Clinical and Laboratory-based Diagnosis in Cases Suspected with COVID-19; An Updated and Comprehensive Systematic Review Study

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Abstract

Objectives
A novel coronavirus disease 2019 (COVID-19) was identified in Wuhan, China, which quickly involved majority of the countries all around the world. Due to the high rate of mortality and morbidity, needless to say the importance of accurate and early diagnosis, especially in suspected and asymptomatic cases.
Hence, in this article, authors tried to provide practical and standardized diagnostic approaches for cases suspected with COVID-19 infection.

**Material and Methods**

Data of this review study were collected from 7 search engine/databases, commencing from December 2019 to June 2021 by using 6 keywords according to Medical Subject Headings (MeSH) terms and our inclusion/exclusion criteria.

**Result**

Due to various clinical manifestations of COVID-19, and high potential for mutagenicity, identification of suspected patients is of great importance for effective control of infection, and improvement of clinical decisions. Therefore, medical history of the patients, clinical signs and symptoms, chest computational tomography, serological and molecular diagnosis can be effective in faster identification of mentioned patients. In spite of the fact that molecular tests have been considered as the gold standard for diagnosis of COVID-19, but there is still high rate of false-negativity. Then, combinative usage of the complementary tests can reduce any misinterpretations for suspected cases.

**Conclusion**

Screening for suspected cases in the shortest possible turnaround time is dependent on the appropriate diagnostic approaches. Subsequently, this allows physicians immediately provide proper medical interventions for suspected patients who are at greater risk for developing more serious complications than COVID-19 like severe nosocomial infections.

**Keywords:** Coronaviruses, COVID-19, Diagnosis, Detection Rate, NAAT, Suspected patients

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**1. Introduction**

From December 2019, a burgeoning rate of population have been involved in a highly contagious type of a severe respiratory disorder (*Coronavirus* 2019) in the *Huanan* seafood wholesale market of *Wuhan, Hubei province, China* [1, 2]. On 11th of February 2020, it was renamed as Severe Acute Respiratory Syndrome related Coronavirus-2 (*SARS-CoV-2*) by International Committee on Taxonomy of Viruses (ICTV) [3-5]. Phylogenetic-based studies proved common features among *SARS-CoV-2*, Severe Acute Respiratory Syndrome-causing Corona Virus (*SARS-CoV*), and Middle East Respiratory Syndrome-causing Corona Virus (*MERS-CoV*) [6-8]. Socioeconomic effects of *SARS-CoV-2* outbreak made World Health Organization (WHO) to announce a rapidly-spreading pandemic from 11th of March 2020 [7-13], and a societal concern for health managers [14]. No hesitate that an integrative collaboration of scientists is necessary for effective management of this situation by the usage of interdisciplinary frameworks [4, 9, 13, 15-17].

Accordingly, *Coronavirus Disease 2019* (COVID-19) in asymptomatic carriers and suspected ones should be immediately diagnosed [14, 18] due to the possibility for rapid developing serious nosocomial infections, and misdiagnosis [7, 9, 19-21]. WHO defines “cases of COVID-19” as “a person with the laboratory confirmation of *SARS-CoV-2* infection irrespective of clinical signs/symptoms” [4, 9, 22]. Trial seventh version of Diagnosis and Treatment Guidelines for COVID-19 issued by the National Health Committee of the People’s Republic of China declares “suspected” cases with one (of four) items of epidemiological history, or two (of three) items of clinical manifestations, or three items
of clinical manifestations with no item of epidemiological history [10, 23], or an acute respiratory tract SARS-CoV-2 infection for less than 14 days, or clinical illness compatible with COVID-19, or asymptomatic carriers in a close contact to the confirmed cases of COVID-19 [24, 25].

Speaking of “suspected cases”, pneumonic people with false results like patients with underlying severe cardiovascular sequelae [26], acute renal disorders, and dead people without ascertained SARS-CoV-2 infection [27-29], pediatrics (as potential carriers of COVID-19 with their imperfect immune system) [30], persons with multi-system inflammation [31, 32], Acute Coronary Syndrome (ACS) [33], and pregnant women undergoing delivery during hospitalization are of great prominence for rapid and accurate diagnosis, too. They are considered as the most problematic challenges for COVID-19, who can spread more viral respiratory co-infections than confirmed cases [7, 11, 16, 34-42].

Diverse clinical signs/symptoms, and similar clinical manifestations with other types of respiratory pathogens (Mycoplasma pneumonia, Chlamydia pneumonia, Adenoviruses, Rhinoviruses, Metapneumovirus, HKU1, NL63, Influenza type A/B, SARS, and MERS) [4, 7, 43] add erroneous results to diagnostic procedures, and adverse clinical outcomes.

