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Correspondence/Letter to the Editor

Detection of COVID-19 RNA: Looking beyond PCR

Dear Editor,

Today, the world is reeling under the deadly coronavirus disease 2019 (COVID-19) pandemic. Lakhs of people worldwide have already succumbed to this highly contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and no halt seems imminent. This havoc that has been wrecked by a positive-sense single-stranded RNA belonging to the Coronaviridae family which transmitted from one person to another by close contact, or through contaminated droplets. Currently, no specific therapeutic option is available for this disease, and the entire focus is on preventing the spread of infection through early diagnosis of the infected individuals and isolating them. But as the disease is spreading to far flung areas, the implementation of this simple approach has off-late become very difficult in India. This failure to contain the spread of infection can be partially ascribed to the lacunae in the currently available detection modalities for COVID-19.

Currently, the standard testing strategy is by nucleic acid amplification testing by real-time reverse transcriptase–polymerase chain reaction. Other than that, multiple immunodetection tests based on immunochromatography (ICT) or enzyme-linked immunosorbent assay (ELISA), either to detect antigens (N protein) or host antibodies (IgM/IgG) are available but cannot be relied on for patient care.

PCR-based detection is highly sensitive and specific, but it is expensive, requires high-end infrastructure and skilled manpower. The transport of samples from areas that lack facilities for conducting PCR to designated COVID-19 testing laboratories and subsequent reporting can take several days, and this delay adversely affects not just patient outcomes but also makes it difficult to contain the spread of infection. Being an expensive test, carrying out a huge number of such tests puts significant strain on the public health infrastructure of states. Although these problems could have been ameliorated by the use of serological tests based on antigen/antibody detection which are extremely easy to perform point-of-care tests and which can provide results without the use of skilled manpower and expensive instrumentation; but they are plagued by their lack of good specificity and sensitivity. The need of the hour is to develop an accurate, simple, low cost, and rapid testing modality.

We believe that an alternate testing modality can be developed by using monoclonal antibodies directed towards viral RNA. Detection of RNA can provide the test, a specificity which is comparable to that of PCR testing; the monoclonal antibodies can subsequently be incorporated into an ELISA/ICT format, which can be conducted rapidly at relatively low cost even in resource poor settings. We base our hypothesis on the fact that antibodies are essentially protein molecules that interact with a wide variety of antigens as a result of formation of multiple non-covalent bonds like hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic forces based on complementarity in shape, hydrophobicity in terms of donor and acceptor groups, charge distribution and the capability to form H bonds. Nucleic acids such as DNA and RNA are known to possess such characteristics, which enable their avid interaction with proteins.[1] This interaction can be highly specific depending on the nucleotide sequence and structure and has now been observed for over 3 decades.[2] This sequence-specific recognition is brought about by the bendability of RNA allowing formation of hydrogen bonding and Van der Waals interactions between protein side chains at lower free energy. The minor grooves of RNA are shallow and broad and can enable penetration by alpha–helices of proteins. In this course, preferential binding of certain amino acids like guanine is seen with uracil base pairs. As a matter of fact, such guanine quadruplexes are present in COVID-19 RNA, which provide binding sites for positive charged pockets of SARS-unique-domain proteins.[3] Special roles in this binding are played by positively charged residue, lysine, arginine and aromatic residues like phenylalanine and tyrosine. Several bioinformatics tools have been developed so far that can assist in predicting the interaction of RNA binding amino acids with RNA sequences. Considering all these facts, binding of antibodies to specific RNAs seems quite plausible (Fig. 1).

Jung et al. have shown in their research, how a monoclonal antibody could recognize a domain of Brain Cytoplasmic (BC200) RNA in a structure and sequence-specific manner.[4] O'Brien et al. have developed two monoclonal antibodies that specifically react with viral RNA intermediates produced during the replication of arboviruses in mosquitoes. These monoclonal antibodies react with dsRNA greater than 30 base pairs in length, which has later been converted into an ELISA or immunofluorescence for demonstration of viral detection.[5]

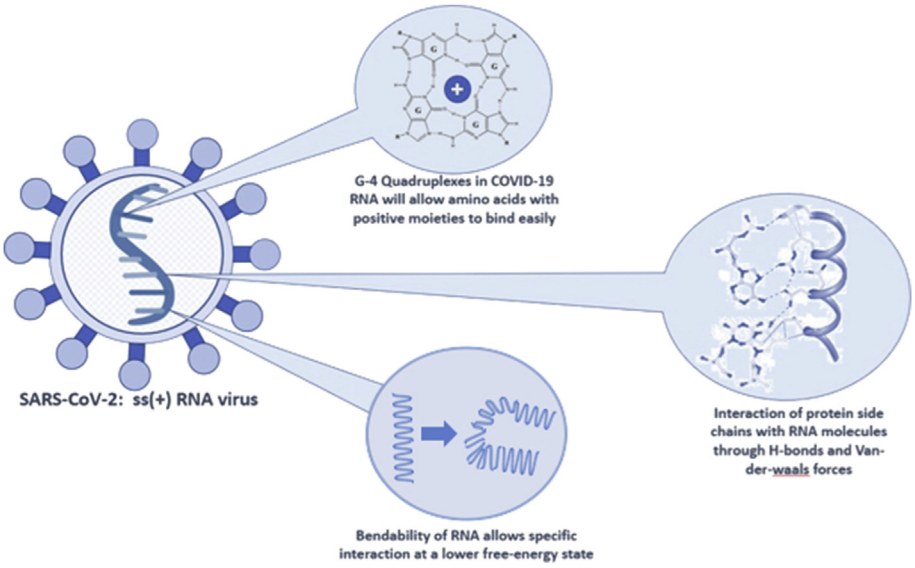

Fig. 1 – A schematic diagram explaining the various factors present in SARS-CoV-2 RNA that would enable its binding with monoclonal antibodies (Image courtesy: Suyash Singh.)

Hence, we believe that antibodies can be created against COVID-19 viral RNA and thereafter ELISA-based testing modalities can be developed, which can carry out the detection in a simpler cost-effective manner.

Although the development of an appropriate method for the preparation of anti–COVID-19 RNA is a matter of thorough experimentation and analysis, before any tangible results are obtained, the bioinformatics tools may come to our rescue. Anti-RNA antibodies can be accessed from Phage-Display Library or other biopanning techniques, their corresponding genes recovered, amplified and cloned into an appropriate vector and introduced into a host. By using modelling programmes, one can determine the various complementarity-determining regions and other epitopes required for epitope binding, which can guide subsequent mutagenesis steps to get the desired antibody DNA. The modified DNA can be injected back into a host, and the antibodies produced isolated. Once these antibodies are synthesized, they can be made into ELISA or other ICT diagnostic forms to enable COVID-19 detection.

This new testing model for COVID-19 by the development of antibodies against COVID-19 RNA seems to be a theoretically sound idea. However, as with any antibody or antigen detection test (here the antigen being viral RNA), this modality may also suffer from relative lack of sensitivity and problems with level of detection. The research on similar lines in the past yielded promising results, thus show the technical feasibility of our proposal. We are optimistic that research focused in this direction will be fruitful and will greatly help in containing this menace of COVID-19 in a simpler and cost-effective manner.

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