A Quick Reference to The Diagnosis Test for COVID-19: Guide to Every Healthcare Professional in Global Emergencies

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Abstract: The outbreak of novel COVID-19 (coronavirus infectious disease) caused by a coronavirus (CoV), which postures a challenge to healthcare professionals including diagnostic laboratories as the virus is widespread than initially thought. The authors aimed to list the various diagnostic methodologies which can be adopted to diagnose COVID-19 in patients. The present work is to bring the currently available tests to diagnose viruses especially SARS/ MARS, and the same can be used to diagnose COVID-19 (its design relying on close genetic relatedness with SARS), and even academic laboratories can serve to diagnose the individuals in emergencies. The writers succeeded in gathering the various diagnostic approaches to identify and confirm the presence of CoV in the serum samples. The present study concludes that with the knowledge of CoV diagnosis even academic laboratories can diagnose and confirm the CoV cases on par with public laboratories when desired.

Keywords: coronavirus, diagnosis, academic, public, emergencies, healthcare

INTRODUCTION

Coronavirus (CoV) infectious disease (COVID-19) caused by a novel CoV [1]. It mostly affects the nose, sinuses and upper respiratory tract [2]. In COVID-19, the letter '19' represents the year of the CoV outbreak [3]. This contamination if takes place in the upper respiratory tract will settle by itself. If the lower respiratory tract is involved medical attention needs to take care of immediately to decrease the death rate [4]. The symptoms that are most commonly associated are cold, cough, sore throat, and fever, these symptoms are very much similar to that of cold flu-like manifestations [5]. As the disease outbreak observed in the Middle East countries so the disease is also termed as Middle East Respiratory Syndrome (MERS) [6]. The disease mainly spreads by inhaling the droplets containing the virus from some infected patients [7]. The mortality rate, before get worsened, it must be diagnosed with fast and reliable tests.

CoV outbreak is widespread than initially thought, and posing a challenge to the healthcare professionals including diagnostic laboratories [8]. As CoV cases increasing, the public diagnostic laboratories are busy, and consuming more time in generating the report (owing to the less number of public laboratories in contrast to the CoV outbreak). During this period the CoV may further expand (as the patient is not confirmed about COVID-19). So, we attempted to bring the various diagnostic tests which can confirm the CoV in serum samples that can be performed even in academic laboratories. This article gives a quick information to the academic healthcare professionals (working in various colleges/universities/research laboratories) about the various approaches in diagnosing CoV, and serving the world in diagnosing CoV in a short span, and can prevent further transmission of CoV.

METHODOLOGY

Laboratory test for identification of viral contamination (like 19-CoV, MERS-CoV, and SARS-CoV2), is illustrated here. In general, the lab tests fall under two categories [9].

Molecular test

It is a diagnostic criterion for the detection of active infection in individuals who are suspected of infection depending on the area of an outbreak for 19-CoV.

- rRT-PCR (Real-time reverse transcriptase- Polymerase chain reaction)
- NAAT test (Nucleic acid amplification test) (Additional confirmation test)
- Radiological computed tomography

Serological test

It contains two screening test

- ELISA & CDC (Enzyme-Linked Immunosorbent Assay & Human cell division cycle)
- One confirmatory test (micro-neutralization)

These tests are elaborated as follows.

Molecular tests

Reverse transcription-polymerase chain reaction (RT-PCR)

The principle involved in this is the conversion of RNA into DNA with the help of enzyme 'reverse transcriptase' leading to the formation of complementary DNA and amplification using polymerase chain reaction (PCR) used to detect the amount of specific RNA by using fluorescence a technique also called real-time PCR or quantitative PCR (qPCR) used for the quantification of viral RNA by 'QT-NASBA' (nucleic acid sequence-based amplification) is the specific test available. This test consists of two processes [10].

- **One-step RT-PCR (**single-step process) (more convenient)
- **Two-step RT-PCR (**two-step process)

Quantification of the RT-PCR is done based on two processes End-point and real-time.

Endpoint RT-PCR

This test is used to measure changes in gene expression in a very little number of samples. The measure of endpoint RT-PCR is done by the use of fluorescent dyes like Ethidium bromide, Labelled PCR products, and Scintillating counting. Endpoint RT-PCR is done by 3 processes [11].

