Title: Development of non-PCR based point-of-care SARS-CoV-2 RNA detection technique

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Rational: Currently, the most accurate method to detect the SARS-CoV-2 infection is the identification of viral genetic RNA by RT-PCR. The viral RNA is usually collected from the upper respiratory tract swab or saliva. However, the requirement of high level of expertise and good infrastructure facility becoming a bottleneck towards widespread application of PCR based testing protocol, a need of the hour. Therefore, there is an urgent need of developing a novel RNA detection technique that will have the same specificity and sensitivity of RT-PCR, but the procedure will much simpler and will be required minimum amount of training. Towards this goal, the current investigation intends to develop a novel Surface-enhanced Raman spectroscopy (**SERS**) spectroscopy-based method that can be operated at any point-of-care facility with minimal instrumentation.

Objective: The current FDA approved COVID-19 RT-PCR test uses three primer and probe sets to detect three regions in the SARS-CoV-2 nucleocapsid (N) gene[1]. This unique RNA segments and subsequent amplification of the corresponding cDNA affords an accurate and sensitive detection of viral gene. It was recently reported that the initial median viral load observed in hospitalized patients in China is 3.3 X 10^6 / ml of saliva samples [2]. This suggests that the concentration of RNA in the collected saliva will be around 10^{-14} M. **SERS** is known to detect sample at a concentration of 10^{-9} M or higher [3]. So, the capture of the RNA in a SERS substrate by employing similar DNA primers currently used in the FDA protocol and subsequent removal of all other non-bound RNA, it may be possible to detect Raman signals corresponding to uracil which is absent in DNA primer employed for capture. Thus, a positive uracil signal will confirm a viral infection. This will also have the same specificity as RT-PCR, as identification of viral genome have same scientific basis, but different detection technique (amplification vs SERS detection). However, this label-free SERS detection may lead to a sensitivity issue with uracil, as uracil is not known to be a strong Raman reporter and actual volume of the sample may not be 1 ml in all cases (experiment need to be repeated) [4]. So, an improvement in the sensitivity may be essential. This can be easily achieved by conjugating a strong Raman reporter to all possible combination of four nucleotide library (there will be 4^4 combinations or 256 sequences). The addition of this library will lead to the hybridization with the RNA in all over RNA sequence (except the primer binding position) of the capture RNA-SERS substrate. Considering the 30 kb size of the SARS-CoV-2, this will effectively increase the concentration of the Raman reporter to 10^{-10} M, which can be easily detected by handheld Raman instrument.

Methodology: We are currently working on the development of a convenient and affordable peptide and gold nanoparticle-based SERS glass substrate. The nanoparticle attached to the glass substrate will be functionalize with thiol modified DNA primer corresponding to SARS-CoV-2 nucleocapsid (N) gene. The final assembly will be reusable substrate for RNA capture. Following this, the tetranucleotide Raman reporter library will be added to the glass substate for hybridization with the genomic RNA. Simple positioning of the handheld Raman laser (which behave similarly to the current IR based handheld temperature device) and

positive presence of Raman reporter will signal viral infection. After the test, the SERS glass substrate can be cleaned with proper solvent and reused for next round of testing.

Outcome: After the optimization of the protocol, the operator only needs to follow a simple RNA separation technique and drop casting and drying of reagent before taking the reading with a handheld Raman spectrometer. It is even possible to reconfigure the spectrometer software just to report positive or negative.

Budget:

Budget for 1 years		
Non recurring	8 Lakhs	Laminar hood Hot Oven Freezer Minor instruments
Recurring	5 lakhs	Consumables, Contingency, Travel and Overhead

References:X1. https://www.fda.gov/media/136151/download.

2. To, K.K.-W., et al., *Consistent detection of 2019 novel coronavirus in saliva.* Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 2020.
3. https://doi.org/10.1038/s41564-020-0713-1.
4. https://doi.org/10.1021/acs.jpcc.6b02753