Currently Used Diagnostic Techniques for The Detection of SARS COV-2 Coronavirus

Muhammad Tayyab1, Adnan Ahmad Khan2, Amir Husain Shahzad1, Muhammad Tahir Zakria1, Muhammad Athar3, Aisha Waheed Qureshi1, Shomaila Sikandar1, Muhammad Aqeel Syuami4, Nasir Iqbal5

ABSTRACT

The corona virus disease being aware commonly as Covid-19 in passing days among common masses is a novel viral illness of the systema respiratorium, causing a sustained pandemic which is in particular characterized by atypical pneumonia with specific symptoms of mild fever, runny nose with secreted fluid similar to flu, hypoxia, myalgia, anosmia, ageusia, shortness of breath, normal or decreased leukocyte count, ground glass opacities, sore throat and cough without phlegm. It is caused by an epidemic referred as SARS CoV-2 with abbreviation of severe acute respiratory syndrome coronavirus-2 nowadays. The procedures to be adopted for the diagnosis of COVID-19 should be based not only on clinical but epidemiological agents also and anyways stand on the link to an assessment and likelihood of the infection. The SARS CoV-2 is diagnosed with different test techniques like serological, molecular and others but most common for it is RT-PCR which is widely used and most reliable one to authenticate the findings of this disease. In the earlier times its detection was too an issue but luckily soon after its threat the scientists and organizations involved themselves and developed strong techniques to get reliable and accurate results with peaceful outcomes. The including such techniques are like immunological assay, amplification technique like RT-PCR (Spin column-and poly amino ester magnetic nanoparticle (pcMNPs) extraction method. Real-time nanopore target sequencing (NTS) and amplification methods) while on the other hands latest in use techniques are Isothermal nucleic acid amplification (as their subcategories include transcription-mediated amplification (TMA), Loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA) and clustered regularly interspaced short palindromic repeats (CRISP), and TMA assay). The novel techniques developed for this particular purpose are Biosensors, Localized surface plasma resonance (LSPR) sensor, the Field effect transistor (FET) and Cell-based potentiometric biosen.

Keywords: SARS-COV-2, Diagnosis, Reliable Techniques, Overdone, Authenticate
INTRODUCTION

