**International Journal of**
**Recent Innovations in Medicine and Clinical Research**
Open Access, Peer Reviewed, Abstracted and Indexed Journal

ISSN: 2582-1075
https://ijrimcr.com/
Volume-2, Issue-2, 2020: 42-49

**Review Article**

# Laboratory Testing of Covid-19: A Brief Review

**Namrata Bhutani[1], Deepak Tangadi[1], Neha Bhutani[*2]**

[1]Department of Biochemistry, Vardhaman Mahavir Medical College and Safdarjung Hospital, New Delhi, India.
[2]ESIC Dental College, Rohini, New Delhi, India.
*Corresponding Author: Dr. Neha Bhutani, Email: nehabhutani09@gmail.com

**Received:** April 9, 2020 **Accepted:** May 10, 2020 **Published:** May 26, 2020

**Abstract:** Coronavirus disease 2019, abbreviated to COVID-19, is an emerging global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As the number of individuals infected with COVID-19 continues to rise globally and healthcare systems become increasingly stressed, it is clear that the clinical laboratory will play an essential role in this crisis, contributing to patient screening, diagnosis, monitoring/treatment, as well as epidemiologic recovery/surveillance. This review article aims to organize relevant available information on laboratory screening, testing protocols, diagnosis, and other general information on covid-19 for laboratory professionals.
**Keywords:** Coronavirus, Covid-19, Laboratory Diagnosis.

## Introduction

At the end of 2019, The World Health Organization (WHO) and China were alerted by a rise in the number of patients with pneumonia of unknown etiology in Wuhan City, China caused by an unidentified causative agent. On January 9, 2020, the Chinese Center for Disease Control and Prevention (Chinese CDC) declared the identification of a novel Coronavirus [1]. A few days later, it was reported that this novel type of coronavirus, termed by the WHO as "novel coronavirus-2019" (SARSCoV-2), was responsible for the outbreak [2]. It was noted that some parts of the genome sequence of SARS-CoV-2 were identical to those of two other coronavirus strains, namely, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) (approximately 79% homology) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (approximately 50% homology) [3, 4]. The current outbreak occurred after two outbreaks of SARS-CoV and one outbreak of MERS-CoV. The first two outbreaks occurred in 2002 and 2003 in the Guangdong region of China and were caused by the viral pathogen SARS-CoV [5,6]. The third outbreak, which occurred in the Middle East, was caused by the microbial pathogen MERS-CoV and led to a respiratory illness epidemic [7].

Coronavirus is member of the family Coronaviridae and subfamily Coronavirinae, which consists of four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus [8]. These four genera were created based on genomic construction and phylogenetic relationships [9]. The SARS-CoV-2 belongs to the Betacoronavirus genus. Zhou et al. [10] suggested that this viral outbreak has probably originated from bats. However, investigators have confirmed human-to-human transmission of this virus. According to recent information, SARS-CoV-2 has spread to different countries, including Thailand, Japan, Hong Kong, Singapore, South Korea, Taiwan, Macau, Malaysia, Australia, France, Italy, Vietnam, Nepal, India, Canada, and the United States.

As such, the current outbreak of SARS-CoV-2 is considered a medical crisis and has been declared as a pandemic by the WHO. This review article describes several aspects of role of laboratory

medicine in diagnosis of this infection including currently available diagnostic assays serological testing, pre-analytical and analytical testing issues,covid-19 detection in different clinical specimens and biosafety guidelines.

**Incubation Period of the Virus**

It is essential to understand the incubation period of a viral pathogen. In general, human coronavirus has an incubation period of approximately 4 days (range, 2-4 days). This incubation period was noted for the human coronavirus that can cause SARS [11]. The incubation period calculated for SARS-CoV was 4-6 days [12]. It has been noted that the incubation period of SARS-CoV-2 is 3-6 days, with the maximum being 14 days [13-15].

**Clinical Features**

On infection with human coronavirus, patients may exhibit signs and symptoms of upper respiratory tract infection, such as sore throat and rhinorrhea. However, clinical signs of SARS-CoV-2 infection include low-to-high fever, non-productive cough, myalgia, dyspnea, fatigue, standard or decreased leukocyte counts, and confirmed evidence of pneumonia on chest radiography. Among 138 hospitalized patients, the most common general symptoms at disease onset included fever (98.6%), dry cough (59.4%), fatigue (69.6%), dyspnea (31.2%), and myalgia (34.8%). Less common symptoms of SARS-CoV-2 infection include headache, abdominal pain, dizziness, nausea, vomiting, and diarrhea. In another study of 41 cases, Hui et al [16] reported several symptoms, including fever (> 90%), dry cough (80%), shortness of breath (20%), respiratory distress (15%), and fatigue. The researchers found that the hallmark signs and symptoms of this disease were stable in the majority of cases. However, investigators detected lymphopenia and leukopenia in these patients.

