DOI: 10.4149/BLL_2021_025

*Bratisl Med J 2021; 122 (1)*
11 – 17

REVIEW

# COVID-19 molecular level laboratory diagnoses

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**ABSTRACT**
AIMS: Aims: The Coronavirus Disease 2019 (COVID-19) caused not only global pandemic, but it also pointed at unprepared health care systems and countermeasures were introduced under the pressure of urgent circumstances. This review is focused on discussion and critical evaluation of instrumental tools for COVID-19 diagnosis that were developed in the last months.
METHODS: Survey of actual literature and scientific reports was made. The most substantial analytical and diagnostical methods were identified and described. Principles and limitations of the methods are described, and actual papers are cited in this review.
RESULTS: Analytical and diagnostical methods like Polymerase Chain Reaction (PCR), Loop-mediated isothermal Amplification (LAMP), Lateral Flow Immunochromatography Assay (LFIA), Enzyme-Linked Immunosorbent Assay (ELISA), biosensors and ChemiLuminescence ImmunoAssay (CLIA) are discussed for assay of viral particles, antigens and specific host antibodies in blood, serum, plasma, nasopharyngeal swab and other samples in order to diagnose COVID-19.
CONCLUSIONS: The Coronavirus Disease 2019 (COVID-19) is an emerging disease that has spread over the world since the end of year 2019. The global epidemic pointed at the necessity to introduce sensitive methods for instrumental diagnosis of COVID-19 and distinguishing it from the other viral diseases. *(Tab. 2, Ref. 96).* Text in PDF *www.elis.sk*
KEY WORDS: antibody, biosensor, COVID-19, Coronavirus Disease 2019, diagnosis, enzyme-linked immunosorbent assay, ELISA, immunoassay, polymerase chain reaction, severe acute respiratory syndrome coronavirus 2, SARS-CoV-2.

## Introduction

The Coronavirus Disease 2019 (COVID-19) is an infection caused by a zoonotic coronavirus taxonomically entitled as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The original local epidemy in China first observed in Wuhan city turned into a global pandemic starting in the 2019 and extensively growing over the world in the year 2020 (1–4). The COVID-19 can be recognized using clinical differential diagnosis; however, there are significant limitations regarding this approach. The fact that many infected patients become vectors for the disease but remain without symptomatic clinical manifestation or with weak symptomatic manifestation without the disease perception is a significant problem. Differential diagnosis becomes inapplicable for these cases (5–9). Another drawbacks of differential diagnosis can be expected for examination of infected children, who have either different or no manifestation than the adult patients and overall impact of COVID-19 on children is fortunately very mild (10). The differential diagnosis of COVID-19 can be further improved by instrumental techniques like computed tomography scan (11–14). Though these instruments do not plausibly confirm the particular disease, they make the diagnosis specific and they can also reveal related pathologies.

Unambiguous confirmation of the disease and final diagnosis are a task for specific laboratory methods where genetic information, unique structures or antibodies specific to SARS-CoV-2 are determined. Various methods are currently available and each of them has its pros and cons in the both laboratory reliability and diagnostical interpretability. The situation about used methods and its specific applications is progressively developing as the disease became a serious world matter and considerable effort is given to resolving of the current problems. This review is focused on survey and discussion of laboratory methods used for COVID-19. Because the disease is new, the diagnostic methods are newly researched and developed on the standard genetical, immunochemical etc. platforms. The newly introduced methods are described in this work.

## SARS-CoV-2 genetic information revealing

SARS-CoV-2 is a single-stranded positive-sense RNA virus and a recognition of specific sequences in the RNA is an impor-

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**Acknowledgements:** This work was supported by A long-term organization development plan "Medical Aspects of Weapons of Mass Destruction" (Faculty of Military Health Sciences, University of Defense, Czech Republic).

**Tab. 1. Basic specifications of COVID-19 and SARS-CoV-2 as outcomes for an instrumental diagnosis.**

| Specification | Description |
|---|---|
| type of virus | single-stranded positive-sense RNA |
| specific parts in genetic information suitable for diagnosis | ORF 1b gene; N protein gene including N1 and N2 genes; S protein gene |
| virion surface proteins | membrane (M), envelope (E), spike (S), and nucleocapsid (N) proteins |
| protein for revealing of SARS CoV-2 by an immunoassay | protein S is the most relevant |
| antibodies produced during COVID-19 | IgA, IgG, IgM |
| time lapse of antibodies production | approximately 1–3 weeks |
| main antigenic structure targeted by the antibodies in the blood of patients with COVID-19 | protein S is the most relevant, most of current test on anti-S1 antibodies |