A) Relative RT-PCR: In this co-amplification of an internal control simultaneously with the gene of interest [12].

B) Competitive RT-PCR: This is used for absolute quantification. It involves the use of a synthetic "competitor" RNA that can be distinguished from the target RNA by a small difference in size or sequence [13].

C) Comparative RT-PCR: It is also involving in targeting RNA competes for amplification reagents within a single reaction with an internal standard of unrelated sequence [14].

Real-time RT-PCR

It is used to measure changes in gene expression on a global scale using four different fluorescent DNA probes SYBR Green; TaqMan; Molecular Beacons; Scorpions [15].

A) SYBR Green: Emits fluorescent by binding to double-stranded DNA [16].

B) TaqMan probes, Molecular Beacons and Scorpions: The creation of fluorescence depend on FRET (Forster resonance energy transfer pairing) of the dye particle and a quencher moiety to the oligonucleotide substrates [17]. The diagrammatic representation of the PCR is illustrated in Fig. 1.

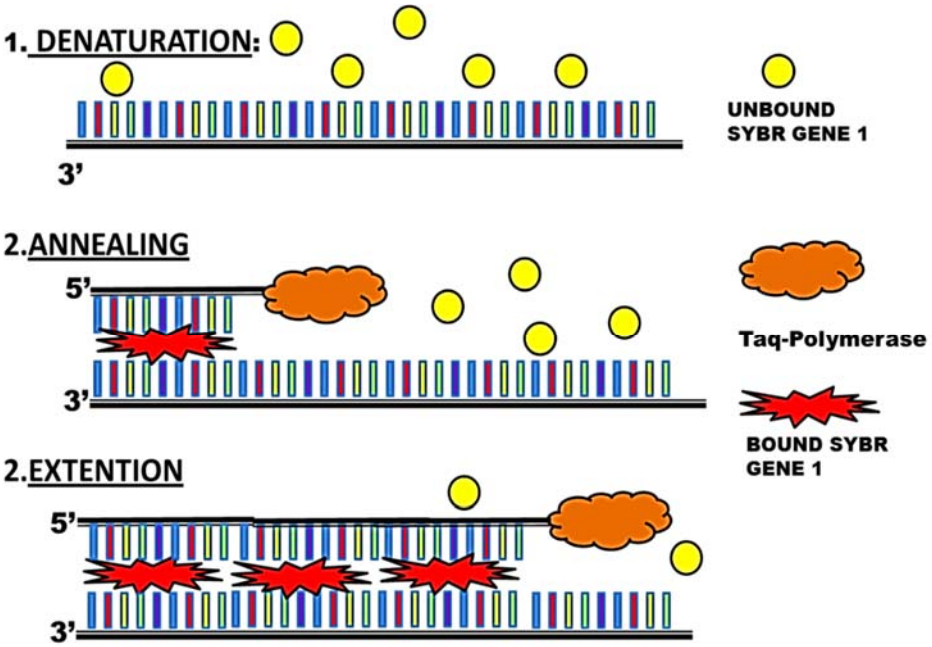

Fig. 1: Steps involved in Polymerase chain reaction

NAAT

It is a three-step method that directly amplifies and detects the genetic material of the viruses [18], these are

- Extraction and purification of the viral nucleic acid
- Amplification of the complementary DNA
- Detection of the amplified products

The major methodologies used to quantify the viral load are:

A) Reverse transcriptase-polymerase chain reaction (RT-PCR) [RT-PCR makes use of an enzyme that synthesizes DNA in an RNA form [19]. This is coded by the pol gene of the retrovirus and by certain elements similar to the retrovirus.]

B) Nucleic acid sequence-based amplification (NASBA) [NASBA is an isothermal process for the *in vitro* amplification of nucleotides [20]. The process involves concomitant action from polymerase DNA, directed by RNA, and ribonucleases and polymerase RNA, directed by DNA, in command to create a huge quantity of an exact sequence of RNA and DNA molecules.

C) Pronged DNA sign magnification analysis [One finish of the bDNA particle is designed to bind to a specific marker [21] whilst the former ending of the particle contains many DNA branches that are considered to connect to a search that is used for signal detection].

