRISPR, such as Cas9, Cas12, and Cas13, were used to detect SARS-CoV-2 infection. Table 1 presents the main characteristics of CRISPR proteins [47-49].

Mechanism of CRISPR/Cas system action

CRISPR is a natural defensive system that helps bacteria and Archaea resist invasion by viruses or foreign plasmids. When a virus infects a bacterium, residues of the viral DNA are incorporated into the bacterial CRISPR gene, acting as a memory. When the same virus infects the bacterium again, it is able to identify the virus using this memory [50]. The whole defensive mechanism consists of three phases: adaptation, biogenesis, and interference. In the adaptation phase, short direct repeats separated by spacers are short variable DNA sequences that constitute the CRISPR sequence, and foreign DNA termed a protospacer is cleaved and incorporated into the CRISPR array. Consequently, these integrated pieces are transformed into new spacers. The second phase is biogenesis, in which the CRISPR array is transcribed to produce precursor CRISPR-derived RNA (pre-crRNA), which is subsequently matured to produce CRISPR-derived RNA (crRNA). Interference is the third phase; crRNA recruits and directs Cas effectors to a particular target for cleaving nucleic acids produced by an invading virus [51].

Table 1. The main characteristics of CRISPR proteins used in COVID-19 diagnosis

Characteristics	Cas9	Cas12	Cas13
CRISPR type	II	V	VI
Protein subtypes	Non	a, b, c, d, e, g1, h1, and i1	a, b, c, and d
Size	1053-1368 a.a	870-1228 a.a	954-1389 a.a
Molecular target	DNA	DNA	RNA
Collateral cleavage activity	No	Yes	Yes
PAM required*	Yes	Yes	Required PFS -PAM like structure-
Function	Endonuclease; that acts on DNA	Endonuclease; that acts on DNA and cuts it in a non-specific way	Endonuclease; that acts on RNA and cuts it in a non-specific way

