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Short Communication

Clustered Regularly Interspaced Short Palindromic Repeats-Based Diagnostics and COVID-19; A Leap Forward in Molecular Pathology

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ABSTRACT

Various diagnostic strategies have also surfaced with the emergence of the COVID-19 threat. Conventionally, the laboratory world used to rely on serological diagnosis, but the availability of molecular techniques, especially "Polymerase Chain Reaction" (PCR), has initially emerged as the front-line test for diagnosing SARS-CoV2 infection. Though defined as the current mainstay of COVID-19 diagnosis, the technology still suffers from less diagnostic sensitivity and specificity and prolonged turnaround times (TAT). The recent emergence of novel techniques, i.e., CRISPR/Cas technologies, in diagnosing COVID-19 infection has been tremendous and provides newer replacements for PCR testing. CRISPR with Cas12 and Cas13 from CRISPR type-V and type-VI has the potential to revolutionize COVID-19 diagnosis due to better diagnostic efficiency, lower limits of detection (LOD), much-reduced turnaround times (TAT) and availability as point of care testing (POCT). Key technologies discussed in this include SHERLOCK, DETECTER, AIOD-CRISPR, PAC-MAN, CREST and others. This short communication briefly conceptualizes CRISPR/Cas, followed by a discussion on currently available CRISPR technologies for COVID-19 infection with an overview classification of most available methods.

Keywords: AIOD-crispr, Cas9, Cas12, Cas13, CREST, CRISPR/cas technology, Detectr sherlock, PAC-man.

How to Cite This Article: *Khan SH. Clustered Regularly Interspaced Short Palindromic Repeats-based Diagnostics and COVID-19; a Leap Forward in Molecular Pathology. Pak Armed Forces Med J 2023; 73(5): 1545-1548. DOI: https://doi.org/10.51253/pafmj.v73i5.7956*

INTRODUCTION

The origin of current times, COVID-19 infection, has taken humankind hostage. It has struck hard the normal functionality of humankind, compromising socialization, economics, and how we now live. Without any question, the response from scientists, researchers and front-line health workers remained appreciable due to the timely development of diagnostics like Polymerase Chain Reaction methods, isothermal amplification techniques and biomarkers to assess disease progression and prognostic evaluation modifying labs to become proactive. The current gold standard in SARS-Cov2 diagnosis remains RT-PCR, but this methodology is not without limitations, and its diagnostic performance also remains questionable in laboratories; increased turnaround times and inter and intra-lab variations have also been common observations.[1] The global COVID-19 crisis is ongoing, and though much-needed help in terms of vaccination has arrived, fears still haunt us about its optimal control due to the appearance of "Variants of Concerns (VOCs)" and ongoing mortality and morbidity.[2] There seems to be an ever-increasing demand for more precise and accurate diagnostic modalities, a much-needed requirement for the timely management of COVID-19 patients. Courtesy of recent plights in biotechnology and scientific data, there are possible replacements for COVID-19 diagnosis where "Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR)" can also lead the race and modify lab-based diagnostic technologies.[3]

CRISPR/Cas technology with its respective nucleases, i.e., Cas proteins, has surfaced as one of the most significant discoveries of our times. CRISPR/Cas originates from studying the ancient bacterial and archeal models, where this naturally occurring method provides these organisms with a unique immune defence mechanism against bacteriophages.[4] This example from "Mother Nature" was later developed in the last decade as a molecular technique with credit attributed to Jennifer Duodona and Emmanuelle Charpentier for editing the genomes of live organisms.[5] CRISPR technologies, since their milestone inception, underwent reprogramming in various modes for use in therapeutic and diagnostic modalities. Unlike RNA interference (RNAi), this newer methodology can cause both inhibition of certain genomic regions and activation.