The theory of life-history can be regarded as a cornerstone in the memoir of evolutionary biology. It fundamentally depends on the predictions of how life stories may develop to boost individual life-span and reproductive successfullness of potential or inherent growth rate. The majority studies done herein concentrate in the life-history theories of simple or complex multicellular organisms. Coronavirus (CoVs) are members of family coronaviridae and order nivovirales with coronaviridae and torovirinae subfamilies. Their name is derived from the Latin word coronam meaning crown as it shows a crown like image under electron microscope because it possesses club-like spikes projections made of proteins on their surface. The genome structure of it consists of non segmented and single-stranded RNA containing virus with positive polarity. The viral genome of coronavirus is alllying with size from 29 to 32 kb length 70-140 nm diameter and 9-12 nm spike size which is packed in envelope of virus with corona-like appearance on surface. The viral genome bears five main open reading frames (ORFs) which are involved in encoding the replicase polyproteins named as ORF1a and ORF1ab, next to that are the spike (S), the envelope (E) and surface membrane (M). Therefore, the nucleocapsid protein (N) belonging to the replicase polyproteins is in a straight line translated from the central viral genome. There are two larger in size polyproteins formally called ORF1a and ORF1b which are not primarily involved in host response modulation. The translation of ORF1a encoding sequence is frequently extended with ORF1ab encoding sequences by one ribosomal frameshift to synthesize ORF1ab polyproteins. Against this, other viral own proteins are translated from sub genomic mRNA molecules which are synthesized by an irregular mechanism of RNA synthesis the bulk of corona viruses are pathogenic agents. Human coronavirus is associated with the respiratory as well as gastrointestinal tract diseases of animals. The corona virus claims severe respiratory, neurological, enteric and hepatic disorders in its hosts. It started with cases appearance of novel coronavirus (covid-19) which causes pneumonia or pneumonia like disease occurred in Wuhan, a city in Hubei province of state of China in November and December 2019. On the base of analyzed data taken from initial 425 confirmed cases of corona disorder primarily occurred in Wuhan, work was done collectively on the epidemiologic characteristics of novel coronavirus infected pneumonia (NCIP). The SARS-CoV-2 is regarded as a family member of the deadly Middle East respiratory syndrome (MERS) as well as the severe acute respiratory syndrome (SARS) corona viruses. The COVID-19 victims are visualized by the symptoms very much relevant to pneumonia like fever, flu, cough and difficulty in breathing and sore throat while other mild ones comparatively are hemoptysis, diarrhea and headache. The respiratory infection like symptoms might possibly be transmitted from animals to humans either directly or indirectly. The general public health dealing authorities have anticipated COVID-19 definitions with collective clinical features e.g. fever, cough along with annihilation of respiratory structures mainly lungs which in severe case may lead the patient to ventilator, linking it with epidemiological factors like frequent visit to a seafood, survey or purposeful journey to animal general bazaar in Wuhan and/or direct visit to a seafood, survey or purposeful journey to animal general bazaar in Wuhan and/or direct contact with with any patient of COVID-19 or suspect one for the sake to improve diagnostic accuracy in performance of tests. Unfortunately, these epidemiological features are not definite and have deprived positive predictive values obtained in the earlier stage of outbreak. The number of CoronaVirus Disease (COVID-19) cases are increasing progressively with the death rate of 4.63%, however, death rate is varied when compared country to country outputs as reported by WHO dated 29 March 2020. It has been wide spread across 201 countries globally with infecting over 658,347 individuals with the general mortality of 30,460 persons across the world (Till the end of June 2020 infection rate increased up to 10.1 Millions while death claims are 502000). Humans are at higher risk of contact with this disease that have been in touch with infected one or with confirmed COVID-19 case or even those who have traveled to the countries where an immense outbreak of this novel disease is being observed. The People who boast flu-like symptoms following trip from those countries or places in last 14 days may be a suspect of COVID-19. The most important point in the reference of detection is the similarity of its symptoms with influenza and common cold so it becomes mandatory as well as highly important to detect the infected individuals to get escape from its spread. The detection of COVID-19 patients in early stages is important for the physicians to avoid complications. The developments of the fast and reliable diagnostic techniques to avoid unnecessary precautions quarantine. The serological tests for the
The detection of COVID-19 include ELISA/CLIA based on the detection of antibody and RNA. In this type of serological testing the magnitude of antibodies is measured, particularly immunoglobulin G and immunoglobulin M which shows presence in the blood when the body reacts against a particular infection or infectious agent. Adapted serological tests for COVID-19 are framed for detection of virus in recently infected individuals instead of that are projected to determine the virus after the infection has become established and mounted an antibody reaction. RT-PCR is being used for the detection of COVID-19 strain corona virus while NAT on the other hand, is used for the detection of genes of COVID-19 which are named like the nucleocapsid protein (N), the Open Reading Frame 1a/b (ORF1a/b) and envelope protein (E) genes etc.\textsuperscript{11} The virus culture and isolation is relatively a time taking and patience process taking up to 96 hours and appearance of virus titer indicates successful culture of its nucleic acids. X-Ray along with Chest CT Scan are also used for the counter checking the conditions of patient at the right time.\textsuperscript{12}

### The Test Diagnosis of SARS CoV-2 Pandemic: Laboratory Safety:

Biosafety as well as protection procedures should be solid ground based with assistance of multiple risk levels of experimental procedures. Personal safety must be taken on priority with the accordance of BSL-3 laboratory protection protocols when collecting zonal specimens, micro linked macromolecular detection and performance of virus culture working.\textsuperscript{13} Personal safety measures in agreement with the set standards of BSL-2 laboratory protection set up should be prerequisite and ought to be managed for microbiological, biochemical, molecular, immunological and all other routine performed laboratory tests. All types of specimens collected from remote areas should be transported to the destination in special transport vessels and boxes which meet the standards set for the sake of biosafety. All laboratory waste should be strictly autoclaved before operations of final clearance.\textsuperscript{14}

