Title: Fast Detection of SARS-CoV-2 Antigen by a Dot Blot

Investigators: Dr. Saugata Saha and Dr. Sudit S. Mukhopadhyay, Department of Biotechnology, NIT Durgapur 713209

Rational: There are two widely accepted detection of SARS-CoV-2 at this moment.The most accurate and reliable detection is done by detection of viral RNA from upper respiratory tract swab or saliva by RT-PCR method. However, given the expertise and infrastructure required for this test and longer time taken to generate the test results, a faster and cheaper alternative is highly required at this time. However, the rapid test method available for the detection of the virus depends on the detection of Antibody raised against the virus. While this method is extremely fast and relatively cheap, it does not differentiate an active infection or an infection in the recent past. Thus a faster and cheaper method to detect an active infection is the requirement of the hour. Towards this goal, the current investigation intends to employ a dot blot detection method to detect virus antigens from the saliva samples. This is simple test based on detection of proteins from the COVID-19 virus in respiratory samples (e.g. sputum, throat swab).

Objective: Earlier studies with SARS-CoV have shown that, the estimated number of viral membrane protein (M) molecules present per viral particle is 800 to 2500 [1]. The similar protein in SARS-CoV-2 is 222 aa long and estimated molecular weight of the that protein is ~25 kDa. It was also recently reported that the initial median viral load observed in hospitalized patients in China is 3.3×10^6 / ml of saliva samples [2]. Thus conservative calculation suggests that, the amount of M antigen in the saliva sample is good enough to be detected by the commercially available highly sensitive Western Blot developing reagents. Commercially available antibody against SARS-CoV M protein and spike (S) protein will be employed [3, 4] initially to detect SARS-CoV-2 M protein from the saliva samples of the COVID-19 patients. The study is mainly aimed at developing, standardizing and optimizing the above reagents in a dot blot setup to make a faster and cheaper detection of active SARS-CoV-2 infection. This is also advantageous due to ease of sample collection as patients themselves will be able to collect their saliva.

Methodology: One type of rapid diagnostic test (RDT) detects the presence of viral proteins (antigens) expressed by the COVID-19 virus in a sample from the respiratory tract of a person. If the target antigen is present in the sample, it will bind to specific antibodies fixed to a paper strip enclosed in a plastic casing and generate a visually detectable signal (chemiluminescence or fluorescence tagged antibody), typically within 30 minutes. The antigen(s) detected are expressed only when the virus is actively replicating; therefore, such tests are best used to identify acute or early infection.

Outcome: Development of faster and cheaper detection method of active SARS-CoV-19 infection.

Budget:

Budget for 1 years		
Non recurring	15 Lakhs	Chemidoc, Minor instruments
Recurring	10 lakhs	Consumables, Contingency, Travel and Overhead

References:X1. Neuman, B.W., et al., *Supramolecular architecture of severe acute respiratory syndrome coronavirus revealed by electron cryomicroscopy.* Journal of virology, 2006. 80(16): p. 7918-7928.

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. Srinivasan, S., et al., *Structural Genomics of SARS-CoV-2 Indicates Evolutionary Conserved Functional Regions of Viral Proteins.* Viruses, 2020. 12(4): p. 360.
4. Wang, Q., et al., *Structural and functional basis of SARS-CoV-2 entry by using human ACE2.* Cell, 2020.