Hence, highly-sensitive and time-preserving diagnostic methods can identify potentially infectious people [44], quantify exact viral load, decrease false-negative results from semi-sensitive tests or from suspected individuals [45-47], reduce the Turnaround Time (TAT) for identification of suspected individuals, immediately provide optimal medical interventions, potentially lower in-hospital spreading of SARS-CoV-2 infection, shorten the length of isolation/medical surveillance for suspected ones, lessen the socioeconomic fear for individuals who were in close contact with them, and help for determination of risk stratification [43, 45, 48-52].

Despite meticulous efforts for the sanitary recommendations, poor level of hygiene in undeveloped countries, and complying with quarantine regulations impose serious socio-medical challenges and extortionate expenditures until complete recuperation. Therefore, accurate and rapid diagnostic strategies can eliminate uncontrolled release of SARS-CoV-2 infection by asymptomatic carriers, and suspected persons [53-55].

Diagnosis of SARS-CoV-2 infection are mainly done by chest Computed Tomography (CT) images, and serological/molecular-based tests [56]. Molecular approaches reveal high analytical accuracy for initial quantitative diagnosis of SARS-CoV-2 infection [49] (like Nucleic Acid Amplification Tests (NAATs) strategies) [57]. Serological approaches (like Enzyme Linked Immunosorbent Assay (ELISA)) are highly dependent on the detection of neutralizing antibodies [9, 54], or viral antigenic proteins after viral exposure [9, 53, 54].

In this systematic literature review study, diagnostic efficacy of those methods for suspected cases with SARS-CoV-2 infection have been analyzed aimed at providing a successful adjustment of laboratory-based data with optimal clinical outcomes, and hopefully increasing life-expectancy for suspected cases with COVID-19.

2. Methodology
This comprehensive systematic review (systematic literature review) study was performed according to Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) statement guideline (https://www.equator-network.org/reporting-guidelines/prisma/) on June 2021 (figure-1).

2.1 Literature Search Strategy and Screening Process
An electronic comprehensive literature review was conducted with time interval commencing from December 2019 to October 2021, by using six main keywords (Figure 1), and four complementary ones (Clinical Diagnosis, AND Laboratory Diagnosis, AND Molecular Diagnosis, AND Serological Diagnosis), based on our inclusion and exclusion criteria.

In order to find potentially-eligible resources, one author independently conducted screening process in three main and one non-electronic backward steps (on the references/supplementary/bibliographies of included articles). Any uncommon points or disagreements were referred to the corresponding author (figure-1).

Figure 1: Search strategy (PRISMA-P extension 2020 statement). Created by Rezaei et al.

2.2 Inclusion and Exclusion Criteria
All of the published (original (experimental/non-experimental), review (mini-review/best evidence/narrative review/systematic reviews (and meta-analysis), comparative/cross sectional/cohort-retrospective/observational/commentary/letter to the editors/editorial/opinion/short (rapid) communication/Randomized Clinical Trials
(RCTs)/case reports/series) articles were considered in the formats of full-text/full-length, abstract, section of book, chapter, and conference papers in English language. Studies involving suspected human subjects aimed at laboratory-based assessment of SARS-CoV-2 infection through usage of biological samples (whole blood, serum, plasma, Nasopharyngeal (NP)/Oral pharyngeal (OP) swabs, Cerebrospinal Fluid (CSF), Bronchoalveolar Lavage (BAL), Tracheal secretions, sputum, and saliva), CT, and High Resolution CT (HRCT) were totally included.

In order to report the impact of the most efficient diagnostic method, studies involving human cases suspected with SARS-CoV-2 infection who had previously used any adjuvants, vaccines, anti-viral/herbal/self-therapeutic regimens all were excluded. Studies with irrelevant/insufficient/ambiguous data, lack of data, undefined diagnostic values/diagnostic methods all were excluded.

2.3 Data Extraction
Five independent authors majorly performed data extraction and made forms to collect study characteristics (author name, publication date, study design, used samples, types/subtypes of diagnosis). In case of overlapping data or several published reports from same studies, the authors tried their best to present the most complete data.

2.4 Bio-statistical Analysis
None

2.5 Ethical Statement
According to the structure and type of study, there is no need to register for Research Ethical Committee (REC). All of the data supporting the findings of this study, are openly available in the context of this study.