Radiological test

Computed Tomography

Upon being suspected of being presented with the symptoms of 19-CoV, researchers believe that radiological studies can come handy in confirmation of the symptoms to be as 19-CoV before the PCR results [22, 23]. The following characteristics are being studied in individuals presented with positive manifestations of 19-CoV.

- Presence of ground+-glass opacity
- Presence of consolidation
- Number of lobes affected by ground glass or consolidation opacities
- Degree of lobes involved in addition to overall lungs “total severity score “
- Presence of nodules
- Presence of pleural effusion
- Presence of thoracic lymphadenopathy (lymph nodes of irregular dimension or morphology)
- Presence of core lung infection such as emphysema or fibrosis or any other thoracic abnormalities.

Chest X-Ray

This involves the use of a minute quantity of ionizing waves to create a photograph of the within of the trunk to assess the lungs in case of positive symptoms of shortness of breath, Pneumonia, Emphysema, and related to 19CoV. Ground glass opacity in the lower corner of the lungs found in the X-Ray of COVID-19 individual [24].

Serological test

ELISA (Enzyme-linked immunosorbent assay)

This method is a very sensitive method for determining the quantity or for testing the presence of antigen or ligand in the specific suspected serological sample. In this test enzyme-linked antibodies are used to bind the analyte on a solid substrate base and convert a colorless reporter molecule to a colored or luminescence product for detection [25]. ELISA test is diagrammatically represented in Fig. 2.

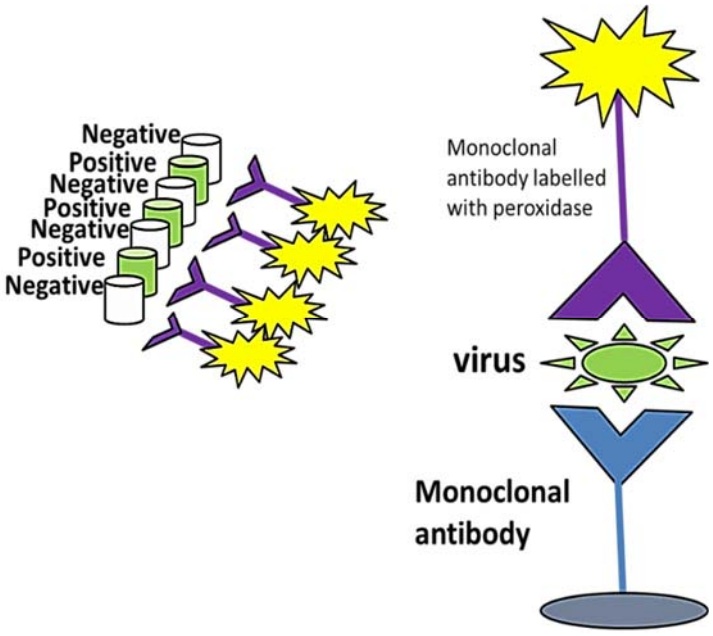

Fig. 2: Diagrammatic representation of ELISA test

Micro –neutralization test

It is a confirmatory test for the detection of viral load in the blood /sera sample of the individual suspected to be encountered with positive symptoms /cases of 19-CoV [26].

The various diagnostic tools for 19-CoV is illustrated below.

MERS-CoV- test kits

A) Based on qRT-PCR

- Power check (Kogene biotech, Korea)
- Diaplex Q (Solgent, Korea)
- Anyplex (Seegene, Korea)
- Loop man RNA amplification kit (Tokoyo, Japan)
- RT-iiPCR

B. Screening: Envelop gene (upE)

C. Confirmation

- ORF1a
- Accupower (Bioneer, Korea)
- Light mix (Roche molecular diagnostics, Switzerland)
- Ultra-fast kits (Nanobiosys, Korea)

SARS-Cov- test kits

- Real-time qRT-PCR
- Antibody-based capture ELISA
- Enhanced real-time fluorescent PCR
- Nested PCR
- Neutralization test
- Western blot assay with N195 protein
- Real-time quantitative RT-PCR modified RNA extraction method 1b region of SARS-CoV
- IFA
- Indirect IFA
- SARS-Cov real-time PCR assay with Taqman minor groove binder probe developed by applied biosystems.