### Worth of The Standardized Serological Tests:

In relation to detection a massive goal is to build up a serological test pattern that may be able to detect any past viral infections, if any was earlier, by trying to find antibodies somebody has already produced to conflict against the infectious agent like virus etc. Such a test will possibly provide a clue to predict the extent of viral stretch within a community and furnish useful public-health related information which may be helpful to counter it. Several prominent groups including Garry’s group (A dignified one) are working appropriately to develop a trust-worthy test. A gaggle of researchers at Icahn School of drugs at Sinai described another one in a review which was still not peer-reviewed at preprint stage which was placed on the medRxiv server on March 18\textsuperscript{rd}, 2020. We can’t expect any hindrance in creating these assays only tinkering to say Garry, a group of researchers in Singapore, worn serological tests to aid in touch detection but at that time this test had not been approximately considered suitable for clinical applications. We think that this come up to adapt first time on this planet where these type of tests are being utilized in this context as were reported by Danielle Anderson, a famous virologist at centre of excellence the Duke-NUS school of drugs in Singapore, in news conference in the month of February.\textsuperscript{15}

### Detection of Serum Antibody:

**ELISA/CLIA:**

ELISA stands for enzyme-linked immunological assay. When an individual is exposed to the virus, in natural defense response, his body develops antibodies which may take several days to over a week. Antibodies are proteins which the body develops when mounting a response against invading germs.\textsuperscript{16} The detection of immunoglobulin M (IgM) antibodies point out recent exposure to COVID-19. Some definite antibodies are produced by the infective body after suffering from SARS-CoV-2 infection. Few methods used for serum antibody detection include colloidal gold immune-chromatography, ELISA, chemiluminescence immunoassay (CLIA) and others.\textsuperscript{17} Positive serum-specific IgM or IgG antibody titer within recovery phase should be more or equal to 4 times above than that which would be found in the prior acute phase and are often used as diagnostic principles for assumed patients with negative macromolecular findings. During the follow-up evaluating tenure IgM is evident within a 10 days span after the symptom inception and IgG is perceptible within 12 days after onset of symptoms. The viral load progressively decreases when the serum antibody intensity rises continuously.\textsuperscript{18} By using this, an expert can’t determine that after how long any immunity will last and when re-infection would be possible. Results from antibody testing should not be used as ultimate because the diagnosis is possible only when the incubation period is over and even it does not tell about the infection status at the right time.\textsuperscript{19}
immunological assays are sensitivity and specificity in the reference of its practical applications. The most utilized proteins taken as target are S, N and receptor binding domains (RBD) in immunological assays in the detection of SARS-CoV-2. The S protein showed lower interaction with MERS-CoV while the higher one was seen with the S protein of SARS CoV. The S1 subunit protein showed only reactivity with SARS CoV. It may be due to S2 subunit but developed methods are more specific with S1 subunit. The RBD region in S protein has revealed cross reactivity between SARS CoV and SARS CoV-2. On the other hand N based ELISA/CLIA method expressed better sensitivity as well as specificity for the detection of SARS CoV-2. On making comparison of three proteins based tests, the RBD and N proteins based tests showed more sensitivity than S1 with mild sickness patients while on the other hand IgG based ELISA/CLIA demonstrated more sensitivity than IgM based ELISA/CLIA but less specificity. The overall sensitivity and specificity was a primary outcome in ELISA/CLIA methods and immunoglobulin class IgG and IgM or both while secondary outcome includes specific sensitivity and specificity inside the subgroup status under given conditions.

Molecular Based Methodologies:

Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR):

Mostly testing in current time for COVID-19 is performed on viral genetic material taken from nose and throat through swabs employing as a workhorse tool of biological investigations called as the reverse transcription polymerase chain reaction (RT-PCR). This test is based on amplifying a selected genetic sequence found within the virus. The short length complementary sequences referred to as primers help to urge the replication but PCR is able to detect viruses present within the sample of the individual. It doesn’t reveal how much it has resolved infections. It has also been seen that sometime it produces false positives if case of reagent contamination during a lab work performance. All over the world Laboratories have modified their PCR tests for SARS-CoV-2 by using different primers designed to target different but specific fragments of the virus’s genetic sequences. Random-amplification and deep-sequencing methodologies have played a vital role in identifying MERS-CoV as well as SARS-CoV-2. For the clinical diagnostic purposes the genetic heterogeneity of HCoVs precludes one “pan-HCoV” (the molecular assay). Some pan-CoV assays employ degenerate primers while some others use multiple non degenerate primer sets. Presently molecular respiratory panels which are able to detect the endemic HCoVs (HCoV-NL63, HCoV-HKU1, HCoV-OC43, and HCoV-229E) use multiple sets of PCR oligonucleotides. SARS-CoV-2 cases which were tested earlier found negative for prevalent HCoVs in their molecular respiratory panels. The oligonucleotide primers and probes for the detection of CoV-19 were selected from different regions of the virus nucleocapsid (N) genes. The panel is meant for specific detection of the COVID-19(two primers are included in a probe set). A further primer/probe set is used to detect the human RNAs P gene (RP) in the taken samples and clinical specimens which are additionally included in the panel. RNA taken as purified one from the upper as well as lower respiratory tract specimens and then is reverse transcribed to cDNA which subsequently is amplified within the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (others may also be used which are in competition like Biorad CFX-96, Rotor Gene etc.) through using SDS version of 1.4 software. During the process, the probe anneals a particular target sequence which is located between the forward and overturn primers. During the performance of extension phase in PCR cycle, the 5 end nuclease action of Taq polymerase ruptures the probe thus causing the reporter dye to interrupt away the quencher dye which generates a fluorescence signal. Within all cycles, additional reporter dye molecules are cleaved from their particular probes, increasing the fluorescence concentration. Fluorescence intensity is watched at each PCR cycle by Applied Biosystems 7500 Fast Dx Real-Time PCR System (or others as indicated above) with SDS version of 1.4 software.