3. Results
3.1 Laboratory Guidelines
Polymerase Chain Reaction (PCR) is one of the most sensitive diagnostic approaches, detecting viral ribonucleic acid of SARS-CoV-2 infection in NP and OP swabs of asymptomatic carriers or suspected cases. Due to false-negative results in PCR, sputum, and saliva are currently-considered specimens with better detection rate in comparison with OP or NP swabs [58]. Because of hurdles in sputum sampling for diagnosis of SARS-CoV-2 infection [47, 59, 60], feces, and blood sampling are also of diagnostic value for increasing detection rate of SARS-CoV-2 infection in suspected persons [61-63].

Therefore, rapid laboratory assessment of appropriate samples from cases suspected with SARS-CoV-2 infection through real-time Reverse Transcriptase-quantitative PCR (rRT-qPCR), and evaluation of other co-respiratory infections will be recommended in order to reduce morbidities/co-morbidities [64], and set up the most practical precautionary or therapeutic approach [65]. Moreover, postmortem sampling through an autopsy should be assessed by PCR for decedent cases with no ascertained SARS-CoV-2 infection. For virological confirmation or exclusion, swab collections of primary bronchi from two categories of suspected persons are evaluated (those with initiative negative NP and OP swabs who represent significant pulmonary findings, and those with initiative positive NP and OP swabs with or without presentation of significant pulmonary findings) [37].
As the number and variety of available tests for detection of SARS-COV-2 infection are increasing, there is an imperative need to more comprehensive understanding on variety of the laboratory-based diagnosis of viral infections, different types of recruited samples, and viral genomic mutations to optimize single-objective diagnostic tests while maintaining the diagnostic sensitivity and specificity. Due to the possibility of repetitive mutations, regular sequencing of the evolved virus can specify structural and functional changes in the primer and probe binding sites, sensibly contributing to decipher complex immunopathogenesis, improving the epidemiological studies, and preventing from more transmission/emergence of recurrent infections.

Moreover, by decreasing the accurate time required for better management of patients with COVID-19, overcoming the challenges associated with the development of rapid Point Of Care (POC) diagnostic should not be underestimated [66, 67]. Due to the ambiguous pathogenesis of newly-mutated Coronaviruses, those two broad diagnostic categories (molecular and serological assessments) will not be interpreted solely [68].

3.2 Molecular Detection of COVID-19

Nucleic acid hybridization, rRT-qPCR, viral genome sequencing, and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have been endorsed as gold standard molecular approaches for the initial diagnosis of SARS-CoV-2 infection [69-71], due to availability, accuracy, and efficiency [53, 72]. Among them, highly-specific multiplex RT-PCR is considered as the most practical test for rapid detection of low concentrations of SARS-CoV-2 RNA [73-78].

There is a co-relation between day of onset of symptoms and viral RNA levels (Cycle threshold (Ct)). As positive results from rRT-qPCR can be still reached 6 to 8 days after the loss of transmissibility, viral viability and transmissibility should not be underestimated for an accurate clinical interpretation. Two consecutively-negative results from RT-PCR separated by at least one day interval, confirm hospital discharge for previously-confirmed patients, and discontinuation of quarantine situation for cases suspected with SARS-CoV-2 infection [79-82].

Targeted genes for molecular detection of SARS-CoV-2 infection include Nucleocapsid (N), Envelope (E), Spike (S), Membrane (M), and RNA dependent RNA polymerase (RdRp) genes [83-85]. For clinically-confirmed patients with COVID-19 and negative results in molecular tests, these tests should be consecutively repeated to be synchronized with clinical manifestations [86, 87]. Results from investigation of cases suspected with SARS-CoV-2 infection and similar symptoms between COVID-19 and other common co-respiratory infections, highlight the necessity of differential diagnosis through RT-PCR, supporting efficient anti-viral therapy and patient care [88-92].

Alteri C et al., [45] conducted viral quantification through a droplet digital PCR (ddPCR)-based assay (targeting RdRp and host RNAse P) on NP swabs of 55 cases suspected with SARS-CoV-2 infection and negative rRT-qPCR results. Among them, 19 persons (34.5%) showed positivity for SARS-CoV-2 infection, and IgG through ddPCR, and chemiluminescent microparticle immunoassay, respectively. Chest CT images in 73.7% of that population showed severe COVID-19 manifestations, introducing ddPCR as a sensitive, and complementary diagnostic approach.
for cases who have low viral copy number in very early stages of viral replication, and negative results in contextual PCR [45].

He J-L et al., [93] serially conducted RT-PCR tests on 82 individuals clinically-suspected with SARS-CoV-2 infection outside of Wuhan city. They reported 79% sensitivity, and 100% specificity for individuals being initially diagnosed positive with RT-PCR. Additionally, CT images revealed 77% sensitivity for positive patients, and 96% specificity among studied population. They concluded that an initial RT-PCR followed by CT images, can potentially reduce false-negativities in RT-PCR results for cases suspected with SARS-CoV-2 infection in the regions out of epidemiologic center [93].