Other diagnostic tests for the confirmation the viral infection

Few more diagnostic tests are available for testing COVID-19 are listed below [27, 28].

Pulse oximetry

It is a secondary diagnostic test that tends to measure the oxygen levels (oxygen saturation) in the blood, which tend to confirm the presence of the infection based on the symptoms present alongside (i.e., shortness of breath). The normal values of pulse oximetry are as follows.

Oxygen saturation 75-100 mmHg = normal

Oxygen saturation <60 mm Hg =Abnormal (supplemental oxygen required).

Pulse oximetry 95-100 % = Normal

Pulse oximetry <90% = Abnormal

Arterial blood gas (ABG)

It is also a differential diagnosis test that supports the primary diagnosis upon confirmation of the COVID-19 symptoms. The experiment processes the acidity (pH), the level of oxygen and carbon dioxide in the blood by, withdrawing blood from the artery by puncture of an artery, this test gives an idea of how the lungs works, as the lungs are a major effected organ in case of SARS-CoV2/ 19-CoV. The normal values of arterial blood gas are as illustrated below [29, 30].

pH: 7.35-7.45

Partial pressure of oxygen (PaO2): 75-100 mm Hg

Partial pressure of carbon dioxide (PaCO2): 35-45 mm Hg.

Coagulation screen test

It is a mixture of screening test which is being designed to provide specific information about blood or haemostatic problems which gives a brief idea about the lung function, the basic screen test includes [31, 32]:

Platelet count: 150,000-450,000 cells/µl (normal); <150,000 (thrombocytopenia)

Bleeding time: 2-7 min (normal)

Prothrombin ratio: 11-13.5 sec

Activated partial thromboplastin time: 60-80 sec

Procalcitonin test

It's a differential diagnosis test used for the confirmation of the symptoms, this test measures the levels of inflammatory biomarkers whose levels rise in the blood in cases of heavy bacterial or viral load [33]. The normal ranges are illustrated in table 1.

Table 1: Blood procalcitonin levels and its interpretation

Range of procalcitonin (ng/ml)	Inference
<0.10	No systemic inflammatory response
0.10-0.49	Minor local infection
0.50-1.99	Moderate risk to systemic infection
2.00-9.99	Increased risk to systemic infection
>10.00	Septic shock

C-reactive protein

It is a differential diagnosis test contrast to the symptoms, it is a protein secreted by the body in response to inflammation anywhere in the body which is a result of invasion by the virus [34]. The range of C-reactive protein in blood is <10mg/L, whereas >10mg/L, indicates Abnormal (serious infection).

Troponin T

It is the part of the differential diagnosis parameter which is used to confirm the presence of the viral infection. It is an ingredient of the troponin compound which is used for the contraction of the muscles including cardiac muscle [35]. Troponin-T ties to tropomyosin and helps arranging it on the actin, the troponin T levels are elevated in the circumstances like cardiac injuries (owing to exacerbation by chronic obstructive pulmonary disease), which is observed as a symptom of 19CoV. The value below 0.04ng/ml indicates normal and above indicates the heart attack.

Serum Lactate Dehydrogenase (LDH)

LDH is an enzyme-bound in almost all tissues of the body acts as a vital character in the metabolic process but in cases of tissue damage due to lack of oxygen supply, this impetus is unrestricted into the blood-stream [36]. The elevated levels of LDH are being observed on a hike in a patient with lung infections and extrapulmonary disorders. The normal ranges are shown in table 2.

Table 2: The normal ranges of LDH on various stages of life

Stage	LDH value (units/L)
New-born	160-450
Infant	100-250
Children	60-170
Adults	100-190

Serum Creatine Kinase (CK)

It is a kind of diagnostic criteria to confirm the presence of infection based on the viral infection symptoms. It is produced by the muscles of the heart, brain, skeletal muscles and other tissues [37], which is released into the bloodstream in case of exacerbation of COPD which is in turn related to infection and shortness of breath related to 19CoV. 22-198 units/L indicates the normal and >198 indicates abnormal.

CONCLUSION

The present study concludes that with the knowledge of Coronavirus diagnosis even the academic laboratories can diagnose and confirm the Coronavirus cases on par with public laboratories when desired during global emergencies.

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