tant way in revealing infected patients (15–19). Polymerase Chain Reaction (PCR) and Loop-mediated isothermal Amplification (LAMP) can be mentioned as the two significant methods suitable for identification SARS-CoV-2 based on recognizing its genetical information. Other techniques common in molecular biology are available as well and sequencing of isolates can be also performed for identification purpose and origin of the virus revealing (20). The tests for detecting SARS-CoV-2 genetic information are not typically suitable for point-of-care diagnostic; on the other hand, plausibility of the results is the major advantage. Blood, saliva, oropharyngeal samples and faeces can be mentioned as applicable specimens for the test (21, 22). The use of nasopharyngeal swabs is the most common way how to acquire samples for a genetical analysis (23–25). In the study by Lin and co-workers, sputum specimens were more suitable for PCR tests than throat swabs (26). The use of swabs seems to be optimal for sampling in genetical test considering overall effectivity, reduction of time necessary for a patient examination, patients' comfort and safety of medical personnel. Basic specifications of COVID-19 and SARS-CoV-2 as outcomes for diagnosis are summarized in Table 1.

PCR tests are available and most of clinical laboratories testing samples during the COVID-19 pandemic are equipped with PCR analysers beside the other standard techniques. Many commercial PCR test have been developed and introduced in the market since the start of COVID-19 pandemics (27). Quantitative (also known under synonymic real-time) reverse transcriptase PSR (qRT PCR) is the particular technique for laboratory testing (28–30). Typical qRT PCR is focused on N1 and N2 genes coding viral nucleocapsid, this target is widely recommended by Center for Disease Control and Prevention (Atlanta, USA) (28, 31). Other primer sets for PCR are focused on spike and envelope genes and they have valid emergency use authorization (EUA). For instance Altona Real Star (Altona Diagnostic, Hamburg, Germany) qRT PCR focused on envelope and spike genes and SHC EUA test (Sandford Health; Sioux Falls, South Dakota, USA) qRT PCR for envelope gene were compared with the N1, N1 targeting qRT PCR and great agreement between the results was achieved, when clinical samples were measured (32). In another paper, qRT PCR focused on open reading frame (ORF) 1b gene was described and compared to the assay focused on the N genes (33). The ORF 1b exerted a lower sensitivity as only 62.5 % of positive patients (total tested 23) were recognized while diagnostics by N gene was fully successful. Nevertheless, the authors concluded their work by the statement that the qRT PCR for ORF 1b gene can be an alternative to the other types of PCR for SARS-CoV-2.

Reverse transcription LAMP (RT LAMP) for SARS-CoV-2 is simpler, cheaper, faster and thus more available for in and out laboratory diagnosis than qRT PCR and a simple coloration as an outputting signal is an advantage for a fast and simple measurement as well (34–43). On the other hand, LAMP has typically a lower sensitivity compared to PCR, this specification is the major drawback of the LAMP assay, when it is considered as an alternative to PCR. The lower sensitivity can result into false negativity, when COVID-19 is diagnosed. In the study by Thi and co-workers, a colorimetric RT LAMP was compared to qRT PCR and the authors stated that the both methods were in a good mutual correlation (44). The RT LAMP had a good specificity (99.5 %) though it had a little lower sensitivity (86 %), when compared to 30 cycles of qRT PCR. On the other hand, the tested RT LAMP was easier for performance and even RNA isolation was not necessary as a direct use of swab as a sample was possible. The RT LAMP appears to be a relevant competitor to qRT PCR and both methods have their advantages and disadvantages. In another experiment, RT LAMP was tested on samples from 223 patients with COVID-19 (confirmed by qRT PCR) and other 143 patients suffering from other respiratory diseases (45). The authors claimed that the limit of detection for the RT LAMP was equal to 42 copies of RNA per a reaction. The colorimetric assay was suitable to be evaluated by a naked eye and the assay had a quite low false negativity comparing to qRT PCR: 212 respective 219 from the total 223 positive samples were recognized by an assay lasting 60 respective 90 minutes. No false positivity occurred, when the samples from 143 patients suffering from other respiratory diseases were tested. The described RT LAMP can be performed on various samples like saliva, throat swab samples, but the best results were obtained using nasopharyngeal swab samples. Samples like blood, serum or plasma from blood can be also used. Considering all aforementioned studies, RT LAMP appears to be a promising method for the surveillance of SARS-CoV-2 presence in humans. Though it is less sensitive than qRT PCR, it can be easily distributed and performed nearly as a point-of-care diagnosis and thus help to manage emergency situations like the COVID-19 pandemic is.