Zhu W et al., [94] in their study, investigated 116 individuals clinically-suspected with SARS-CoV-2 infection outside of Hubei province (Anhui), and reported 32 clinically-confirmed patients with SARS-CoV-2 infection by chest CT images on admission to Emergency Department (ED). 67%, and 40% of negative cases were coping with fever, and Ground-Glass Opacity (GGO), respectively. They demonstrated that a combination of epidemiological features, laboratory tests, and chest CT findings confirm viral infection in the cases suspected with SARS-CoV-2 infection [94].

In a cross-sectional study conducted by Datta A et al., [95] diagnostic value of HRCT with RT-PCR was evaluated on 114 clinically-suspected cases with SARS-CoV-2 infection in Bangladesh. 91.22% of patients showed GGO with no consolidation in chest CT images. Bilateral chest findings, and vascular thickening were reported for 94%, and 66.66% of the patients, respectively. 96 of 114 patients showed positivity in their RT-PCR results. 90 people out of 96 cases revealed positive chest findings. Among 18 persons with negative results in RT-PCR, 14 people showed positivity in chest CT findings, demonstrating that chest CT could be of high sensitivity for primary diagnosis of SARS-CoV-2 infection when early detection or early clinical decision is prioritized [95].

Kuzan T.Y et al., [96], compared the diagnostic efficacy and accuracy of chest findings to RT-PCR results in first admission to the hospital among routine laboratory-confirmed (69 clinically-suspected cases with SARS-CoV-2 infection and dry cough, fever, bilateral multi-lobate involvement, and patchy shapes), and clinically-diagnosed patients with COVID-19 (51 cases with dyspnea, bronchial wall thickening, and GGO). Due to the high sensitivity and low specificity of chest CT, a combination of clinical features, chest CT images, and laboratory tests are highly recommended to differentiate any similarities between results from laboratory, and clinical investigations [96].

In a recently-conducted retrospective study, positive results of molecular tests were reported in 12 out of 28 cases suspected with SARS-CoV-2 infection. As there were no significant differences for laboratory results, and chest CT results (pure/mixed GGO, bilateral lung involvement, and rounded/patchy/linear opacities) among the studied population, RT-PCR tests are still efficacious for confirmed diagnosis of SARS-CoV-2 infection [97].

In a multi-center retrospective study, Miao C et al., investigated diagnostic criteria for chest CT images, and RT-PCR test (on NP/sputum samples) of cases
suspected with SARS-CoV-2 infection [98], reporting 76 cases as negative group, and 54 cases as positive group (or initially-negative and subsequently-positive group (with one day interval)).

Despite similar clinical manifestations and low sensitivity, combination of common features (GGO with bilateral pulmonary distribution (57.4% of positive group), GGO with pleural distribution (55.6% of positive group), GGO with bilateral pulmonary and peripleural distributions (48.1% of positive group), and GGO with bilateral pulmonary distribution, the crazy-paving pattern, and pleural distribution (22.2% of positive group)) led to increased specificity (approximately 99% for GGOs with the crazy-paving pattern and bilateral pulmonary distribution), and reduced overlaps in the clinical manifestations common with viral pneumonia. Hence, repeated sampling for molecular tests, and immediate isolation for successful differential diagnosis of SARS-CoV-2 infection are recommended [98]. Case report/series studies on cases suspected with SARS-CoV-2 infection are collected in table 1.

