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LETTER TO THE EDITOR

LAMP Assay: Could it be a Boon for the Molecular Diagnosis of COVID-19?

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Dear Editor,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a major threat for mankind and poses a great challenge to global economy. On 31st December 2019, the World Health Organization (WHO) was informed about a cluster of pneumonia cases in Wuhan, China [1]. Subsequent investigations identified a completely unique sequence of coronavirus which differed from existing type SARS-CoV known as SARS-CoV-2. Initially, it was observed within the Wuhan province of China but later spread fast to rest of the world, which forced WHO to declare COVID-19 as pandemic. So far there is a total of 10,302,867 confirmed cases, with 505,518 global deaths, while 5,558,161 are recovered cases. In India, a total of 5,68,473 confirmed cases out of which 2,15,838 of active cases and 3,35,656 of recovered cases also 16,919 of the death cases are reported till date. The SARS-CoV-2 is a single-stranded, positive-sense RNA virus [2] with its complete genetic sequence been decoded and made available to the Global Initiative on Sharing All Influenza Data (GISAID) platform on January 10, 2020. This provided an opportunity for several R&D companies and research institutes to design specific primers targeting COVID-19 which further lead to the development of RT-PCR diagnostic kits (NCBI Reference Sequence: NC_045512.2).

Currently there is search for the development of highly sensitive and specific molecular diagnostic kits in line with real time PCR (RT-PCR), easy to use and cost-effective which is vital in combating the evolving SARS-CoV-2 pandemic. Timely identification of individuals infected with SARS-CoV-2 will enable rapid isolation of infected patients and track their contacts to screen and also to prevent further spread of the virus. Till date, the majority of molecular diagnostic test kits have utilized the RT-PCR technology targeting different genomic regions of SARS-CoV-2 for detection in line with other serological techniques for aiding the accurate diagnosis of infection [3]. PCR-based methods can detect very low levels of viral RNA, so are highly sensitive and can enable early diagnosis of COVID-19 within few days of infection. However, they require highly skilled personnel and sophisticated, often expensive equipment, with turn-around times landing in excess of 24 h (including shipment of samples) meaning they are not suitable for rapid, point-of-care situations. Moreover, issues with poor sampling technique can also cause a relatively high rate of false negatives. Reagent shortages are causing bottlenecks in many countries, limiting the amount of tests that can be performed day-to-day. RT-PCR requires multiple temperature changes for every cycle, involving high end thermal cycling equipment.

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Hence, Isothermal amplification method is an alternate strategy that permits amplification at a single temperature and eliminates the necessity for a thermal cycler. Loop-mediated isothermal amplification (LAMP) is a rapid and accurate isothermal amplification technology [4–6], which has been applied for the detection of virus, bacteria and fungi. The LAMP reaction generally takes place during a constant temperature, and therefore the target regions are often amplified within 30 min. The LAMP method uses 4 to 6 primers to bind six regions of a target gene with high specificity. Since the LAMP method only needs a single constant temperature for amplification and simple device to detect the amplified signal there by excluding the use of any sophisticated equipments. There is commercially available reverse transcriptase enzyme (New England Biolabs, UK) which makes it possible to combine both reverse transcription and LAMP technology in single reaction. Since SARS-CoV-2 is a RNA virus with the length about 30 kb [7], the single reaction of reverse transcription (RT) and LAMP together can significantly shorten the reaction time without the DNA purification step from RT, thus a rapid detection of SARS-CoV-2 can be achieved [8]. The RT-LAMP primer set enables highly sensitive detection of the SARS-CoV-2 virus genome that induces a strong colorimetric reaction, due to this intensity in color change, point-of-care LAMP-based diagnostics requires only simple detection instruments. This technology could overcome such cost restriction of RT-PCR and still detect nucleic acids from the pathogen. Loop mediated isothermal amplification methods can prove to be economically profitable and can be applied for point-of-care testing. The intercalating fluorescent dyes used are compatible with LAMP reaction so that amplification can be read in real-time [9, 10]. Since, the efficiency of target amplification in a LAMP reaction is high, changes in reaction mixture components make it possible to detect the result with colorimetric detection methods such as colour change of positive and negative amplicon [5, 10] which can be read by using simple spectrophotometer. LAMP technology has the advantage to be utilised in resource limited lab settings and due to its simplicity and robustness can be adapted in remote areas as well.

In conclusion, the ultimate goal is to develop a simple molecular assay that can be used to screen as many individuals for SARS-CoV-2 virus at the point-of-care situations without compromising the sensitivity and specificity of the assay. Further, it could be beneficial in terms of reducing running cost, infrastructure and skilled manpower. In addition, the RT-LAMP could be used as a rapid and large-scale screening confirmatory molecular assay where susceptible asymptomatic cases can be easily identified at early stage of the disease in our clinical settings.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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