Note: *Cas9 used G-rich PAM sequences whereas Cas 12 used T-rich PAM sequences

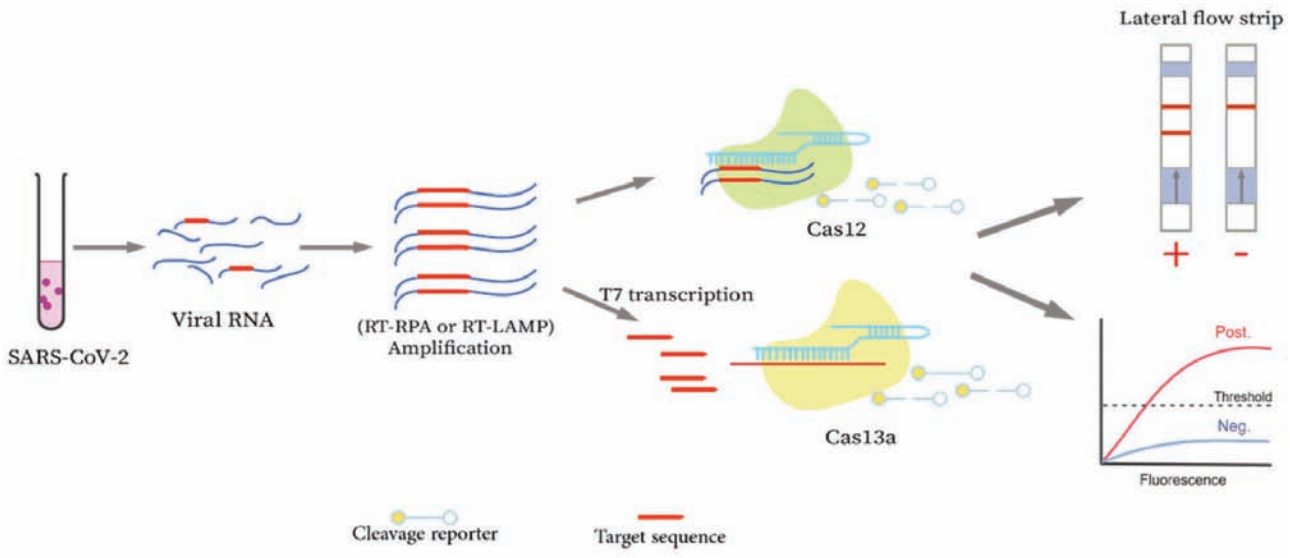

Fig. 3. Detection protocol for SARS-CoV-2 based on the CRISPR/Cas platforms

On a technical level, CRISPR-based diagnostic methods have been created, utilizing the nucleases Cas13 and Cas12a, which operate on the collateral cleavage activity principle. Similar to Cas9, the Cas12a nuclease binds to the target area of the genome using a gRNA and makes the break. In practice, the difference between Cas9 and Cas12a is that when Cas12a starts to cut the target DNA, it also starts to cut nearby ssDNA nonspecifically. Cas13 nuclease has a similar job to Cas12a, but it works on RNA sequences instead of DNA. A CRISPR/Cas13-based method is designed to identify RNA sequences of SARS-CoV-2 that the Cas13 nuclease can attack via a gRNA and cut the specific region to destroy the virus. However, by using this property, "trans-cleavage" or "collateral cleavage" fluorescent ssDNA/RNA reporters can be cut around the target genome and visible bands can be seen via the lateral flow strip [52, 53], as shown in Fig. 3.

CRISPR/Cas9 for COVID-19 detection

The Cas9 protein and sgRNA are the components of the CRISPR type II system. Cas9 acts as a nuclease that triggers a double-strand break (DSB) in the DNA molecule, while sgRNA can identify the target site, specifically through homologous recombination of the 20-bp DNA sequence. Thus, the gRNAs direct the Cas9 nuclease to a specific DNA site with PAM that corresponds to the gRNA. Then, the Cas9 nuclease with the HNH and RuvC nuclease regions breaks the dsDNA and produces a DSB. The HNH nuclease region splits the strand of DNA complementary to the gRNA sequence, whereas the RuvC nuclease region splits the DNA strand [54, 55], as shown in Fig. 4.

Depending on the mechanism of the CRISPR/Cas9 system, many diagnostic platforms, such as FELUDA and TL-LFA, have been developed. In addition to its ability to modify genes, Cas9 is utilized in the evolution of biosensors because it has good DNA identification capabilities with no trans cleavage activity. However, researchers concurrently use a mutant form of Cas9, dead Cas9, to achieve the role of viral identification in the COVID-19 diagnostic. The mutant form is produced by mutating the HNH

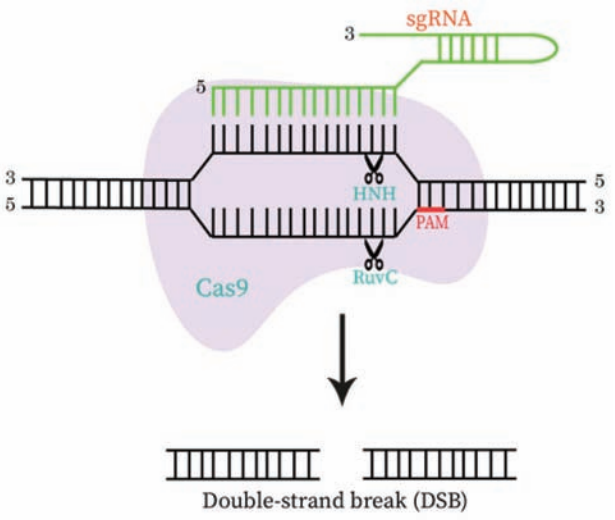

Fig. 4. Mechanism of CRSPR/Cas9 action

Table 2. The CRISPR/Cas9-based SARS-CoV-2 detection platforms

CRISPR protein	Platforms	Molecular target	Amplification assay	Specificity/ Sensitivity	Readouts	Time	Pots	LoD (copies)*	Refs
Cas9	TL-LFA	E and ORF1ab	RT-RPA	97/100%	Fluo.	<1 h	2	100	[58]
	FELUDA	N	RT-RPA or RT-PCR	N/A	Lateral flow	45 min	2	N/A	[59]

Note: N/A – not applicable; Fluo – Fluorescence; LoD – Limit of detection; each study has a different unit

and RuvC1 domains of the Cas9 protein at the same time. As a result, dead-Cas9's endonuclease function is completely lost, leaving only gRNA's ability to direct into the genomic [56]. The dead-Cas9/gRNA complex was used to detect SARS-CoV-2 using a colorimetric assay. In which the presence of viral RNA identical to gRNA results in a color change [57]. In contrast, the TL-LFA platform was created to target two genes in the SARS-CoV-2 RNA, E and ORF1ab, using Cas9/sgRNA [58]. Table 2 summarizes the SARS-CoV-2 detection platforms based on CRISPR/Cas9 system.

