COVID-19 Laboratory diagnosis methods

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Abstract:
Coronavirus-19 is caused by “Severe acute respiratory syndrome coronavirus-2” (SARS-CoV-2), COVID-19 pandemic, has led to millions of confirmed cases and deaths worldwide. Efficient diagnostic tools are in high demand, as rapid and specific testing plays an important role in patient management and decelerating disease spread. The current technologies used to detect COVID-19 in clinical laboratories will be classified into main three categories, I- The polymerase chain reaction (PCR) method, II- Immunological or serological methods (Antigen and Antibody Detection), III- Biochemical and hematological methods which include I- Inflammatory biomarkers (Elevated and decreased group), 2. Multi-organ damage/failure (Cardiac, Hepatic, and Renal).

Keywords: COVID-19, SARS-CoV-2, PCR, Immunological methods, Biochemical and hematological methods.

Introduction:

80% of patients infected by COVID-19 may be asymptomatic or only mildly symptomatic, but around 10% develop severe respiratory symptoms that evolve into acute respiratory distress syndrome (ARDS)(4). The coronavirus disease 2019 (COVID-19) pandemic, has led to millions of confirmed cases and deaths worldwide. Efficient diagnostic tools are in high demand, as rapid and specific testing plays an important role in patient management and decelerating disease spread. This paper reviews current technologies used to detect COVID-19 in clinical laboratories, these current technologies will be classified into main three categories, I- The polymerase chain reaction (PCR) method, II- Immunological or serological methods, III- Biochemical and hematological methods.

I- The polymerase chain reaction (PCR) method:
The direct detection of SARS-CoV-2 RNA through nucleic acid amplification tests (NAATs) is the most prevalent method for diagnosing COVID-19, most often RT-PCR from the upper respiratory tract (5). The current gold standard for the etiological diagnosis of SARS-CoV-2 infection is rRT-PCR on a variety of clinical specimens, including bronchoalveolar lavage fluid, fiber bronchoscope brush biopsies, sputum, nasal swabs, pharyngeal swabs, feces, or blood (6).
The polymerase chain reaction (PCR) characterized by high specificity, sensitivity, and rapid detection is considered the “gold standard” among the nucleic acid tests for the detection of some viruses. Real-time RT-PCR as a simple and specific qualitative assay is of great interest today for SARS-CoV-2 detection(7). The collecting of suitable respiratory samples from suspected patients, the use of predetermined primers and probes, the Ct value, the evaluation of fluorescence curves, and the use of suitable controls are all factors that affect the outcome of an RT-PCR test. A negative control is used to examine sample cross-contamination, whereas a positive control is used to assess the dependability of the primers, reagents, and probes(8).

In a systematic review and meta-analysis by Mustafa Hellou et al., the pooled sensitivity for SARS-CoV-2 detection from 29 studies was 96.2%, and the pooled specificity was 98.1% (9).

II - Immunological methods:

1- SARS-CoV-2 Antigen Detection

Rapid diagnostic techniques for SARS-CoV-2 antigen detection have been developed, however, they face some performance and accessibility issues. Quick control, preventative measures, and early identification of viral outbreaks are all impacted by selecting the right test for the detection of COVID-19. Early viral diagnosis reduces the likelihood of transmission, enabling the quick treatment of illnesses. The COVID-19 Ag-RDT is a highly sensitive and specific antigen test for nasopharyngeal swab-derived SARS-CoV-2 Ag(10).

Ag-RDTs are inexpensive, do not require infrastructure, are easy to perform, and quality results are achieved in minutes. Ag-RDT is strongly supported by Ricks et al. for the evaluation of symptomatic individuals making it practical and cost-effective(11).

Rapid diagnostic tests are small, affordable, and portable tests that use the lateral flow testing concept to determine whether samples of saliva, blood, or a nasal swab are positive or negative. When the material put on the membrane travels along the membrane by capillary action in this test, gold nanoparticle labeled antibodies (Au-Ab) including membrane and capture antibodies display two different lines(12).

Mertens (2020) conducted a retrospective study on 328 pharyngeal samples in Belgium for the detection of nucleoprotein antigens through the immunochromatographic method. They found that COVID-19 Ag Respi-Strip represents a useful rapid antigen assay for the SARS-CoV-2 virus for the initial diagnosis of COVID-19 in 15 min when the pandemic is at its peak(13).

