

Review Article

## A NOVEL CRISPER/CAS9-13 BASED DIAGNOSTIC TOOL FOR COVID-19: A REVIEW

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### ABSTRACT

COVID-19 is a viral disease caused by the SARS-CoV-2 virus and now it becomes a severe global pandemic. Its atrocity can be imagined by its world-wide lockdown and WHO monitoring at the special focus. This mutant corona 2019 viral strain is emerging as a disaster on earth in the last 20 years. Diagnosis is the first step in the detection of any diseases either native or pandemic level before the treatment through medication or vaccination. Early and fast diagnosis is the primary step for rapid spreading pandemic contagion infections. In this review, we have discussed recently developed CRISPR–Cas-based diagnosis detection tool from the respiratory swab RNA extracts for SARS-CoV-2. It is a rapid, specific and sensitive technique using nowadays due to its robustness than pervasively PCR, ELISA based diagnosis methods.

### 1. INTRODUCTION

A severe outbreak of COVID-19 starts within a local level in China (Wuhan) and then spread at the jet level at the world (1). Earlier, last 20 years several viruses' attacks spread and affected mass public such as influenza H1N1 virus, Ebola virus, Zika virus at different zones on earth but due to the efforts of governments and world health organisation and private N.G.O, they became controlled (2). This time SARS-CoV-2 which was earlier detected as pneumonia-like disease become uncontrollable.

In India, total cases 10.1 million affected and worldwide 76.8 million cases according to recent data given by government organisations for public and it is increasing day by day until vaccination or specific drug therapy will not come to effective against this pandemic virus [3, 4]. At the initial stage, infectious symptoms that appear are modest fever, dry cough, vertigo & if serious symptoms arise and disease progress then difficulty

in breathing or shortness of breath, chest pain or pressure loss of speech or movement. Immediate medication required after that [6, 7, 8]. As we discussed earlier, the diagnosis is the major problem aroused when evoking of the pandemic because of asymptomatic cases. All assays based on spike protein/RNA based detection but sometimes false-positive result arises.

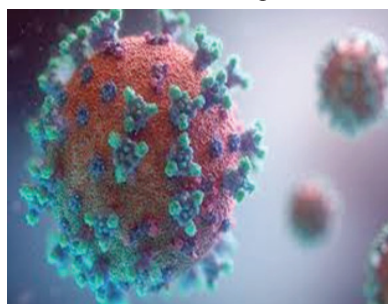


Fig. 1: Corona virus, Source: NCIRD

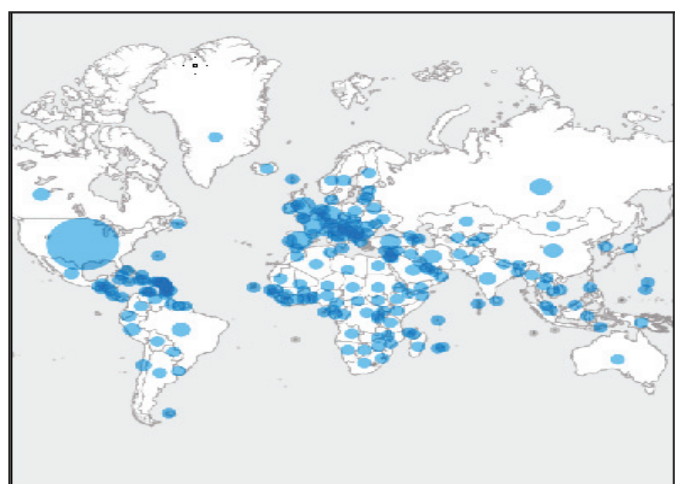
Today's scenario, RT-PCR assay is extensively used but still, cases are increasing. So, few scientists from core science decided to develop a new way to detect fast COVID19 infection [9-13]. CRISPER/Cas has recently become boon and achievement in genetic engineering & it had given accurate results of test when experimentally designed to experimental pathology (14,15). Further, if approval granted for CRISPER rooted diagnosis by united state of food and drug administration (US FDA) then it can generate a reliable and trusted report by this next generation inevitable technology.

## 2. WORLD SCENARIOS

The organisation involves in testing, Health & food programs, and communication to develop good medical devices and vaccines e.x. Moderna's COVID-19 vaccine, Pfizer-BioNTech, Russian vaccine. These are approved vaccines and recommended & few are in clinical trials such as AstraZeneca's COVID-19 vaccine, Janssen's COVID-19 vaccine [16].

