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Molecular diagnostic for COVID-19: literature of Review

A report

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Introduction

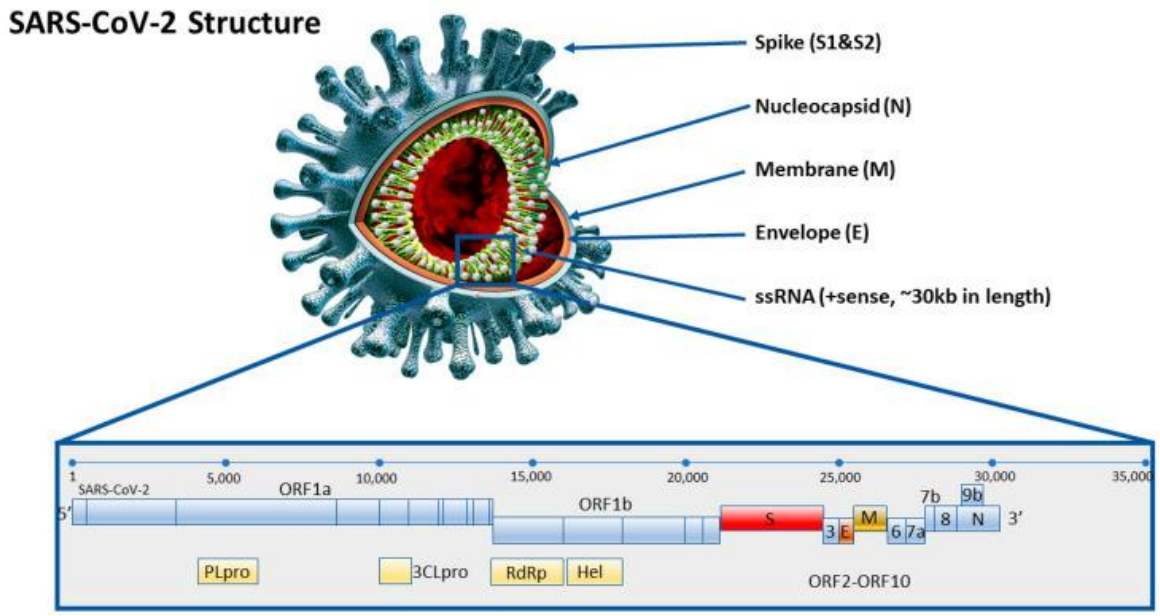
Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus. Most people infected with the COVID-19 virus will experience mild to moderate respiratory illness and recover without requiring special treatment. Older people, and those with underlying medical problems like cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop serious illness. (*World Health Organization ,2019*).

sdThe disease caused by Coronaviruses are enveloped, positive-sense single stranded viruses ((+)ssRNA virus) belonging to the family Coronaviridae. Most coronaviruses have 8-10 open reading frames (ORFs). ORF1a and ORF1b are translated

into polyprotein 1a (pp1a) and pp1ab, which are processed by viral proteases to produce 16 non-structural proteins containing RNA-dependent RNA polymerase enzyme (RdRp).

The viral RNA is replicated through transcription of a minus-strand template by RdRp. During replication, coronaviruses generate 6-9 subgenomic mRNAs (sgmRNAs), which lead to translation of accessory and structural proteins from downstream ORFs (*Sola, etal* , 2015) .

Spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins, necessary for completion of a viral replication cycle, are translated from sgmRNAs (Fehr and Perlman , 2015)



Diagnosis is a major aspect in tackling the consequences of any deadly contagious diseases. Diagnostic tests demonstrate the presence or absence of an infectious agent.

Early and better diagnosis has helped in limiting fatalities due to highly infectious and contagious diseases in the past (Caliendo *et al*., 2013).

Whole genome sequencing of SARS-CoV-2 led scientists to design testing protocols to detect the pathogen in the affected people and also provided an insight in the phylogenetic study of the virus. It was elucidated that SARS-CoV-2 belongs to the family of beta corona virus, which include SARS-CoV and Middle East Respiratory Syndrome (MERS) viruses (Zhou, *et al*, 2020a)

Currently, two major categories of diagnostic assays are commercially available for diagnosing SARS-CoV-2. The first group of assays identifies the viral RNA using molecular techniques that are based mostly on polymerase chain reaction (PCR) or nucleic acid hybridization. The second group are immunological assays that detect either antibodies that are produced in response to the infection or antigenic proteins. Laboratory-based SARS-CoV-2 molecular assays are currently the reference standard for the diagnosis of this infection (*CDC's*, 2020)