### Table 1: Case studies on cases suspected with SARS-CoV-2 infection

<table>
<thead>
<tr>
<th>Author</th>
<th>Case Suspected with SARS-CoV-2 Infection</th>
<th>Signs/Symptoms, Clinical and Paraclinical Results before admission to ED</th>
<th>Signs/Symptoms upon admission to ED</th>
<th>Clinical Results from thoracic CT images or Bronchoscopy or transbronchial biopsy upon admission to ED</th>
<th>Paraclinical Results from molecular or routine laboratory tests</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harkin et al.</td>
<td>A 34-year-old anesthesiologist</td>
<td>Fever, cough, and dyspnea due to a confirmed diagnosis of Influenza A virus, and no other any clinical manifestations, a delayed diagnosis of SARS-CoV-2 infection.</td>
<td>A rapid onset of fever, chills, rigors, dry cough, and shortness of breath after re-hospitalization.</td>
<td>Ill-defined nodule in the right mid-lung until first day of hospitalization. A rounded opacity in the right lower lobe until the second day of hospitalization.</td>
<td>A severe leukocytosis, lymphopenia, negative results of viral respirator disorders from NP swab until Bronchoscopy combined with molecular tests on BAL specimen for increasing diagnostic sensitivity especially</td>
<td>Bronchoscopy combined with molecular tests on BAL specimen for increasing diagnostic sensitivity especially</td>
<td>[99]</td>
</tr>
<tr>
<td><strong>Enlargement of the right lower lobe opacity surrounded by a large new GGO, a new rounded opacity in the left lower lobe, and a new multi-lobulated opacity in the right upper lobe until the sixth day of hospitalization.</strong></td>
<td><strong>Negative results of NP swab until the second and seventh day of hospitalization.</strong></td>
<td><strong>Positive results of BAL until ninth day of hospitalization.</strong></td>
<td><strong>An invasive fungal infection until seventh day of hospitalization. Alveolar tissue with patchy chronic inflammation, consistent with acute lung injury, until ninth day of hospitalization.</strong></td>
<td><strong>for who were intubated with negative results from their upper respiratory tracts.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Hao Q et al.,

<p>| A 34-year-old man with an epidemiological link to Wuhan (with no history of contact with a confirmed or probable case of COVID-19). | An intermittent fever, chills, dry cough with no other abnormal results in physical examination. An initially negative, and a subsequently positive results from OP samples during quarantine situation. Negative results for other respiratory pathogens. | Increased levels of Lactate Dehydrogenase (LDH) and C-Reactive Protein (CRP), normal rate of procalcitonin, lymphocytes, and lateral atypical infiltrates. | A mild GGO in the right upper lobe in the chest CT. Enlarged lesion in the second chest CT image during first day of rehospitalization. Further expanded inflammatory infiltration in the third chest CT image on the fifth day of rehospitalization. Completely improved outcomes on the thirteenth day of rehospitalization. | Two consecutively negative results from rRT-PCR with one-day interval, leading to patient discharge. Recurrent fever, dry cough, fatigue, and routine blood tests indicating SARS-CoV-2 infection during first day of rehospitalization. Two consecutively positive results with one positive results False-negative results acquired from upper respiratory tracts at the early stage of SARS-CoV-2 infection, can make rapid spreading of infection in the community and delayed confirmatory diagnosis of SARS-CoV-2 infection. Repetitive multi-site sampling for rRT-PCR assessment combined with dynamic CT images. | [100] |
| Khodamoradi Z et al. | Four pneumonia cases. | Pneumonia symptoms. | --- | Non-specific chest CT findings for viral pneumonia in all of the studied population, All identified as co-infection of SARS-CoV-2 (positive results acquired from assessment of both NP and OP samples through rRT-PCR) and Influenza A virus in the initial days of affliction. | Due to the co-emergence of SARS-CoV-2 and other respiratory disorders, chest CT imaging cannot be a differential diagnostic approach. Molecular tests is necessitated for confirmation of OVID-19 infection, [101] | could decrease false-negativity, especially in progressive stages of SARS-CoV-2 infection. |
| <strong>Hornuss D et al.</strong> | An adult man as a construction worker. | No significant sign/symptoms or pathological findings, except than a seven-day long fever with cough and previously diagnosed hypertension. | Lateral atypical infiltrates. | Increased levels of LDH and CRP, normal rate of procalcitonin, and lymphocytes, negative RT-PCR results reported from three times of sampling on deep OP swabs. Then, positive results co-infections, and prescription of the most appropriate medication for empirical therapeutic regimens. Repetitive negative results of OP swabs, makes us rule out for sputum, BAL, tracheal secretions, or stool in order to make the most effective clinical decision for treatment, discharge and/or further isolation. [102] |</p>
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Description</th>
<th>Findings</th>
<th>Recommendations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen L-D et al.</td>
<td>A young newly-arrived passenger from Wuhan city.</td>
<td>Several GGO in the right lung.</td>
<td>Positive results reported from fifth time of sampling for molecular investigations.</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Multiple negative rRT-PCR results, no notable clinical signs/symptoms, and no results from routine laboratory tests, chest radiography, and NP swab, except than a six-day long unexplained fever.</td>
<td>Multiple negative results from molecular tests should be ruled out as quarantine persons without discharge until further confirmation. A combination of exposure history in the regions out of clinical images and repetitive sampling for molecular tests, can pave the road for</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hase R et al.,</strong></td>
<td>A 35-year-old passenger from Wuhan to Japan.</td>
<td>----</td>
<td>No respiratory symptoms.</td>
<td>Pneumonia.</td>
</tr>
</tbody>
</table>
Giannitto C et al., [105] investigated 337 cases suspected with SARS-CoV-2 infection with initially-negative results from their molecular tests on BAL/NP samples to weigh up diagnostic value of pneumonic manifestations acquired from chest CT scans. Accordingly, in a time-interval shorter than five days, patients underwent sampling for first NP/OP swabs, CT, and then second BAL or NP swabs. Multiplex real-time RT-PCR assay for simultaneous detection of N, E, and RdRp target genes showed negativity for 87 cases in their first NP sampling (excluding 19 cases with no second round of sampling). Of 68 main participants, 48 cases showed negativity in their second sampling. Among them, there were 24 cases suspected with pneumonia in analysis of CT images. GGO, and positivity in second round of sampling were reported in more than 50% of lung patterns, and 58% of this population. Sensitivity, specificity, and accuracy of CT images combined with second round of molecular tests, were reported 100%, 79%, and 85%, respectively. Then, combinative interpretation of CT-based approaches with molecular tests, provides differential diagnosis of SARS-CoV-2 infection from other viral or bacterial pneumonia, and early identification of false-negative patients [105].