CRISPR/Cas12 for COVID-19 detection

Cas12 is a member of class II of CRISPR proteins that may be guided to attach to ssDNA and dsDNA sites by using guide RNA [60]. Because of its unique characteristics, including the capacity to target T-rich sequences and the lack of a trans-crRNA, Cas12 has appeared as an alternate to Cas9 [61]. For these reasons, the two best known Cas12 subtypes (a and b) are used in COVID-19 diagnosis [49]. Furthermore, many Cas12-based diagnostic platforms, such as ENHANCE, DETECTR, and CASdetec, are used.

Cas12-based diagnostic platforms

Cas12a (Cpf1) was discovered first [62]. Following that, researchers identified Cas12b (also known as C2c1) and other Cas proteins in the CRISPR system [63]. The DETECTR technique was the first to employ Cas12a in the genomic identification of human papillomavirus (HPV) [52]. However, a group of researchers developed the SARS-CoV-2 DETECTR platform, a Cas12a-based method that enables the easy and quick identification of SARS-CoV-2 RNA, which was isolated from an oro-/nasopharyngeal patient's swab within 40 min. Following RNA isolation, reverse transcription and isothermal amplification of RNA performed by RT-LAMP. Subsequently, after identifying the target viral sequence and cleavage reporter molecule using the Cas12a-gRNA complex, the detection of SARS-CoV-2 was proven over 30-40 min in clinical samples by using a fluorescent detector or lateral flow strip. A FAM-biotin reporter molecule and lateral flow strips made to catch labeled nucleic acids. A signal is produced by random Cas12 cleavage activity at the second detection line (test line), while uncut reporter molecules are caught at the first detection line (control line). Furthermore, this process identifies the N and E genes of SARS-CoV-2 in about 10 copies per µl of SARS-CoV-2 nucleic acids [64].

On the other hand, Cas12b is utilized in the STOP-COVID.v2 technique, which detects the SARS-CoV-2 N gene using AapCas12b as a thermostable Cas protease and the LAMP reagent-dependent amplification. STOP-COVID.v2 was created to be more sensitive and specific in the diagnosis of COVID-19. The researchers used the magnetic pellets to concentrate SARS-CoV-2 RNA into a single pot mixture, enabling RNA detection from the whole swab sample. Furthermore, STOP-COVID.v2 could be performed within one hour with only a few tools and at a fixed temperature [65]. Table 3 summarizes the SARS-CoV-2 detection platforms based on the CRISPR/Cas12 protein.

CRISPR/Cas13 for COVID-19 detection

Cas13 is a CRISPR protein of type VI, class II, that targets RNA rather than DNA [60]. Cas13 is divided into four subtypes (a, b, c, and d), each with its own unique features [81]. However, Cas13a (C2c2) is the first and most used Cas13 subtype, and it has been used for many Cas13-based diagnostic purposes [82]. Furthermore, like Cas12, Cas13 displays non-specific cleavage activity when activated by a ssRNA sequence that complements its crRNA re-

Table 3. The CRISPR/Cas12-based SARS-CoV-2 detection platforms

CRISPR protein	Platforms	Molecular target	Amplification assay	Specificity/ Sensitivity	Readouts	Time, min	Pots	LoD. (copies)	Refs
Cas12a	DETECTR	E and N	RT-LAMP	100/95%	Lateral flow	45	2	10	[64]
	AIOD-CRISPR	N	RT-RPA	N/A	Naked eye	20	1	5	[66]
	CFVD	N, E, and ORF1ab	RT-LAMP	100/100%	Lateral flow	40	1	20	[67]
	SENA	ORF1ab and N	RT-PCR	99/99%	Fluo.	–	–	2	[68]
	Opv-CRISPR	S	RT-LAMP	N/A	Naked eye	45	1	5	[69]
	iSCAN	E and N	RT-LAMP	100/86%	Fluo./ lateral flow	40	2	10	[70]
	ITP–CRISPR	E and N	RT-LAMP	100/93.8%	Fluo.	30-40	2	10	[71]
	CRISPR-FDS	ORF1ab	RT-RPA	100/100%	Fluo.	15	1	<0.05	[72]
	CRISPR/ Cas12a-NER	E	RT-RAA	N/A	Fluo.	45	2	10	[73]
	MeCas12a	E	RT-RAA	100/100%	Naked eye	45	2	5	[74]
	ENHANCE	N	RT-LAMP	N/A	Fluo./ Lateral flow	40-60	2	3-300	[75]
	CRISPR-ABC	ORF1ab	RT-PCR	99.2/91.2%	Fluo.	30-40	2	1.1	[76]
	OR-DETECTR	N and RdRp	RT-RPA	N/A	Fluo.	50	1	2.5	[77]
	COVID-19 CRISPR-FDS	N and ORF1ab	RT-RPA	71.4/100%	Fluo.	50	2	2	[78]
Cas12b	CASdetec	RdRp	RT-RAA	N/A	Fluo.	60	1	10	[79]
	iSCAN	E and N	RT-LAMP	100/86%	Fluo./ Lateral flow	40	1	10	[70]
	STOPCovid.v1	N	RT-LAMP	N/A	Lateral flow	50	1	100	[80]
	STOPCovid.v2	N	RT-LAMP	98.5/93.1%	Fluo./ Lateral flow	15-45	1	33	[65]