**Diagnostic Testing of Covid-19**

Laboratory medicine plays an essential role for diagnosing and managing many human pathologies [17] thus including infectious diseases and COVID-19 [18]. The diagnostic accuracy of (real time) reverse transcription polymerase chain reaction (rRT-PCR) this technique shall be considered a foremost prerequisite as currently it is the most widely used test [19-21].

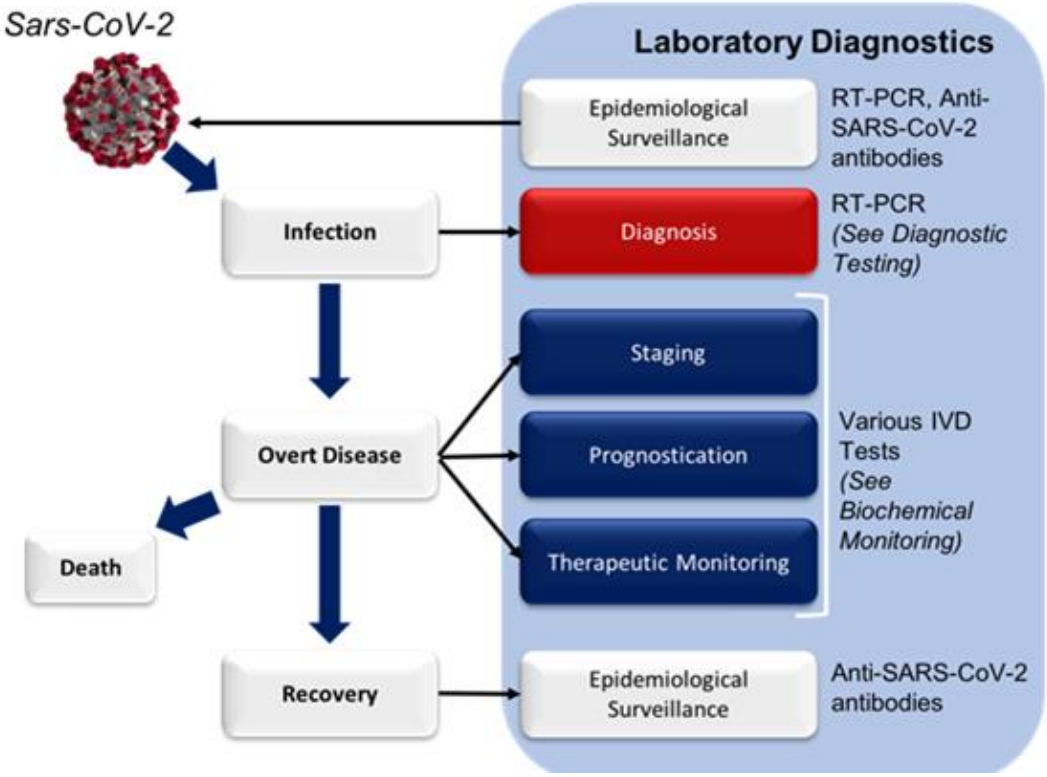



**Figure 1. Role of Laboratory Medicine in Covid-19 Pandemic[17]**

**Patient Screening**

According to the World Health Organization (WHO) guide for global surveillance for COVID-19 caused by human infection with COVID-19 virus, [22] a suspect case is defined as:

- ✓ A patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath), and a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset; **OR**

- ✓ A patient with any acute respiratory illness and having been in contact with a confirmed or probable COVID-19 case (see definition of contact) in the last 14 days prior to symptom onset; **OR**

- ✓ A patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath; and requiring hospitalization) and in the absence of an alternative diagnosis that fully explains the clinical presentation.

**A probable case is defined as:**

- ✓ A suspect case for whom testing for the COVID-19 virus is inconclusive; **OR**

- ✓ A suspect case for whom testing could not be performed for any reason.

**A confirmed case is defined as:**

A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms.

**Diagnostic Testing: Analytical and Clinical Aspects**

Upon confirmation of a suspected case, specimens should be rapidly collected and tested. The Centers for Disease Control and Prevention (CDC) Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (updated April 8$^{th}$) [23] recommends collecting an upper respiratory specimen for initial diagnostic testing.