Primers and probes for the described PCR is in descending sequence and are given as follows; 1.2019-nCoV-N1 2.2019-nCoV-N2 3.2019-nCoV-N3.

Diagnosis Based on Nucleic Acid Detection:

Macromolecular testing is the well-liked technique for diagnosis of SARS-CoV-2 infection. The adapted testing process including kit instructions is as follows: Specimens are pre-processed for the sake of virus lysis and therefore the virus is lysed to extract out its nucleic acids. The three definite genes of SARS-CoV-2 as aforesaid are the Open Reading Frame 1a/b normally called (ORF1a/b), the nucleocapsid protein called (N) and envelope protein known as (E) genes. Now these are amplified...
by real-time quantitative PCR technique. The amplified genes are detected on the behalf of their produced fluorescence intensity. Criteria of positive macromolecule result are: ORF1a/b gene and/or N gene/E genes are detected and their detection makes results positive.\textsuperscript{33} The combined detection of nucleic acids from multiple sorts of specimens taken for this purpose can improve the diagnostic accuracy. Among the patients with confirmed positive results of macromolecule in tracts about 30% - 40% of those patients showed detection of viral macromolecule within the blood and about 50% - 60% of patients have shown detection of viral macromolecule in feces. However, the positive rate of macromolecule testing in urine samples is a kind of lesser output. Combined testing with specimens from tract, feces, blood and others is useful for improving the diagnostic sensitivity of suspected cases with their monitoring, treatment and efficacy therefore the management of post-discharge isolation is considered as adoptable measures.\textsuperscript{34}

**Virus Isolation and Culture procedures:**

The isolation of the virus was made from oropharyngeal as well as nasopharyngeal sites for the samples collection from patients of putative COVID-19. Samples of oropharyngeal site were made diluted with viral transfer medium enriched with nasopharyngeal swabs containing antibiotics (Nystatin, penicillin–streptomycin with 1:1 dilution) at 1:4 ratio and were incubated for one hour at 4°C done prior inoculation onto the Vero cell line. Virus culture should be performed in the laboratory not less than qualified grade 3 (BSL-3).\textsuperscript{35} The process is briefly described as follows: Fresh samples of sputum, feces, etc. taken from the patient are first of all collected and then is inoculated on Vero-E6 cell type for the purpose of virus culture. Their cytopathic effect (CPE) is detected after the time span of 96 hours. Diagnosis of viral nucleic acids in the used culture medium expresses successfulness of the culture. Virus titer is measured after diluting the virus stock concentration by an element of 10 serial for the sake to adopt the TCID\textsubscript{50} method. The viral viability is determined by a plaque forming unit (PFU). If the cells show changes, known as cytopathic effects then the culture is considered as positive.\textsuperscript{36}