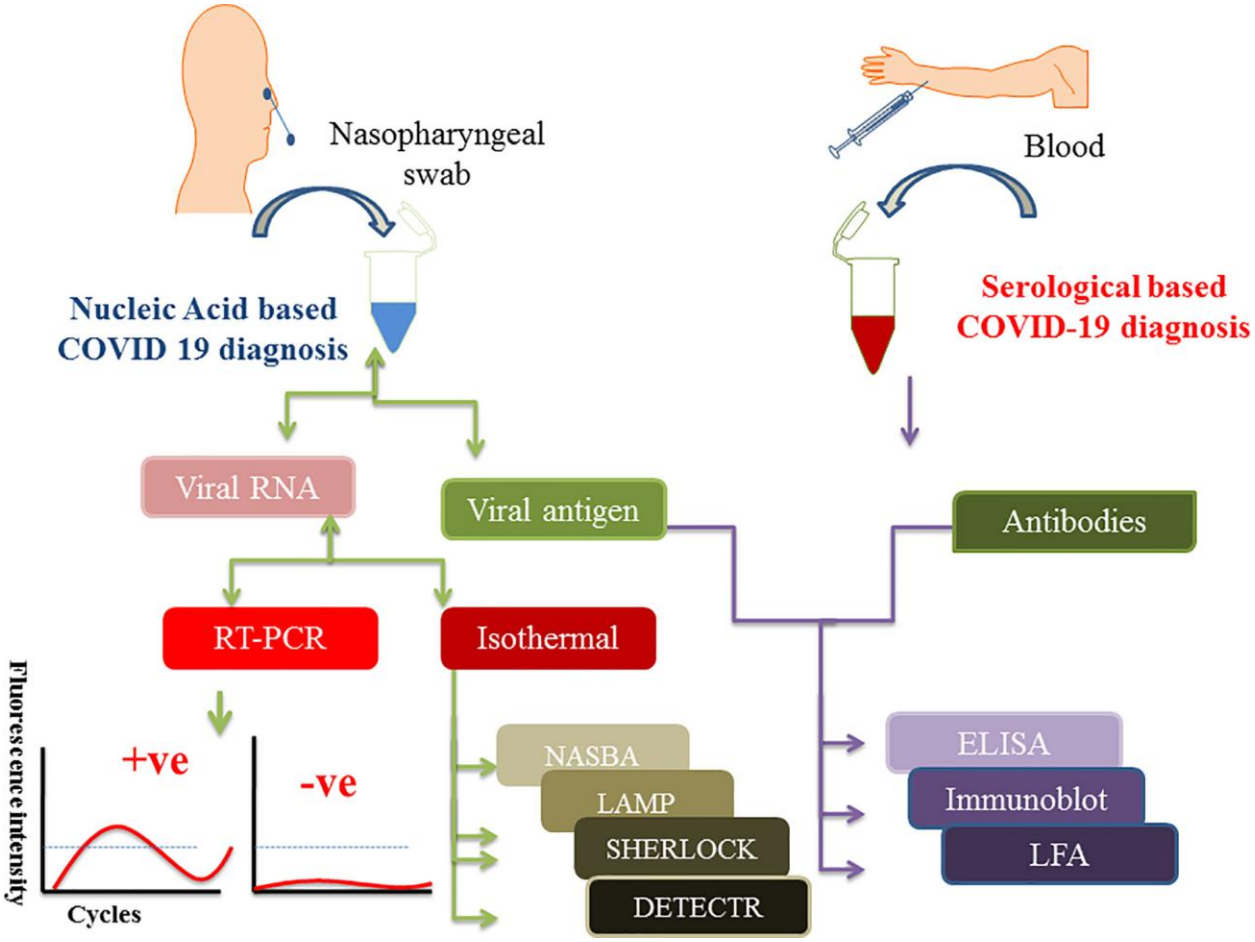
Samples collection:

The samples collection depend on primary clinical suspected diagnosis of covid19 Clinically the case definition of COVID-19 is persons presenting with a sudden onset of acute respiratory illness and at least one of the following symptoms: cough, shortness of breath, sore throat and fever (≥ 38 °C), or a history of fever, irrespective of admission status. According to a previous simple clinical signs the diagnosis must be confirmation with laboratory diagnosis For an early diagnosis of COVID-19, nasopharyngeal or oropharyngeal swabs are recommended (Zou, *et al.,* 2020).

