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Short Communication

Modern Approaches for Diagnosis of COVID 19: A REVIEW

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Coronavirus, presently coined as the 2019 novel coronavirus, appeared from Wuhan and give rise to a challenging outburst in many cities in China and extended internationally, including Thailand, United States, Japan and India. The disease is formally termed as Coronavirus Disease-2019. It is also coined as Severe Pneumonia with Novel Pathogens by the Taiwan CDC and is a remarkable transmissible disease of the fifth category. COVID-19 is inherently a zoonotic disease with low to moderate (estimated 2%-5%) mortality rate [1].

The recent outburst of Coronavirus disease 19 (COVID-19) due to SARS-CoV-2 virus providing an opportunity to enlarge the scope of several diagnostic approaches including FELUDA and SHERLOCK that make an alteration in the ongoing public health emergency throughout the world. In accumulation of general social distancing, detection of infected individuals and showing their interactions for possible quarantine measures is one of primary steps for dropping community transmission of this virus31-33. Now a days quantitative Real-Time (qRT) PCR is measured as a gold standard test for identifying active COVID19 cases, such examinations are expensive, have required long times and need a devoted qRT-PCR machine, due to limited utility in handling an emergency of this scale [2-4]. It is pursued to repurpose FELUDA as a lateral flow assay (LFA) for the identification of SARS-CoV-2 that is cheap, does not require complex instrumentation, with precision and accuracy in diagnosis. To permit such a diagnosis on commercially obtainable paper strips the chemistry of capturing RNP-bound biotinylated substrate molecules on a different test line of the paper strip is enabled by using FAM labelled chimeric gRNA. By utilizing an augmented single step Reverse Transcription-PCR protocol trailed by FELUDA, an assay that can sense SARS-CoV-2 sequences from RNA samples within an hour was developed. Almost up to 21 targets across the SARS-CoV-2 RNA genome was tested and two regions were significantly detected (in the viral N and S genes) and described minimal number of mutations in publicly available datasets. Through wide-ranging optimization of PCR and reaction components, FELUDA touched a limit of detection (LOD) of nearly around 10 copies of purified viral sequence. Regular dilution of patient RNA, FELUDA and qRT-PCR both were proficient to recognize samples up to the similar dilution range. Meanwhile visual discovery can occasionally have an operator-bias, particularly when the signal is very dim, a smartphone app TOPSE (True Outcome Predicted via Strip Evaluation) was developed to assist finding by inveterate a predictive score depending on background correction.

SHERLOCK that is known for specific high sensitivity enzymatic reporter unlocking technique. It hires an enzyme named Cas13a

as an effector and was embattled to the S and ORF1ab genes of SARS-CoV-2. Cas12a and Cas13a enzymes expressed a collateral cleavage activity which can be elucidated as cleavage of an additional RNA non-specifically succeeding the cleavage of target RNA. Cas13 remains sedentary if two or more divergences are found in target RNA and competently differentiates between SARS-CoV-2 and other identical viruses [5-7]. As per one report, it was reported that quenched fluorescent ssRNA reporter was exploited in SHERLOCK technology. Its sensitivity has been improved by utilizing recombinase polymerase amplification (RPA) or reverse transcriptase-RPA (RT-RPA) to intensify specific DNA or RNA prior to the commencement of the reaction. The test may be performed by utilizing from extracted RNA of patient samples, as mentioned in qRT-PCR, and delivered out with a dipstick within an hour without the need for any additional equipment. As a consequence, SHERLOCK for SARS-CoV-2 detection is noticeably faster than qRT-PCR and showed marked sensitivity [8]. Further in addition to the traditional approaches, from SHERLOCK to progress "STOP" that signifies "SHERLOCK Testing in One Pot" for post operative diagnosis of COVID-19, named as "STOP Covid". From a survey it was reported that "SHERLOCK" identification tool helps to detect a total of 534 clinical samples [9].

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