2- SARS-CoV-2 Antibody Detection or serological methods:

Antibody tests identify the presence of antibodies produced as part of the immune response due to the viral pathogen infection. For SARS-CoV-2 infection detection, the commonly used serological assays include Enzyme-Linked Immunosorben Assay, neutralization test, rapid diagnostic determinations, and chemiluminescent immunoassay. Serological testing for COVID-19 is the analysis of serum, plasma, or whole blood for immunoglobulins (antibodies) detection, particularly immunoglobulin M (IgM), IgG, and IgA that are specific for antigens of SARS-CoV-2, including the nucleocapsid proteins and spike glycoproteins(14).

Serological testing might be helpful for the confirmation of suspected cases, particularly in patients tested in the late phase of COVID-19 or with mild to moderate illness, not identified with molecular assays (15).

The traditional viral infections immune response involves the development of first IgM, frequently followed by the advent of immunoglobulin A, and then to immunoglobulin G production(16). Current research on COVID-19 is contradictory, with some groups finding that IgM is produced first and others claiming that IgM and IgG are produced simultaneously (17).

III - Biochemical and Hematological markers methods:

1- Inflammatory biomarkers:
Severe coronavirus-19, which suggests an immunochemical pattern resembling a "cytokine storm," has been linked to several inflammatory biomarkers. In conclusion, it has been shown that pro-inflammatory cytokines, notably interleukin (IL)-6 and TNF-, are significantly associated with mortality in patients with severe disease(18,19), this support the hypothesis that severe coronavirus-19 cases are characterized by immense pro-inflammatory responses or cytokine storm, which can lead to MOF (multiple organ failure) in severe cases. Laboratory test findings can help in patient diagnosis, prognosis, and monitoring through the identification and measurement of numerous biomarkers, with COVID-19 infection the level of Inflammatory biomarkers increased or decreased in blood samples so can be divided into the Elevated group and Decreased group.

A-Elevated group:

**White blood cell count (WBC):**

Blood leukocytes are an important part of the body’s defense system, and infection status can be predicted by measuring WBC levels(20), Huang et al., 2020 reported high WBCs count in all 13 ICU cases (21), but in mild COVID-19 infection total WBCs were normal in 63.5%, low WBCs count in 7.9%, and high WBCs count in 28.6% (22).

**Neutrophils count:**

Neutrophils are immune cells that are well known to be present in various lung diseases, including viral respiratory disease (23), Studies have suggested that neutrophil recruitment may exacerbate COVID-19 immunopathology(24), high neutrophils count or neutrophilia has a sensitivity of 77.8% as a biomarker for COVID-19 diagnosis or prognosis in mild infected COVID-19 patients (Outpatients and patients under home observation)(22).

**CRP:**

C-reactive protein (CRP) is an acute-phase reactant that is produced by the liver in response to inflammation. A study including over 50,000 participants noted a significant association between elevated serum or plasma concentrations of CRP and ischemic vascular disease(25). Serum CRP is a simple and effective prognosticator which casts light on potentially critical patients, Serum CRP has a predictive value of COVID-19.

CRP levels were positively correlated with mild COVID-19 infection of lung lesions with a sensitivity of 90.2%. CRP levels could reflect disease severity and should be used as a key indicator for disease monitoring(26).

**Ferritin:**

A cytokine storm might be brought on by elevated ferritin levels because of their direct immunosuppressive and pro-inflammatory actions(27). The levels of ferritin, a crucial immune response mediator, increase in severe Covid-19 cases(27). high serum ferritin levels(71.4 %) In mild COVID-19 infection, which was a significant biomarker for COVID-19 diagnosis(28).

**IL-6:**

Increased IL-6 levels (up to 100 pg/mL) and higher mortality were correlated with serum viral load in critically ill patients(29).

**ESR:**

Erythrocyte sedimentation rate ESR is affected by the size, shape, and concentration of red blood cells and plasma characteristics(30). ESR was reported by several studies to be significantly elevated and was considered a predictor of COVID-19 infection severity(31).