However, a single nasopharyngeal swab is a method of choice for health practitioners because patients can easily tolerate it and is safe for handling. To obtain a

proper nasopharyngeal swab specimen, the swab must go deep into the nasal cavity eliciting tears in the patient. Collected swabs should be immediately transported using transport media to the diagnostic laboratory, ideally in refrigerated conditions. Patients with severe COVID-19 pneumonia have shown high viral loads in bronchoalveolar lavages, however, nasopharyngeal swabs were not compared in the particular study (Wang *et al.,* 2020).

These patients have also shown high viral RNA in fecal samples as well Thus the preferred method of collecting samples from advanced COVID-19 patients is from the stool or the rectal swabs. Sample collection for protein-based diagnosis like IgG/IgM and LFA, requires patients' blood samples. Figure 1 shows the schematics of specimen/sample collection for COVID-19 diagnosis as well as various nucleic acid and protein-based diagnostics approach. (Zhang W. *et al*., 2020).



Molecular diagnostic:

To detect this novel coronavirus, molecular-based approaches are the first line of methods to confirm suspected cases. Nucleic acid testing is the main technique for laboratory diagnosis. Other methods such as virus antigen or serological antibody testing are also valuable assays with a short turnaround time for the detection of novel coronavirus infection (Chen *etal,*2015).

As with other emerging viruses, the development of methods to detect antibodies and viral antigens are started after the identification of the viral genome. In general, the specific genetic regions selected as the target in RT-PCR diagnostic assays are very important. Assays targeting the E gene, which has been identified to be similar to that of other coronaviridae strains, have been shown to have the highest sensitivity (Corman *etal*,2020).

On the other hand, the low homology of the RdRp, N, and S genes in SARS-CoV-2 with those in other batrelated viruses makes these genes specific targets. Multiplexed assays targeting multiple geness imultaneously or detecting different regions in the same target gene have been used in various laboratories to increase the sensitivity of detection (chan *etal,*2020).

Digital PCR, the template is isolated into single molecules by sub dividing the reaction mixture into thousands of microscopic partitions with the ultimate goal of each partition containing, on average, less than a single copy of the template of interest. The quantity of the template in the sample is subsequently calculated using Poisson statistics based on the overall number of compartments that are either amplification positive or -negative (*Salipante*, 2020).

The advantages of digital PCR over quantitative PCR include quantification without the need for calibration curves, higher precision, and less susceptibility to artifacts that

may arise from sub-optimal amplification efficacy because of PCR inhibitors or primer/template mismatch (Hindson *etal*, 2013).

In addition, digital PCR has a higher analytical sensitivity due to its ability to partition the samples; this leads to decreased competition between various targets for the amplification reagents (*Quan*, 2018).

It also has a higher multiplexing capability compared with quantitative PCR (Whale AS, 2016). Nonetheless, the complicated workflow, which requires more expensive instruments and consumables and also longer hands-on time and higher staff costs, is a major disadvantage of this method that results in higher per-test cost compared with quantitative PCR (*Kuypers* J, 2017). In a study of 77 suspected COVID-19 patients, digital PCR was shown to have a higher sensitivity (94% vs 40%), negative predictive value (63% vs 16%), and accuracy (95% vs 47%) compared with RT-PCR. This advantage of digital PCR over RT-PCR was corroborated by other independent studies (*Falzone* L, *et al*.)

An investigation of 55 suspected COVID-19 cases who had had previous negative RT-PCR test results showed evidence of SARSCoV-2 genome in the NP samples of 35% of the tested individuals when they were retested with digital PCR (*Alteri* C, Cento V, Antonello M, *et al*)

False positive results

Globally, most effort so far has been invested in turnaround times and low test sensitivity (ie, false negatives); one systematic review reported false negative rates of between 2% and 33% in repeat sample testing.4 Although false-negative tests

have until now had priority due to the devastating consequences of undetected cases in health-care and social care settings, and the propagation of the epidemic especially by asymptomatic or mildly symptomatic patients,1 the consequences of a false-positive result are not benign from various perspectives (panel), in particular among health-care workers.

There are many potential causes of a false positive result, including the following:

1 Mislabeling at the point of collection and at the point of processing.

2 This can be guarded against by robust processes such as rigorous sampling and laboratory protocols.

Contamination during sampling and processing.2 Having skilled and well-trained personnel is crucial to keeping this type of error rate low. Additionally, having stricter standards imposed in laboratory processes and testing including external quality assessment schemes and internal quality systems may help reduce the risk of this happening to a minimum.

Low-level reactions in the PCR process, which may be generated for several reasons.2 Results with a single positive gene at low level (Ct>35) should therefore be treated with caution. Clear evidence-based guidelines on interpretation of low-level positive results should be developed for clinicians to become familiar with. Also, laboratories should report the details of the result to facilitate better interpretation at the bedside.

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