The following specimens can be collected for swab-based testing: Nasopharyngeal specimen (preferred), Oropharyngeal specimen, Nasal mid-turbinate specimen and Anterior nares specimen. Lower respiratory tract specimen testing is also recommended by the CDC, if the specimens are available.

**Nucleic Acid Amplification Tests (NAAT)**

Real-time reverse transcription polymerase chain reaction (rRT-PCR) is the current gold standard for diagnosing suspected cases of COVID-19. rRT-PCR is a nucleic acid amplification test (NAAT) that detects unique sequences of the virus that causes COVID-19 (SARS-CoV-2) in respiratory tract specimens. The N, E, S, and RdRP are the viral genes currently targeted (WHO, Laboratory testing for coronavirus disease (COVID-19) in suspected human cases) [24].

A validated diagnostic workflow for detecting SARS-CoV-2 has been recently published by Corman and colleagues [25] as follows: (a) First line screening: E gene, (b) Confirmatory screening: RdRP gene, and (c) Additional confirmatory screening: N gene.

Table 1 presents criteria for a case to be considered as laboratory-confirmed by validated NAAT assays according to the WHO [24]:

**Table 1. WHO criteria for a case to be considered as laboratory-confirmed by validated NAAT assays [24]**

| Case Location | Confirmation Criteria |
|---|---|
| Area with known COVID-19 virus circulation | ✓ A positive NAAT result for a single discriminatory target in the SARS-CoV-2 genome |
| Area with no known COVID-19 virus circulation | ✓ A positive result for at least two different targets, one of which is specific for the SARS-CoV-2 virus (preferably).<br>✓ A positive NAAT result for betacoronavirus and identification of the SARS-CoV-2 virus by partial or whole genome sequencing (note: sequence target must be larger or different from the assay amplicon used). |

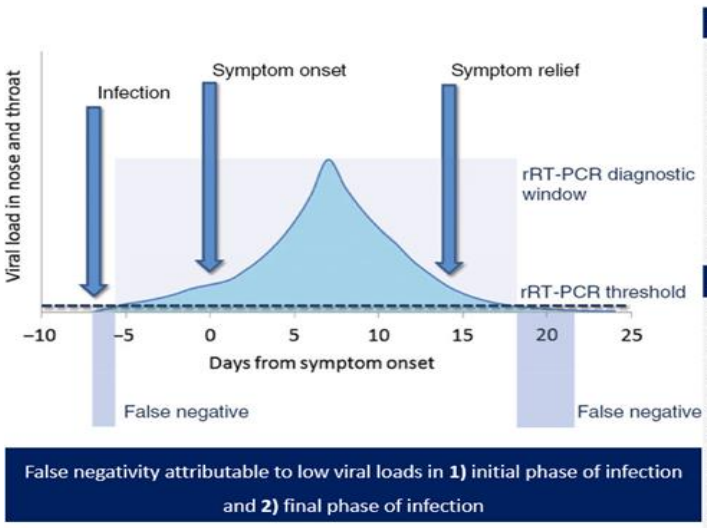
In some cases, a negative result may be returned for a suspected case with a high likelihood of COVID-19 infection. If the negative result was concluded based on only an upper respiratory tract specimen, a lower respiratory tract specimen should be subsequently tested. Additional specimens eligible for testing include blood and stool (WHO, Laboratory testing for coronavirus disease (COVID-19) in suspected human cases) [25].

**Currently Available Diagnostic Assays**

Several in-house and commercial assays are currently being developed and optimized. Links to currently available in-house protocols can be accessed here via the World Health Organization. Countries who have no testing capacity or national COVID-19 laboratories with limited experience on COVID-19 testing are encouraged to send the first five positives and the first ten negative COVID-19 samples to WHO reference laboratories providing confirmatory testing for COVID-19 [26-29]. Additionally, the WHO also released a Laboratory Assessment Tool (LAT) which is designed to assess the capacity of existing laboratories which aim to implement COVID-19 testing.