**LDH:**

Several reviews and meta-analyses have reported the prognostic value of lactate dehydrogenase (LDH) for the severity of COVID-19 and other inflammatory diseases. A metabolic and prognostic biomarker for immune surveillance is serum LDH. In CoVID-19 patients, a high level of LDH has been linked to respiratory health and used as a prediction of respiratory failure. When it is elevated in the serum, people who are immunocompromised have poorer results(32). Serum Lactate dehydrogenase LDH level is a good biomarker of infection in COVID-19.
Outpatients and patients under home observation increased by 67.7% (33).

**Procalcitonin**
High levels of procalcitonin PCT have been found, indicating the development of bacterial co- or supra-infection in seriously sick individuals (34). PCT is also often raised due to the inflammatory cascade caused by a cytokine storm in COVID-19 patients (35). Multiple studies have mentioned the prognostic significance of PCT in patients with COVID-19 (36).

**B- Decreased group:**

**Lymphocytes:**
Lymphocytes play a crucial role in maintaining immune homeostasis during virus infection, especially SARS-CoV-2 (37). Several cohort studies have reported that lymphopenia can predict prognosis in COVID-19 patients (38), also in mild COVID-19 patients (Outpatients and patients under home observation) lymphopenia has a sensitivity of 73%, and it was a significant biomarker for COVID-19 diagnosis or prognosis (22).

**Eosinophils:**
A significant decrease in the peripheral eosinophil counts was found to correspond to the increase in COVID-19 severity. Eosinopenia is significantly more frequently present in patients with severe COVID-19 (39).

**Platelets:**
Platelets may directly interact with SARS-CoV-2 and have been shown to carry the SARS-CoV-2 virus. In COVID-19 patients, platelet count differs between mild and serious infections. Patients with mild symptoms have a modestly elevated platelet count, whereas severe COVID-19 infections are characterized by thrombocytopenia (40). But a study in Egypt showed Platelet count was normal, and Platelet was not a significant biomarker for COVID-19 diagnosis or prognosis in out-hospitalized patients (Outpatients and patients under home observation) (41).

2- Multi-organ damage/failure:

**A. Cardiac:**
Reports suggest that severe COVID-19 patients have a significant cardiovascular impairment (42). Patients with increased troponin levels have higher chances to be admitted to the ICU with an increased rate of hospital mortality (43), It has been reported that Covid19 was associated with hemostatic abnormalities and markedly elevated D-dimer levels were observed in those non-survivors (44), also high plasma D-dimer levels in 36.4% of mildly infected patients was a significant biomarker for COVID-19 diagnosis in out-hospitalized patients (Outpatients and patients under home observation) (45), some researchers analyzed the clinical and laboratory findings of COVID-19 and found that severe patients often had prolonged PT, increased D-dimer levels, low fibrinogen, and DIC (44)

**B-Hepatic:**
Many large-scale hospital investigations have found elevated liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl-transferase (GGT) (46).

**C-Renal biomarkers:**
COVID-19 has been linked to a higher risk of acute kidney damage (AKI), Blood Urea Nitrogen BUN and blood creatinine are both common indicators of kidney damage (47), These findings suggest that in the therapy of COVID-19, early diagnosis and prevention of renal deterioration, which includes maintaining adequate physiological balance and limiting kidney-toxic medications, may be essential, and that more frequent creatinine testing or other kidney indicators may be required. Total protein and serum and urine albumin may be useful prognostic indicators in COVID-19. Similar to liver failure, the pathophysiology and causes of renal disease in coronavirus-19 carriers are unclear and may be complicated (48).

**Conflict of interest:**
There are no conflicts of interest.
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I - The polymerase chain reaction (PCR)
- SARS-CoV-2 Antigen
- SARS-CoV-2 Antibody

II - Immunological or serological methods

III - Biochemical and hematological methods

1- Inflammatory biomarkers

A - Elevated group
- WBC
- Neutrophils count
- CRP
- Ferritin
- IL-6
- ESR
- LDH
- Procalcitonin

B - Decreased group
- Lymphocytes
- Eosinophils
- Platelets

Cardiac
- Troponin
- D.Dimer
- PT
- CKMB

Hepatic
- AST
- ALT
- GGT
- ALP

Renal
- BUN
- Creatinine

Figure (1): COVID-19 Laboratory diagnosis methods
References