Note: N/A – not applicable.

gion. Activated Cas13, in contrast, cuts all adjacent ssRNAs rather than DNA [83]. *In vitro*, this feature has been used for extremely specific diagnostics [83, 84].

Cas13-based diagnostic platforms

Similarly, Cas13a is utilized as Cas12a but with a few minor differences. First, because RNA targets stimulate Cas13a proteins, so following amplification, extra T7 transcriptions are required to transform DNA amplicons into RNAs. However, this process may be combined with the amplification and Cas13a test to be performed in one pot. Second, as active Cas13a cuts ssRNA, reporters must be created with ssRNA, as opposed to ssDNA reporters utilized in Cas12a systems [84].

However, the first use of Cas13a in nucleic acid detection was by the SHERLOCK assay. This assay

Table 4. The CRISPR/Cas13-based SARS-CoV-2 detection platforms

CRISPR protein	Platforms	Molecular target	Amplification assay	Specificity/ Sensitivity	Readouts	Time, min	Pots	LoD. (copies)	Refs
Cas13a	DISCoVER	N	RT-LAMP	100/93.9%	Fluo.	35	2	25	[87]
	SHERLOCK	N, S, and ORF1ab	RT-RPA	100/100%	Fluo.	35-70	2	10	[86]
	SHINE	ORF1ab	RT-RPA	100/90%	Lateral flow/smartphone	50	1	10	[88]
	CRISPR-COVID	ORF1ab	RT-RPA	100/100%	Fluo.	40	2	7.5	[89]
	AF-CRISPR	E and N	No need	N/A	Fluo.	30	1	100	[81]
	CREST	N	MiniPCR	98/97%	Fluo.	50	2	10	[90]
Cas13d	SENSR	N and E	RT-RPA	100/–	Lateral flow/Fluo.	120	2	100	[91]

Note: N/A – not applicable.

has high accuracy and sensitivity for detecting target RNA instead of DNA [38]. As a collateral activity of its action, the Cas13a protease destroys target and non-target RNA [85]. Thus, dependent on this activity, particular RNA can be detected *in vitro* [83]. The SHERLOCK assay utilizes the RT-RPA technique to isothermal amplify RNA and then detects the target genes using Cas13a. Further, this assay targets Orf1ab, S, and N genes by using an RPA primer and crRNAs [86]. Table 4 lists several Cas13-platforms used to detect SARS-CoV-2.

CRISPR as a novel antiviral tool

There are many therapeutic strategies, such as drugs, plasma therapy, and vaccines, which are used to combat COVID-19 [92]. Moreover, CRISPR/Cas13 was used in the war against COVID-19 as a new technology. Strikingly, the CRISPR method focuses on detecting and destroying the viral particles inside cells and their resultant viral mRNAs [93]. In this regard, the PAC-MAN method was created as a new therapeutic strategy based on the Cas13d-sgRNA complex. However, there are many features that make Cas13d the proper choice to use, such as its strong cleavage efficacy in human cells, its tiny size (967 a.a), and high specificity. Consequently, in epithelial cells of the human lung, the PAC-MAN method employs Cas13d for viral suppression, which can efficiently destroy RNA from the influenza A virus (IAV) and SARS-CoV-2. In regard of SARS-CoV-2, Cas13d targets N and RdRP proteins, which are required for replication and activity of the virus. Thus, targeted suppression of these proteins might have a large impact on inhibiting virus generation and activity, as well as reducing the spread of the virus by destroying the viral genome [94].