## Detection of antigens

Direct recognition of surface structures of SARS-CoV-2 would be another way to recognize the pathogen although the aforementioned genetic tests would be probably more sensitive and applicable for practical tests in clinical laboratories. Similar samples like in the PCR and LAMP test can be used for the antigen determination. The SARS-CoV-2 virion particles contains three major

group of proteins containing specific areas but also parts containing homologies with other relative virus families. Membrane (M), envelope (E), spike (S), and nucleocapsid (N) proteins can be mentioned (46–48). S protein appears to be the most convenient for an immunoassay because it is a unique protein responsible for virus interaction with angiotensin-converting enzyme receptor on host cells (49–52). The studies on surface antigenic structures are necessary not only for analytical respective diagnostical purposes, but also for the identification of structures that would serve for development of a vaccine (53–56). The N and S proteins (57) and protein glycations (58) can be studied as specific determinants. Similarities between SARS-CoV-2 proteins and proteins from the other viruses should be taken into consideration. For instance, homologies between SARS-CoV-2 S protein and dengue virus were reported (59). These homologies can cause a false positive diagnosis, when an antigen is measured. Moreover, there can be also cross-reactivity between the antibodies produced in an organism hence a diagnosis based on antibodies can also have a false positivity. Basic specifications of COVID-19 and SARS-CoV-2 as outcomes for diagnosis are summarized in Table 1.

Simple immunoassays for a SARS-CoV-2 antigen can be an alternative for diagnosis based on antibodies. For instance, a microfluidic immunoassay for the detection of serum antibodies IgG and IgM specific to SARS-CoV-2 and immunodetection of antigen isolated by pharyngeal swabs was successfully developed and reported by Lin and co-workers (60). Another approach was proposed by Mavrikou and co-workers, who developed a membrane engineered cell cowered human chimeric anti spike – S1 antibody and interacting with S1 spike S protein from SARS-CoV-2 (61). The interaction resulted in a generation of a membrane potential, which was measured as an outputting signal. The assay exerted the limit of detection 1 fg/ml and semi-linear response between 10 fg/ml and 1μg/ml during an assay lasting 3 minutes. Biosensor for the detection of SARS-CoV-2 S protein was also developed by Seo and co-workers (62). The biosensor was based on field-effect transistor coated with graphene sheets with an antibody against SARS-CoV-2 S protein. The assay was suitable for the detection of virus in nasopharyngeal swab and virus growing in cell cultures. The assay was able to detect at least 16 PFU/ml in cell cultures and 242 viral copies in nasopharyngeal swab, the limit of detection for pure S protein was equal to 1 fg/ml. There are also developed tests working on Lateral Flow Immunochromatography Assay (LFIA) principle. The commercially available BIOCREDIT COVID-19 Ag test suitable for proving of SARS-CoV-2 in nasopharyngeal swab sample can be mentioned as a relevant analytical tool. In the study by Mak and co-workers, the BIOCREDIT COVID-19 Ag test was compared to qRT PCR (63). The researchers stated that qRT PCR was 100,000 times more sensitive to SARS-CoV-2 than the BIOCREDIT COVID-19 Ag test and the risk of false negativity was the main disadvantage of BIOCREDIT COVID-19 Ag test. On the other hand, BIOCREDIT COVID-19 Ag test could be used easily outside of laboratories and could be provided in a point-of-care. The LFIA assay cannot replace the tests like qRT PCR or RT LAMP, but it could be used as a simple test for screening of population for COVID-19 and finding of infected communities.

**Diagnostic based on antibodies**

Antibodies are an important and specific marker of infectious diseases. Concentration of immunoglobulins is quite high in the blood. The most common antibodies from the group of immunoglobulins (Ig) G, IgM and IgA reaches typically 7–16 mg/ml for IgG, 0.4–2.3 mg/ml for IgA or 0.4–2.3 for IgM in serum, but the level can be significantly increased due to some pathological processes including infectious diseases (64–67). Amplification of an stimuli is the major advantage of diagnoses based on antibodies assay, it means that a relatively low amount of antigen causes a significantly higher production of antibodies and not the original antigen, but the propagated antibodies are detected. All the isotypes, IgG, IgM and IgA, are increased and applicable for COVID-19 diagnosis, which was for instance reported by the study on 21 Croatian patients, where serum samples were tested by standard Enzyme-Linked Immunosorbent Assay (ELISA) (68). The overall high level of antibodies in combination with the extensive change in antibodies concentrations make them an ideal biological marker. In the case of COVID-19, production of antibodies has not been researched enough to make a single conclusion. While humoral immunity is studied and certainly take place in the infected people (69), some studies proved that the immunity is quite naïve for SARS-CoV-2 and some people have a quite limited response. For instance, Bahar and co-workers revealed that only 17 patients from a group of 33 people suffering of COVID-19 had an adequate neutralizing antibodies level (70). Therefore, the results from diagnoses focused on antibodies measuring should be considered as suspicious until the whole issue is cleared. The production of antibodies in an organism is a time dependent process and the antibodies cannot be detected immediately after an infection transmission into host. In the work by Chughtai and co-workers, samples from COVID-19 patients were analysed for a specific antibodies production (71). They revealed seroconversion 68 % at day 7 post-symptom onset. The percentage reached 88 % for 14 days and 100 % for 21 days after symptoms onset. For the reason, the conclusion about immune system naivety for COVID-19 should consider a time span of infection starting.