**Pre-Analytical and Analytical Testing Issues**

There are various pre-analytical and analytical issues that can affect diagnostic testing for COVID-19 infection. Some pre-analytical issues include improper collection, handling, transport and usage of swabs, as well as collection of inappropriate or inadequate material, interfering substances, and sample contamination. A common analytical issue is testing outside of the diagnostic window, in addition to active viral recombination and inadequately validated assays [30].




| Preanalytical Factors: |
|---|
| Lack of identification/misidentification |
| Inadequate specimen collection, handling, transport and storage |
| Collection of inadequate material for quality or volume |
| Presence of interfering substances |
| Manual (pipetting) errors |
| Sample contamination |
| Testing in patients receiving antiretroviral therapy |
| **Analytical Factors:** |
| Testing carried out outside of the diagnostic window |
| Active viral recombination |
| Use of non-adequately validated assays |
| Lack of harmonization of primers and probes |
| Instrument malfunctioning |
| Insufficient or inadequate material |
| Non-specific PCR annealing |
| Misinterpretation of expression profiles |

**Figure 2. Various pre-analytical and analytical issues that can affect diagnostic testing for COVID-19 infection [30].**

**Serological Testing**

There has been much debate regarding the current value of serological testing in COVID-19 diagnosis and monitoring. Serologic based tests are not currently recommended by the CDC, NHS or other health organizations. There is general concern regarding their use in the acute phase of infection as they detect infection too late in the course of illness (usually more than 7-10 days), they also may cross-react with serologic responses to seasonal coronaviruses and the rate and kinetics of serological response has not been clearly defined so far. However, there is anticipated value in using improved serological testing in the future for public and occupational health monitoring and assessment.

**Biosafety Guidelines for the Clinical Laboratory**

It is of the utmost importance that proper biosafety guidelines are followed by clinical laboratories when handling samples from suspected COVID-19 patients. Interim guidelines from the World Health Organization on laboratory biosafety guidance related to coronavirus disease were updated on March 19th, 2020. Excerpted highlights include:

- ✓ All procedures must be performed based on risk assessment and only by personnel with demonstrated capability, in strict observance of any relevant protocols at all times
- ✓ Initial processing (before inactivation) of all specimens should take place in a validated biological safety cabinet (BSC) or primary containment device
- ✓ Non-propagative diagnostic laboratory work (for example, sequencing, nucleic acid amplification test [NAAT]) should be conducted at a facility using procedures equivalent to Biosafety Level 2 (BSL-2)
- ✓ Propagative work (for example, virus culture, isolation or neutralization assays) should be conducted at a containment laboratory with inward directional airflow (BSL-3)
- ✓ Appropriate disinfectants with proven activity against enveloped viruses should be used (for example, hypochlorite [bleach], alcohol, hydrogen peroxide, quaternary ammonium compounds, and phenolic compounds)
- ✓ Patient specimens from suspected or confirmed cases should be transported as UN3373, "Biological Substance Category B"

**Efficacy of Masking**

The efficacy and necessity of surgical grade or N95 masks in the clinical laboratory and the general public has not been well defined. In a recent paper in Nature Medicine by Leung and colleagues [31], surgical face masks were shown to significantly reduce the detection of influenza virus RNA in respiratory droplets and coronavirus RNA in aerosols, with a trend toward reduced detection of coronavirus RNA in respiratory droplets. In light of increasing evidence towards the efficacy of masking, the WHO has reversed its initial recommendation and now supports government initiatives that require or encourage the public wearing of masks, marking a major shift. The CDC has also encouraged general masking, highlighting the importance of masking for all healthcare workers whether patient-facing or not.

**COVID-19 Detection in Different Clinical Specimens**

An important consideration in biosafety is the detectability of COVID-19 in clinical specimens. A recent JAMA [32] article investigated biodistribution among different clinical specimens of inpatients with COVID-19. Study findings are summarized below:

| Clinical Specimen | Positive Rate |
|---|---|
| Bronchoalveolar lavage fluid | 93% (14/15) |
| Sputum | 72% (72/104) |
| Nasal Swab | 63% (5/8) |
| Fibrobronchoscope brush biopsy | 46% (6/13) |
| Pharyngeal swabs | 32%(126/398) |
| Feces | 29% (44/153) |
| Blood | 1% (3/307) |
| Urine | 0% (0/72) |

**Figure 3. Bio-distribution among different clinical specimens of inpatients with COVID-19**

**COVID-19 Detection in Domestic Animals**

A recent publication in Science [33] investigated the susceptibility of domestic animals, including dogs, cats, chickens, pigs, and ducks. Their findings demonstrated that cats are susceptible to airborne infection while COVID-19 replicates poorly in dogs, pigs, chickens, and dogs. Healthcare workers should keep this in mind when interacting with domestic pets.