Cas13d is also used to target SARS-CoV-2 proteins in the lab. Likewise, by targeting the S and ORF1ab proteins of the virus, it might be potential to restrict its capacity to replicate [95]. Before these methods can be used, however, they need to be studied more. One way to do this is to use CRISPR/Cas13 as an antiviral tool and test the activity and specificity of the crRNA in model animal cells to stop the virus from spreading.

Limitation and Future perspective

As a result of the speedy spread of COVID-19 worldwide, there is a vital need for rapid, simple, and accurate tests. CRISPR technology offers a high potential to fight outbreaks of the COVID-19 pandemic by providing a fast, affordable, precise, and particular test for SARS-CoV-2 diagnosis. In addition, many CRISPR proteins were used and many CRISPR-based platforms were developed. Despite its numerous benefits the growing CRISPR technique has some drawbacks and limitations. Firstly, the extraction step of RNA/DNA molecules is the largest snag of using CRISPR in point of care test (POCT). Simultaneously, the majority of the isolated RNA/

DNA must be filtered prior to amplification to remove possible inhibitors that might interact with the end application [96]. Also, the source of the samples, because the extraction process differs depending on the sample type. Besides, some samples such as mucus and blood require dilution before being used to detect viruses. Other samples like saliva require sufficient virus molecules to be detected [97]. Many of the diagnostic techniques mentioned previously still wouldn't be able to fulfil clinical diagnostic criteria without the nucleic acid extraction technique due to decreased specificity and sensitivity.

Therapy relying on the CRISPR system might be a possibility for handling COVID-19. But the off-target effects can't be avoided in therapy. Notably, there are different ways of dealing with off-target effects, such as improving sgRNA design and enhancing the selectivity of CRISPR proteins [56]. Another drawback of the CRISPR system is its applicability. CRISPR is not commonly utilized in humans yet. The majority of present research has been done on animals, and this constraint should be addressed one day.

Importantly, the COVID-19 pandemic offered a significant challenge in all aspects, particularly health and economics. Besides, COVID-19 changed our concept of our relationship with microorganisms and how to deal with them. Accordingly, the evaluation of our health-care capacities regarding research and treatment centers is necessary in order to get to previously dealing with such an outside pandemic.

Conclusion. From this review of almost all the CRISPR platforms, we can understand the most important parts of CRISPR RNAs and Cas effector proteins. Also, reviewing and clarifying all platform parts, principles of work, and readout signal techniques. Hence, the genome editing technique CRISPR can be used for both quick virus detection and as a possible treatment for the COVID-19 pandemic. This technology provides additional benefits since it is accurate, affordable, and simple to use. Scientists believe that the CRISPR technique has a promising future since it can be used to treat genetic defects and destroy viruses by specifically targeting their genetic material. Albeit the CRISPR/Cas system's clinical applications are still in their youth, its development opens up a wide range of opportunities for the field of biomedicine. Furthermore, since the CRISPR system is easily reprogrammable, several guide RNAs can be leveraged to make sure that the specific sequence is detected even if the virus mutates. Also, in addition to its ability as a coronavirus therapy, CRISPR is racing to develop testing and diagnostics and have a long-lasting and effective impact on the COVID-19 epidemic and possibly on any up-coming pandemics. In the future, with ongoing in-depth study and clinical verification, we believe that the use of the CRISPR/Cas system could play a vital role in the control and prevention of rising contagious diseases. Finally, we hope that this research will help future researchers learn more about this technology and use it as a guide.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http:// ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

CRISPR ЯК НОВА ТЕХНОЛОГІЯ ДЛЯ ДІАГНОСТИКИ COVID-19

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До теперішнього часу захворювання людини на COVID-19, що виникає внаслідок тяжкого гострого респіраторного синдрому, спричиненого коронавірусом 2 (SARS-CoV-2), залишається гострою проблемою для усього світу. У січні 2022 року зареєстровано понад 300 мільйонів випадків інфікування та понад 5 мільйонів смертей від COVID-19. Щоб протистояти такому швидкому поширенню, вкрай необхідні ефективні та недорогі методи діагностики, які допоможуть контролювати небезпеку пандемії. Технологія CRISPR довела свою ефективність у виявленні COVID-19 завдяки своїй простоті, специфічності та високій чутливості. У цьому огляді розглянуто сучасний стан розробки платформ CRISPR для діагностики та лікування COVID-19. Обговорюються обмеження та можливості CRISPR з точки зору методів аналізу нуклеїнових кислот для діагностики вірусних інфекцій.

Ключові слова: COVID-19, CRISPR, SARS-CoV-2, протеїн Cas, RT-PCR.

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