To sum up, for having a validated molecular detection of SARS-CoV-2 infection, at least one of the following criteria must be met: 1) positive NAATs results for at least two different genomic locations of COVID-19, 2) verification of at least one genomic site for COVID-19, being proved by sequencing. If cases suspected with SARS-CoV-2 infection have consecutively-negative results in two days (with one day interval) from molecular investigations, they can be discharged. In other words, for suspected cases with a history of close contact with clinically-
confirmed patients, molecular detection of SARS-CoV-2 should be considered repeatedly at least two times from appropriate sample [106] in the proper time/anatomical site for reducing any misinterpretations, and qualifying therapeutic approaches for SARS-CoV-2 infection [50, 91, 107-109].

3.3 Serological Detection of COVID-19
Serological tests (authorized by Food and Drug Administration (FDA)) can detect specific Immunoglobulin M (IgM) and IgG antibodies against structural units of SARS-CoV-2 [110]. They are wildly used for verification of molecular tests to raise the sensitivity and accuracy of laboratory-based diagnosis especially for cases suspected with SARS-CoV-2 infection [111, 112].

Serological tests identify individuals with therapeutic/prophylactic antibodies, and determine the immune responses to the possible causes of infection [113]. Figure 2 indicates a schematic presentation of serological tests, using plasma, whole blood, or serum specimens for cases suspected with SARS-CoV-2 infection [72, 114-117].

Figure 2: Serological detection of SARS-CoV-2 infection. Created by Rezaei et al.

Because of high abundance of N antigenic protein, involvement of S proteins in the attachment to host cell surfaces, and high specificity of the antibodies against Receptor-Binding Domain of S (RBD-S), detection of both antibodies against those antigens results in a highly-sensitive serological diagnosis of SARS-CoV-2 infection. Those serological tests mainly screen the donors of convalescent plasma, specify asymptomatic carriers, determine mortality rate (estimate population exposure rates), demonstrate previous infection, and complementarily verify molecular tests. Therefore, combination of
molecular and serological-based tests, may consistently raise the sensitivity for laboratory diagnosis of SARS-CoV-2 infection up to 80–90%, and improve clinical outcomes [81, 118-121]. Simultaneous negativity in IgM and IgG antibodies in cases suspected with SARS-CoV-2 infection, should be followed by repetitive molecular detection or high-throughput viral genome sequencing [122].

H.Zeng et al., [112] investigated the diagnostic value of combinative molecular and serological tests on 71 cases suspected with SARS-CoV-2 infection. They reported increased sensitivity for combined detection (63.38%) compared to solely-measured out molecular tests (46.48%), and serological tests (42.25%), opening promising windows for reduction of false-negativities in cases suspected with SARS-CoV-2 infection [112].

In another retrospective study [123], Jia X et al., investigated 57 cases suspected with SARS-CoV-2 infection. For 24 cases with positive molecular results in primary NP/OP sampling (the first one was confirmed with two additionally-repetitive nucleic acid detection), positive diagnostic rate for combinative antibody detection was 87.50% (more than single-antibody detection). For 33 persons with negative molecular results in primary NP/OP sampling, positive diagnostic rate for combinative antibody detection was 72.73% (more than single antibody detection). Among routine blood tests, only hs-CRP, and Aspartate aminotransferase (AST) showed a statistically-significant difference between studied groups. Regardingly, combinative antibody detection with nucleic acid assessment, and CT images provides an accelerated diagnostic approach with reduced risk of false-negativities among cases suspected with SARS-CoV-2 infection [123]. Negativity in NAAT test, strong epidemiological association, and validated serological assessment in the acute/recovery stages of infection (accompanied by CT-imaging) can support an accurate diagnosis for cases suspected with SARS-CoV-2 infection. Serum samples of asymptomatic patients with the history of close contact with clinically-confirmed patients with SARS-CoV-2 infection, can be stored for sero/epidemiological surveillance [7, 124, 125]. Although interactions with other coronaviruses can be challenging in diagnostic approaches, commercial and non-commercial serological tests are currently underway [82, 126].