**Table 1: Word scenario of Covid-19 pandemic support organization**

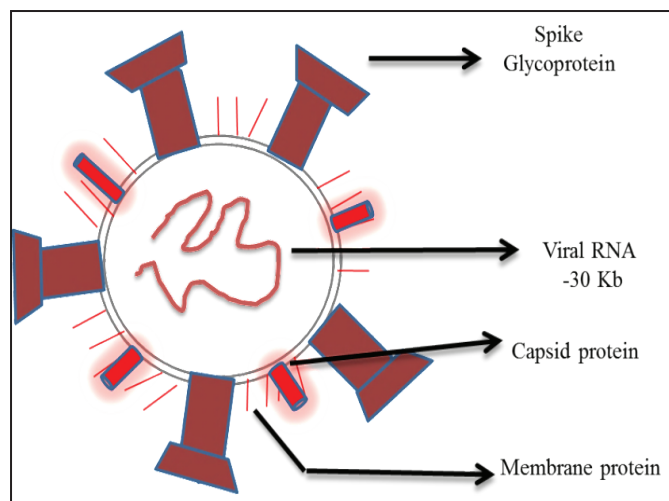
Organisation	Efforts
W.H.O	Medical aid monitoring
Bill and Milinda gates	Medication and treatment
Akshaya Patra Foundation	Food and safety
Russia, Israel, china, india, U.S, Britain government	Vaccination programs



**Fig. 2:** W.H.O COVID-19 pandemic map worldwide.

*(The blue dots show hot spot area recognised based on survey and diagnosis of SARS-CoV-2)*

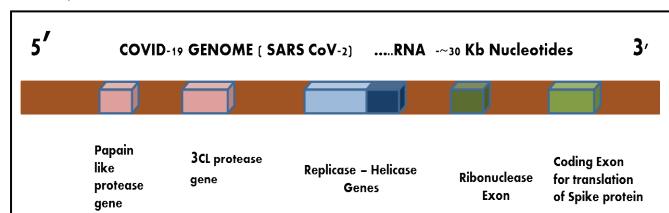
To a major concern, the World health organisation taking COVID19 as a major challenge and guidelines were released immediately (5). As you can see, viral pandemic behaviour from Asia to central Asia and continued to the western world where it has gone atmospheric survival conditions to spread and infect at disaster level. Most important it can survive outside in droplets/sputum in few hours to few days.



**Fig. 3:** Systematic representation of SARS CoV-2 structure

## 3. GENETIC FRAME OF COVID-19

At the initial level, it was detected and reported as unknown flu virus but soonly, researchers got knowledge that it is a new mutant strain which starts in china and spread quickly by contact to contact in humans. For unknown pathogen, there is always a need to know the structural features so that treatments can apply and preventions can be improvised. The De-nova drug designing needs structural and functional data so that drugs can be targeted and vaccines can be developed. This novel COVID-19 mutant strain is circular shape contains spike proteins which are majorly targeted by the scientific community for vaccines and diagnosis [17]. This virus has ~30 kb RNA genome (29,674 bases)



**Fig. 4:** Exon representing the expressing units on genome of SARS CoV-2 Virus

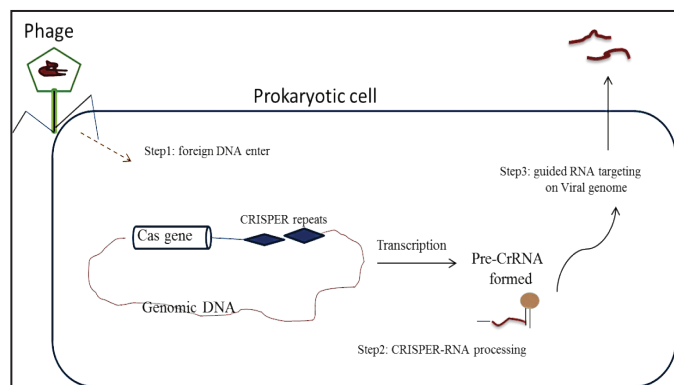
The genome has protease expressing genes which are required for cleavage activity. Indeed, other coding regions are there to viral survival but mutating by environmental factors. As you can see, data suggested that it contains also membrane proteins and capsid proteins but its virulence is suspected by these proteins but is part of the research. Further, structure and crystallography reveal that targeting helicase and spike part is benefited for drug targeting and vaccination.

## 4. CRISPR/CAS SYSTEM

### 4.1 Gene-editing technology: mechanism

Genome editing technology innovations perceive and achieve upgrade technology.

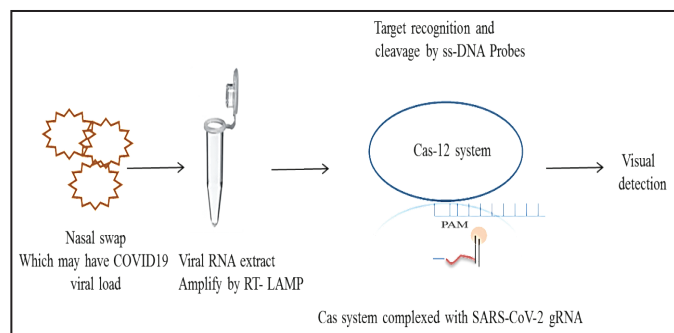
This clustered regularly interspaced short palindromic repeats are innate immune system found in bacteria and archaea. This system based on nuclease activity of CAS enzyme which targeted on PAM sequences which is present on the gene to target. This technology is recently applied in spite of tedious cloning for editing and insertion of genes because only Guided RNA required to target gene i.e Cr-RNA::TracrRNA(Cas9 System) (18-20).