CRISPR/Cas Concept

Though no longer a newer innovation, it is still Greek in many parts of the world. A brief conceptual understanding of the methodology seems mandatory for the audience. The technology works simply on the

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Received: 29 Dec 2021; revision received: 03 Apr 2022; accepted: 04 Apr 2022

principle of a "cut and paste" mechanism. In primitive Archeal and bacterial systems, whenever a bacteriophage invades their organisms, small DNA fragments from the invader organisms are clustered and placed with the help of guide RNA (gRNA) in a way that a crRNA/gRNA is generated. On any future exposure to similar bacteriophage, the crRNA representing the invader DNA is guided by gRNA into the Cas protein to allow Cas nucleases to attack the invading DNA by generating double-stranded nicks, thus neutralising the invading bacteriophage.[6]Figure-1, in brevity, explains the general concept of CRISPR/Cas function. This naturally gifted technology was incorporated as a genome editing tool with specified gRNA with corrected genetic sequences with the Cas proteins through a viral or non-viral vector either by removing specific codon sequences or inserting/replacing DNA codon segments.[7]

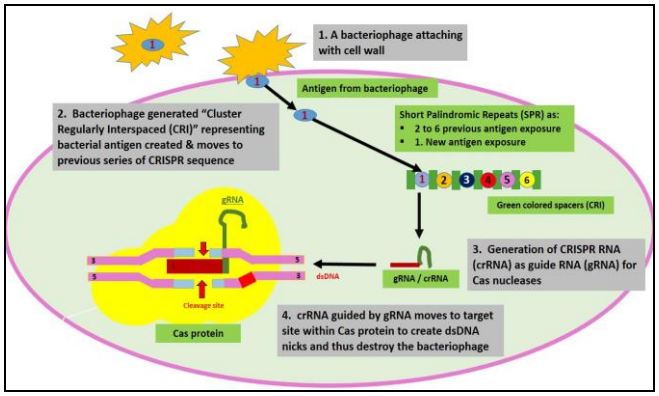

Figure-1: A General Orientation of CRISPR/Cas function with step by step generation of Cluster Regularly Interspaced regions development to final destruction of Phage by the help of gRNA inside Cas Proteins

Crispr Technologies For Sars-Cov2 Diagnosis

CRISPR/Cas technology has multiple roles in both therapeutics as a powerful genome editing method but has also been successfully modified to diagnose both infectious and non-infectious disorders. This short communication provides insight into its possible utility in diagnosing COVID-19 infection within clinical laboratories. Emerging data has identified Cas12 and Cas13 nucleases as well-evolved and better programmable DNA and RNA targeting agents. Current research on using CRISPR/Cas revolves around various miniaturized models of the technique with some add-on features. Starting from the "DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR)", which can be grouped under the umbrella cover of the type-V CRISPR method, replaces the usual Cas9 nucleases with Cas12a(Cpf1).[8,9] Cpf1, being smaller in size, handles some size-related limitations to create double-stranded nicks in DNA coupled with isothermal amplification of various pathogens. Preliminary data suggests this methodology to have this method to have very high sensitivity and negative predictive value along with short turnaround times.[10,11] Gronowski *et al.* have developed a method using CRISPR technology with Cas13a and compared the methodology with metagenomics Next generational Sequencing (mNGS) and RT-PCR to conclude that CRISPR-COVID method has almost 100% sensitivity and specificity.[12]

The first CRISPR-based methodology emerged in 2018 and was called**"**Specific High sensitivity Enzyme Reporter unlocking technology" (SHERLOCK), which used mostly Cas13 but also data suggested the use of Cas12 with turnaround times reduced to as slow as 15 minutes.[13] Another variant of SHERLOCK is termed "STOP", which implies "SHERLOCK Testing in One Pot", which can be useful in field epidemiology.

by reducing turnaround test time by one hour.[14]Another technique termed "FELUDA", a variation CRISPR implying "FNCAS.[9] Editor-limited Uniform Detection Assay", reduces hands-on turnaround times by one hour, uses paper-based strips, and is cost-effective.[15] Tata Group devised This test in India and is being suggested for use in a field with minimal expertise. Another CRISPR technique employing Cas12a has been developed as a point-of-care test (POCT) with a very simple methodology. This technology is abbreviated as "All In One Dual CRISPR" or AIOD-CRISPR. This technology is simple to deploy in the field, sensitive, with a very short turnaround time of less than 30 min.[16] A technique with both therapeutic and diagnostic potential employing a slight variation in the original CRISPR method using Cas13 is "Prophylactic Anti-viral CRISPR (PAC-MAN)". This CRISPR modification can specifically target conserved regions within the SAR-CoV2 virus.[17] A novel biotechnological development incorporating Ca13 uses a "Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids" in Micro-Array format, which has allowed multiplexing and thus can manage an increased workload.[18] A simpler POCT format utilizing CRISPR/Cas12a technique with green fluorescence emission has shown promise as a rapid test with much-improved sensitivity leading to "Naked Eye Readouts" and termed as "CRISPR/Cas12a-NER".[19] Cas3-Operated Nucleic Acid detection (CONAN) is a recent CRISPR modification that

employs Cas3 nucleases which has been used as a POCT and has a very low limit of detection.[20] Two other CRISPR variants, including Antibody And CAS fusion (ABACAS), derived from PAC-MAN and Cas13-based Rugged Equitable Scalable Testing (CREST) have also been devised, which have shown reduced turnaround times and lower analytical sensitivity.[21,22] Figure-2 provides a general classification of various CRISPR/Cas technologies modifications.