The major structurally-specific protein of SARS-CoV-2 reacting with the membrane of host cells is S protein [127], stimulating humoral responses against these proteins which will be measured through western blotting, ELISA, or colorimetric fluorometric outputs (in case of secondary antibody reacting with the bound antibodies). Thus, proper binding of antibody-antigen can justify the folding and spatial shape of the protein, and reducing false-negativities [128]. Moreover, S protein of COVID-19 can be identified by a monoclonal antibody known as CR3022 (for research purposes). In addition, researches in the University of Hong Kong used Coronaviruses to hypothetically identify immunogenic parts of S protein [129].

4. Discussion

Rampant increase in the number of newly-identified patients with SARS-CoV-2 infection makes problematic challenges for occupational health managers, medical staff, economists, socialist, and governors [130, 131]. This highly-rapid spreading
The pandemic is deeply rooted in an improper recovery due to a poor/false diagnosis, and asymptomatic transmission. Totally, to globally curb long-term lung complications, diagnostic tests should be capable of rapid identification of SARS-CoV-2 infection [130, 132], and provide effective patient care among hospitalized patients. From immunopathological aspects, host immune responses of those critically-ill patients with COVID-19 (with pneumonia and severe lymphopenia), will proceed to Acute Respiratory Distress Syndrome (ARDS), shock, and death, necessitating a right frontline diagnostics in the shortest TAT, including laboratory-based diagnosis aimed at reducing falsifying results [130, 132, 133].

Accumulative studies declared that NAATs make a highly-sensitized viral detection by target genes, being majorly prioritized for clinical management of cases suspected with SARS-CoV-2 infection, directly measuring SARS-COV-2 nucleic acid by PCR-based techniques [134, 135]. Despite those mentioned advantages, as shortcomings, molecular diagnosis of SARS-CoV-2 infection cannot detect previous viral exposure, arising the interest of researchers to design clinical studies purposed to increase the sensitivity and specificity of those molecular tests [135-137]. Structurally, the key function of genetic material in Coronaviruses (a single-stranded, positive sense RNA) is encoded by the replicas gene, encoding two major polyproteins named pp1a and pp1ab. Hence, we can improve efficacy and sensitivity of the diagnostic procedures by simultaneous detection of two or more specific sequences through duplex or multiplex real-time RT-PCR test. Although, in case of accuracy, CRISPR and other lateral flow-based diagnostics are competing in a parallel manner with RT-PCR-based diagnosis [130, 133, 138, 139].

Using appropriately-stored samples from proper and various anatomic sites of cases suspected with SARS-CoV-2 infection accompanied with investigation of clinical features, can be effective in controlling viral spreading or disease aggravation, monitoring response to treatment, and assessment of viral infectivity [133, 137, 140]. Although existing viral RNA-based diagnostic tests are primarily qualitative, reliable results were reported from optimization of several synthetic RNAs in the process of UPE and ORF1b-based single-stage RT-PCR diagnostic approaches. Visiting the NCBI site with the code hCov-EmcJX869059 DNA UPE and ORF1b DNA Coronavirus patterns can lead to designation of specific primers and probes for the viral genome in PCR-based molecular testing [141, 142].

Although usage of appropriate samples in RT-PCR tests for cases suspected with SARS-CoV-2 infection have some limitation [143], repetitive (four times of) NP and OP sampling can definitely increase diagnostic sensitivity in mentioned cases upon admission to the hospitals [143].

Despite acceptable specificity and sensitivity of serologic-based tests for COVID-19 detection, false-negative results from reduced viral load after acute phase of disease, false-positive results due to the cross-reactivity of antibodies, and predisposition (autoimmune) diseases, lead us toward a combination of serological and molecular-based diagnosis to increase diagnostic sensitivity and specificity for cases suspected with SARS-CoV-2 infection [144-146].