**Fig. 5:** Phage entry to bacteria leads to activation of CRISPER/ Cas system and gRNA framed which have sequence to target phage genetic material.

#### 4.2 CRISPER technique as a diagnostic tool

Approx. 20 bases of Covid-19 RNA can be detected and identified by CRISPER assay. Firstly the researcher has to amplify sample viral RNA before diagnosis. The method proceeds by synthetic guide-RNA that should be complementary to a gene of interest or target RNA. When gRNA binds to target RNA, the attached Cas enzyme (nuclease) binds to its PAM unique sequences & cuts its nearby single codon (...NNN...PAM.) where N is any nucleotide. The cut sequences bind to fluorescent probes in a test solution. So, when the sample is focused on Fluorescence detector, gives a signal and get visualize. The data should be entered to confirm SARS-CoV-2 virus (21-24). The flow process is given in the figure.



**Fig. 6:** CRISPER/Cas based diagnosis flow diagram

Up to till December 2020, the data published on peer journals given reports that this technology provides one single g-RNA (Cr-RNA) can detect one lakh viral load particles per microliter sample. But if second g-RNA added then it can detect up to at 100th of level virus/UL. This tool is recently introduced in

genetic editing but the impact is so high that it got noble prize within 10 years and patent rights are still in process. CRISPER/ Cas diagnostic tool is also in approval by USFDA, America. The advance technologies are always in trust due to its specificity and sensitivity in detection. Therefore, cr-RNA: endonuclease-based tool should be gone through the test by clinical trials.

**Table 2: Comparison of different diagnosis methods for COVID-19 test:**

Method	Time taken	Sensitivity
ELISA (Antibody IgG based)	5-6 h	<94%
RT-PCR (RNA based)	2–4 h	95%
RT-LAMP (synthetic ssDNA)	45 min	95%
Chest CT as a diagnostic tool	Approx. 1 day	94%
Rapid test (Antigen based)	30 min	96%
CRISPER/Cas system (synthetic RNA)	15 min	97%

While comparing the recent in-process assays for COVID-19, It is seen that Real-time- Reverse transcription loop-mediated isothermal amplification (LAMP) is best choice to detect in laboratories as it is taking only 45 minutes but it requires much handling and procedure. It is a good tool to validate and confirmation of SARS-CoV-2 worldwide (25-33). But specificity & sensitivity and time are taken leads to cases increments day by day. So, scientist analysed gene complementary editing tool rapid and confirm test to detect in COVID-19. Therefore, CRISPER technology comes in place and found sensitive detection without false-negative results.

#### 5. CO-INFECTION CASES: DIAGNOSTIC OBSTACLE

##### 5.1 Re-emerging of Covid-19 SARS-Cov-2 virus

Recently in the clinical diagnosis, it has seen that SARS CoV-2 virus emerging as co-infection with the HIV/AIDS or with diabetes. It leads to global burden again on public economics and especially at the initial stage of reinfection with asymptomatic cases (35-41). After long research, it was found that remdesivir antiviral drug is useful in coinfection cases but treatments can be in the table when specific diagnostic can be provided to the doctor (42,43). Again, we stand on the same road where we started. In these cases, Improved technologies can give us hope to detect disease at earlier stages and progression can be controlled. CRISPER/Cas based genome assay can be a profitable boon in early and easy detections.

#### 6. CONCLUSION

In the twenty-first-century, when science and technology are on the breakthrough level, many significant scientific inventions made by mankind that are helping humans in health-related disorder and pathogenesis. But sometimes humans become vulnerable when SARS-19(COVID-19) virus attacks pandemic. There are millions of deaths & billions of financial losses in last year. Indeed, the researcher had able to control due to advance

technologies in medical sciences. Here, we have shown that in comparison to present COVID-19 diagnostic, newly designed CRISPER/Cas9-13 technology gives a fast and accurate report. But still needs approval by a certified organisation. Within 15 minutes, the result can be shown visually by the fluorescent detector. The next-generation innovation CRISPER is helpful in symptomatic but asymptomatic cases. The delivery of short g-RNA is the minor hurdle in modern therapeutics but can be overcome as of shotgun just as gene therapy targeting methods. Therefore, as soon as the disease confirmed, the treatment can start to control further spread in the endemic area and so many lives can be saved by this novel 21st-century next-generation technology.

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