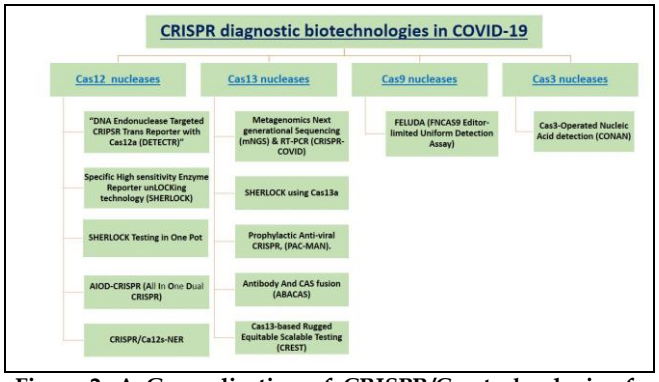

Figure-2: A Generalization of CRISPR/Cas technologies for Diagnosing COVID-19 Infection

DISCUSSION

After a year and a half, COVID-19 infection's third wave remains a much bigger public health threat than anticipated. Vaccinations, in some way, address the issue in some bigger economies with stringent public health measures. However, it is not over yet, with new variants taking over headlines from different parts of the globe, causing increased infectivity and, to some extent, associated higher morbidity and mortality.[22] Timely diagnosis remains a pivotal step for the physician where serious COVID-19 case delays cannot be afforded. Similarly, timely triage through a POCT device with much enhanced diagnostic characteristics and reduced turnaround time stands the moot point for triage and stopping further exposure from a COVID-19 patient. CRISPR technologies offer great relief by doing tests with very short turnaround times.[16-18]

Current gold standard technology in COVID-19 infection, i.e., PCR, though robust and cost-effective in some ways, still needs long turnaround times, associated with low diagnostic performance, lack of standardization and multiple steps, including cumbersome extraction steps.[1] More so, there could be a possible variation resulting from primer selection from different regions of SARS-CoV2, which can create some degree of ambiguity.[23] The clinical requirements for COVID-19 management have been evolving since this global pandemic with needs tailored to shorter turnaround times, the lower limit of detection (LOD), higher analytical performance and biotechnological up-gradation as the point of care formats. Recent evolution of CRISPR/Cas technologies, especially incorporating Cas12 and Cas13, have allowed real-time translation of aforementioned clinical needs.[16-22] Detectr, sherlock and aiod-crispr and related CRISPR innovations have allowed an opportunity to detect infections with better clinical efficiency in a shorter period, which can help emerge in future as the backbone of the fight not only against COVID-19 infections but other similar threats in future.

Provided potential benefits, the CRISPR technologies still need to be versant with clinicians and laboratories due to obvious limitations of trained human resources, minimal research and development culture, especially regarding newer molecular sciences and possibly economic reasons. The need of the time is to move on towards the futuristic requirements to combat better biothreats and related potential of CRISPR therapeutics, for which awareness among healthcare experts from all walks of life needs to be highlighted. Knowledge is power, and this theory applies directly to the current pandemic scenario.

CONCLUSION

Current molecular diagnostics may need up-gradation and innovation by adopting smarter, more efficient methods with reduced turnaround times for COVID-19 and future bio hazards where CRISPR technologies, especially Cas12 and Cas13 nucleases, can fill the gaps in PCR-related diagnostics. There is an ever-surfacing need for translating bench side to clinic translation of CRISPR technologies in evolving economies with more research and development. Undeniably innovative molecular methods, including CRISPR, will be the mainstay of future healthcare hazards.

Conflict of Interest: None.

Author's Contribution

Following author has made substantial contributions to the manuscript as under:

SHK: Conception, study design, data acquisition, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

Author agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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