There are validated diagnostic RT-PCR kits to detect viral presence in the biological samples [147]. As noted earlier, CT-based approaches can be practically...
recruited for reducing ambiguities in early clinical symptoms of COVID-19 patients, or asymptomatic carriers being exposed with clinically-diagnosed patients with COVID-19, or clinically-confirmed patients, or clinically-suspected cases with SARS-CoV-2 infection in the epidemic areas [94, 148-151]. As negative result of chest CT cannot exclude SARS-CoV-2 infection, clinicians and laboratory specialists should pay attention to the alternative/complementary strategies (repeatedly-over time test based on the assessment of each patient's clinical status) [152-154]. For instance, chest ultrasonography was reported for confirmation of positive results acquired from RT-PCR (being associated with Lung Ultrasound Score (LUSS), abnormal AST findings, and fever with an overall accuracy of 91%) in cases suspected with SARS-CoV-2 infection, aimed at reducing irradiation risks in CT-based approaches, and false-negativities in RT-PCR tests [155]. Moreover, triage-based strategies like fever in early screening of cases suspected with SARS-CoV-2 infection and negative results in their second nucleic acid test point out the establishment of fever clinics for reduction of misdiagnosis [156]. Moreover, Liver function damages, crazy-paving pattern, leukopenia, lymphopenia, elevated inflammatory factors can make indisputable roles for precise and rapid diagnosis aimed at early screening of cases suspected with SARS-CoV-2 infection [157-161].

Although, the role of CT for evaluation of cases suspected with COVID-19 infection is still yet to be specified [162], CT images can alternatively help differential diagnosis, early screening of COVID-19 or other pulmonary disorders, and initial evaluation of cases with non-specific clinical symptoms [162-165]. Due to lowered diagnostic specificity, it is worthy to mention that CT should not be used as a first-line screening approach for patients with COVID-19 and left-sided pneumonia [166], persistent indications of inflammation (despite receiving antibiotic regimens), repetitive negative results from NP swabs, and positive results from BAL samples in RT-PCR tests [166].

Accordingly, CT-based approaches can be initially of diagnostic specificity and quarantine necessity for cases suspected with SARS-CoV-2 infection and repetitive negativity in their RT-PCR tests on NP, OP, or BAL samples [155, 167]. Thereafter, combinative usage of chest CT and RT-PCR testing for febrile cases suspected with SARS-CoV-2 infection in EDs is recommended to reduce the possible false-negativities [168-172]. Additionally, COVID-19 Reporting and Data System (CO-RADS), as a categorical assessment scheme for prediction of infection rate and pulmonary involvement, increases specificity and diagnostic accuracy for cases suspected with SARS-CoV-2 infection [173-175].

5. Conclusion

According to the high risk of viral transmission from cases suspected with COVID-19, an accurate POC diagnostics can validate monitoring therapy of symptomatic patients, and prevent from other loaded nosocomial infections, as well. Correspondingly, “Precision/Personalized/Individualized Medicine”, as a missing piece in the puzzle of targeted diagnosis, can provide predictable outcomes for cases suspected with SARS-CoV-2 infection, and the most efficient vaccine with maximum immunogenicity with consideration of predisposition factors and immunogenetics of appropriate patients. Additionally, it remarkably minimizes negative predictive value of diagnostic tests, psychological burdens on health-care professionals, and exorbitant expenses of
hospitalization imposed on patients. So, further pre-clinical investigations will be unquestionably needed to clarify the most efficient diagnostic protocol for SARS-CoV-2 infection. Moreover, scientific collaboration among specialists in the fields of internal/infectious diseases, pulmonary disorders, personalized medicine, immunology, medical microbiology/virology, medical biotechnology, medical genetics, medical laboratory, sciences, basic medical sciences, nursing, epidemiology, disease-specific biomarkers, and health system coordinators is highly recommended.

**Declaration Statement**

**Ethical Approval and Consent to Participate/for Publication**

Not Applicable.

**Author Contribution**

Supervision and verification of the last version before submission, N.R.; Conceptualization, Validation, and Formal analysis, N.R., and N.B.; Main methodology, Search Strategy, and Data Curation, Academic/Scientific/Grammatical revision for important intellectual content, Preparation and main designation of the final draft of the manuscript, N.R., and N.B.; Major Conception, Data extraction, Interpretation, and preparation of final sections related to clinical diagnosis of the study, M.A., and N.B.; Preparation of first draft of manuscript related to laboratory diagnosis of the study, M.M., P.AE., SH.A., F.R., E.N., P.F., M.M., A.M., H.K., M.SH., M.B., S.R., M.J., S.S., Z.D., S.L., S.M.M.K., SH.J., and S.E.; Visualization, Image/Table Designation, N.B. and E.N.; All of the authors attest to the validity and legitimacy of data, receiving an electronic copy of the final version, and published version of the manuscript.

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**Conflict of Interest**

None

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for Corona Virus Disease (COVID-19).


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