**Detection Indicators In The Inflammatory Response:**

It has been given recommendations for the performance of C-reactive protein tests, procalcitonin, ferritin, D-dimer, subpopulations and total of lymphocytes like IL-4, IL-6, IL-10, TNF-\(\alpha\), INF-\(\gamma\) with even other indicators showing the inflammatory and immune status which may be helpful in the evaluation of clinical progress. The alerts should be circulated on a regular basis for severe and significant tendencies to provide a base for the formation of treatment strategies. Majority of the patients with COVID-19 have shown a traditional level of procalcitonin together with significantly increased levels of C-reactive protein.\textsuperscript{37} A quick and considerably prominent C-reactive protein level indicates a chance of occurrence of secondary infection. Levels of the D-dimer are notably elevated in severe conditions which is a potential risk factor of the poor prognosis. Patients showing total number of lymphocytes at the time of onset of the disease generally have a lower level prognosis. The serious patients indicate a progressive decrease in the number or level of peripheral blood lymphocytes. The appearance levels of defense cells called IL-6 and IL-10 in severe patients increase extensively. Monitoring levels of IL-6 along with IL-10 may be helpful in having assessment to the risk determination of progression of coronavirus towards the level of severe situations.\textsuperscript{38}

**Use of Imaging Technique in Finding of COVID-19 Patients:**

The imaging of thoracic is the great concern in the field of diagnosis, monitoring, therapeutic efficacy with analysis and patient assessment of COVID-19 to be discharged. X-Ray as well as high-resolution CT scan are highly preferable choices in this context to go towards formulation of ultimate decisions. Portable chest X-rays are especially helpful in the case of those patients who are critical as well as immobile.\textsuperscript{39} On the other hand CT scan is beneficial for baseline evaluation of those patients of COVID-19 and it is typically performed on the admission day in hospital or in case of ideal therapeutic efficacy is not provided then it may be re-performed after the gap of 2 to 3 days time span. After it, if still its symptoms stably exist with persistency or show bit improvement after getting treatment then the chest CT scan can often be reviewed after a gap of 5 to 7 days time span. Daily routine based portable chest X-rays are suggested ordinarily for the critically sick patients. The patients suffered from COVID-19 at the early stage often represent multifocal patchy signs shadows or ground glass opacities observably located in the lung periphery, sub- pleural area and both lower lobes of the chest CT scans.\textsuperscript{40} The long axis of the lesion(s) is mostly seen on the parallel pattern to the pleura. The thickening in Interlobular
septal as well as intralobular interstitial thickening present as subpleural reticulation formerly named as “crazy paving” pattern which is detectable in some ground glass opacities. A lower number of cases may exhibit solitary, local or nodular/patchy lesion distributed along the side of bronchus with small peripheral ground glass opacities.\textsuperscript{41} Disease successions happen mostly in the time period of 7-10 days with symptoms of inflamed and increased density of seen lesions which are compared with previous images and consolidated lesions with air bronchogram signs. Critical cases may show further extended consolidation with the entire lung density presenting increased levels of opacity which is sometimes referred to as “white lung”. When the situation is relatively relieved, the bottom glass opacities are most often entirely absorbed and few consolidation lesions will leave fibrotic stripes or subpleural reticulation there.\textsuperscript{42} Patients showing multiple lobular collaboration particularly for those who are found with extended lesions may be observed for the disease exacerbation while those with typical CT scan pulmonary manifestations should be isolated and follow continuous nucleic acid tests even the nucleic acid test of SAR-CoV-2 is found negative as well.

Figure: Imaging Findings of the COVID-19 Patients

The Typical features of the COVID-19 taken from CT Scan:
Figure A, Figure B: showing patchy ground glass opacities.
Figure C: showing patchy exudation along with nodules.
Figure D, Figure C: showing multifocal consolidation lesions presence.\textsuperscript{43}

CONCLUSION

Conclusion  Effective diagnostic testing is not only worthy but vital as well for the successful detection of the novel coronavirus disease. As the world moved ahead, after the initial missteps, towards the state of emergency in the health sector, it became the need of the hour to counter such mishaps accurately. Timely guideline issuance for policy making and smooth practices should be made possible within the short span of initial response. There exists the potential in ELISA/CLIA to be taken as creditable in this context and easy to perform. RT-PCR can also be a time conserving with good sensitivity and specificity outcomes. The public health and medical communities dealing with health issues must identify the necessity to change the policy along with parameters effective and suitable in changing scenarios to support and compassionately resolve problems. It will prove a base to get escape from the losses and damages in future that may be a cause of repent. In the future it may be expected that development of more latest, advanced, reliable, easier and reproducible systems would be made to cope with such problems. System with more efficient results will help health professionals to make informal decisions to discharge people from hospital and home quarantine.

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