ELISA is the standard and probably the most common method for the measuring of antibodies level in blood, blood serum and blood plasma and the detection of antibodies against SARS-CoV-2 is currently used as well (72–76). LFIA in the form of test strips is another standardized and evolving method for anti-SARS-CoV-2 antibodies detection (77–79). Beside the methods common in clinical praxis, other immunoassays, immunosensors, and immunoblotting tests are applicable for the antibodies detection (80–82).

An ELISA test for COVID-19 diagnosis is typically focused on antibodies specific to surface proteins, the anti-S1 protein antibodies appears to be suitable for the diagnosis (83, 84). Beside the standard diagnosis of the COVID-19, ELISA is also a routine method for measuring the antibodies level in people that underwent vaccination or clinical trials in development of an anti-COVID-19 vaccine and efficacy of seroconversion due to vaccination is thus determined (85). Research and characterization of developed monoclonal antibodies can be done by ELISA as well (86).

**Tab. 2. Overview of samples and methods for COVID-19 instrumental diagnosis.**

| Marker | Methods | Samples |
|---|---|---|
| viral RNA including RNA in intact virions | Polymerase Chain Reaction (PCR), Loop-mediated isothermal Amplification (LAMP) | saliva, throat swab samples, nasopharyngeal swab samples blood, serum, plasma |
| surface S and N proteins including proteins on intact virions | Lateral Flow Immunochromatography Assay (LFIA), Enzyme-Linked Immunosorbent Assay (ELISA), biosensors and ChemiLuminescence ImmunoAssay (CLIA) | saliva, throat swab samples, nasopharyngeal swab samples blood, serum, plasma |
| antibodies against SARS-CoV-2 | LFIA, ELISA, CLIA | blood, serum, plasma |

ELISA is convenient for measuring of trace level of antibodies so it can serve for the purpose of epidemiologic studies and it can retroactively identify patients with a mild manifestation of the disease that were not revealed during the disease (87). Kovac and co-workers. tested commercial tests based on chemiluminescence immunoassays principle (CLIA test; Roche, Rotkreuz, Switzerland) and ELISA (IgG and IgA ELISA test; Euroimmun, Lubeck, Germany) for haemolyzed blood samples and reported them as the optimal for antibodies testing in the laboratory conditions (88). They placed the ELISA and ECLIA test above LFIA. In another paper, CLIA test (Abbott SARS-CoV-2 CLIA IgG; Abbott Laboratories, Illinois, USA), ELISA tests (Euroimmun Anti-SARS-CoV-2 ELISA IgG/IgA; Euroimmun) and LFIA tests (LFIA NG-Test IgG-IgM COVID-19; NG Biotech, Guipry, France) were tested on 293 specimens and the researchers compared them mutually (89). They reported 100 % sensitivity for all methods, when samples from patients were taken 14 days after symptoms onset. Overall specificity for IgG was above 98 % for CLIA and LFIA and 95.8 % for ELISA. The authors concluded their study by a statement that all the methods are reliable and accurate enough to be performed in clinical laboratories. The diagnoses based on antibodies can be further supported or confirmed by the analysis of cytokines and evaluation of cellular immunity by e.g. flow cytometry (90-96). Basic specifications of COVID-19 and SARS-CoV-2 as outcomes for diagnosis are summarized in the Table 1. Overview samples and methods for COVID-19 diagnosis described in the text above are written in the Table 2.

## Conclusions

Instrumental diagnosis of COVID-19 can be made by a wide number of techniques allowing a reliable identification of pathogen or markers and the disease can be plausibly confirmed or neglected by these techniques. Combination of genetic test like PCR and revealing of specific antibodies is adequate for most of the scenarios. On the other hand, there are other methods and some of them are applicable in point-of-care diagnosis though they are less sensitive than the standard laboratory test. Development of new methods on COVID-19 instrumental diagnosis can be expected because of extensive and intensive research. Significant changes in the standard laboratory methods for COVID-19 instrumental diagnosis are not expected, but development of portable devices, kits etc. is probable.

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Received September 11, 2020.
Accepted December 2, 2020.