**Conclusions**

The COVID-19 pandemic is spreading across the globe at an alarming rate. It has caused more infections and deaths as compared with SARS or MERS. Based on R0 values, it is deemed that SARS-CoV-2 is more infectious than SARS or MERS. Elderly and immunocompromised patients are at the greatest risk of fatality. The rapid spread of disease warrants intense surveillance and isolation protocols to prevent further transmission. No confirmed medication or vaccine has been developed. Current treatment strategies are aimed at symptomatic care and oxygen therapy. Prophylactic vaccination is required for the future prevention of COV-related epidemic or pandemic.

**Conflict of Interest:** The authors declare that they have no conflicts of interest.

**References**

1. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. The Lancet. 2020;395(10223):470-3.
2. Bravo D, Solano C, Giménez E, Remigia MJ, Corrales I, Amat P, Navarro D. Effect of the IL28B Rs12979860 C/T polymorphism on the incidence and features of active cytomegalovirus infection in allogeneic stem cell transplant patients. J Med Virol. 2014;86(5):838-44.
3. Gralinski LE, Menachery VD. Return of the Coronavirus: 2019-nCoV. Viruses 2020;12:pii: E135.
4. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020;395(10224):565-74.
5. Zhong NS, Zheng BJ, Li YM, Poon LL, Xie ZH, Chan KH, Li PH, Tan SY, Chang Q, Xie JP, Liu XQ. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. The Lancet. 2003;362(9393):1353-8.
6. Drosten C, Günther S, Preiser W, Van Der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. New England J Med. 2003;348(20):1967-76.

7. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. New England J Med. 2012;367(19):1814-20.

8. Müller H. The Coronaviridae. The Viruses, Stuart G. Siddell, H. Fraenkel-Conrad, RR Wagner (Eds.), Plenum Press, New York-London (1995), Urban & Fischer; 1996; pp. 1-418.

9. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. 2019;17(3):181-92.

10. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3.

11. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DA. Incubation periods of acute respiratory viral infections: a systematic review. The Lancet Infect Dis. 2009;9(5):291-300.

12. Meltzer MI. Multiple contact dates and SARS incubation periods. Emerg Infect Dis. 2004;10(2):207-209.

13. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The Lancet. 2020;395(10223):514-23.

14. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Yu T. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 2020;395(10223):507-13.

15. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, Fang C, Huang D, Huang LQ, Huang Q, Han Y. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res. 2020;7(1):4.

16. https://doi.org/10.1016/S0140-6736(20)30920-X

17. Clin Chem Lab Med. 2020 Feb 18. pii:/j/cclm.ahead-of-print/cclm-2020-0114/cclm-2020-0114.xml. doi: 10.1515/cclm-2020-0114.

18. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med. 2020. doi: https://doi.org/10.1515/cclm-2020-0198

19. Lippi G, Plebani M. The novel coronavirus (2019-nCoV) outbreak: think the unthinkable and be prepared to face the challenge. Diag. 2020;7(2):79-81.

20. Pang J, Wang MX, Ang IY, Tan SH, Lewis RF, Chen JI, Gutierrez RA, Gwee SX, Chua PE, Yang Q, Ng XY. Potential rapid diagnostics, vaccine and therapeutics for 2019 novel coronavirus (2019-nCoV): a systematic review. J Clin Med. 2020;9(3):623.

21. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, Fang C, Huang D, Huang LQ, Huang Q, Han Y. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res. 2020 Dec 1;7(1):4.

22. World Health Organization. Global surveillance for COVID-19 caused by human infection with COVID-19 virus: interim guidance, 20 March 2020. World Health Organization; 2020.

23. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

24. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance

25. Euro Surveill. 2020 Jan 23; 25(3): 2000045. doi: 10.2807/1560-7917.ES.2020.25.3.2000045

26. https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

27. https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/announcements/interim-order-importation-sale-medical-devices-covid-19/guidance-medical-device-applications.html#a24;

28. https://eur-lex.europa.eu/legal-ontent/EN/TXT/PDF/?uri=CELEX:52020XC0415(04)&from=EN

29. https://www.finddx.org/covid-19/dx-data/

30. https://doi.org/10.1515/cclm-2020-0285

31. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020;323(18):1843-4.

32. https://doi.org/10.1038/s41591-020-0843-2

33. Science. 2020 Apr 8: eabb7015. Published online 2020 Apr 8. doi: 10.1126/science.abb7015

**Citation:** Bhutani N, Tangadi D, Bhutani N. Laboratory Testing of Covid-19: A Brief Review. Int J Rec Innov Med Clin Res. 2020;2(2):42-49.