Systematic Review

# A Review on Applicable and Available Paraclinical Methods for Diagnosis of Coronavirus Disease-19

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**Abstract**
**Background:** The recent outbreak by a novel coronavirus originated from Wuhan, China in 2019, and is progressively spreading to other countries. Timely diagnosis of the coronavirus disease 2019 (COVID-19) improves the survival of the patients and also prevents the transmission of the infection. In this study, we reviewed the applicable and available methods for the diagnosis of COVID-19.
**Methods:** For the review, we systematically searched Web of Science, PubMed, and Iranian articles that were published about COVID-19 diagnostic methods with a combination of the key terms: laboratory, radiological, tests, coronavirus.
**Results:** Although the current gold standard diagnostic test for this virus is real-time reverse-transcriptase polymerase chain reaction (RT-PCR), the occasional false-negative and the low sensitivity of the test should not be underestimated. A chest computed tomography (CT) scan is another diagnostic test for COVID-19, with higher sensitivity but low specificity. A combination of sensitive RT-PCR with a chest CT scan together with the clinical features are highly recommended for the proper diagnosis. Notably, there are some other sensitive and low-cost tests for evaluation of COVID-19 infection, but their validation should be approved.
**Conclusion:** Since early and accurate diagnosis of the viral disease could improve the survival rate of the patients, and halt the transmission chain, it is not surprising that tremendous attempts should be made to reduce the limitations of the tests leading to the false-negative results and to find a rapid test for the diagnosis of COVID-19.
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## Introduction

The outbreak of novel coronavirus (2019-nCov) started from Wuhan in Hubei province, China. From December 2019 to of May 12, 2020, there were 4 256 583 confirmed cases of coronavirus disease 2019 (COVID-19) and 287 354 deaths (https://www.worldometers.info/coronavirus/). The main diagnostic criteria for COVID-19 are (1) history of contact with patients, (2) positive nucleic acid test, (3) clinical symptoms like fever and cough, (4) lung lesions, and (5) laboratory findings.[1] Koch's postulates, immunological methods, protein microarray, and micro-neutralization are considered to be conventional methods for virus detection.[2] However, their lack of sensitivity and specificity leads to the urgent need for more valid, more reliable, as well as more rapid tests to properly diagnose the disease and prevent its spread.[2,3] Also, confirmatory tests should be performed for differential diagnoses such as the respiratory syncytial virus, adenovirus, influenza, parainfluenza, as well as bacterial infections.[4] In this study, we reviewed the acceptable and available, sensitive, specific and rapid paraclinical methods for diagnosis of COVID-19.

## Materials and Methods

For the review, we examined 60 published articles related to the diagnosis of 2019-nCov infection. The search process was performed in international medical databases, including ISI, PubMed, and Scopus, along with Iranian databases like SID. The search strategy was to include articles from 2019 (beginning of the coronavirus outbreak) or those articles about the virus detection-related methods of Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). The keywords for the search included 'coronavirus', '2019-nCov', 'COVID-19', 'diagnosis', 'detection', 'test', 'laboratory', 'radiology', 'sample', 'laboratory findings'. We included and evaluated

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the studies of laboratory and radiological methods with the ascertained results along with reported sensitivity.

## Results

Selected studies were investigated and, as will be shown in the following, various methods with different sensitivity were identified for diagnosis of COVID-19.

### Molecular Methods
#### PCR-Based Methods

As the traditional and gold standard method, isolation of virus from infected samples as well as the detection of the viral genome are confirmative diagnostic methods for viral infections.[5] Although a viral culture of infectious samples is the gold standard method for the laboratory-based diagnosis of respiratory infections, the accuracy and sensitivity of this method for human coronaviruses are low due to the difficult growth of the virus in the cell culture conditions.[6] Given this, it seems that the polymerase chain reaction (PCR)-based techniques, including reverse-transcriptase (RT) PCR, nested PCR, real-time PCR, multiplex real-time RT-PCR and TaqMan-based real-time RT-PCR could be more effective for the diagnosis of this virus, as these methods have been developed for compensating the lack of sensitivity and accuracy of the cell culture techniques.[6-8]

For the first time, 2019-nCov was discovered by metagenomic next generation sequencing (mNGS) and it was established that this virus belongs to the beta-coronavirus group and is 79% similar to SARS.[9,10] Today, RT-PCR and mNGS are two common molecular diagnostic approaches for 2019-nCov.[11] In RT, cDNA is synthesized from the extracted RNAs from the samples and then, in the presence of the designed and targeted primers for specific sequences (like 2019-nCov specific sequence), amplification of the designated sequence is accomplished by thermocycler.[12] In order to confirm that the amplified products are the targeted sequence, gel electrophoresis or sequencing methods could be performed.[13] Since RT-PCR is a time-consuming and costly method, real-time

RT-PCR can be used instead as a more sensitive and less expensive approach for quantitative detection of a designated sequence, in particular the viral genome of coronavirus.[14] However, to improve the technique and to tackle some of the basic problems of real-time RT-PCR like genetic variation and contamination, which lead to the false-negative and false-positive results, modifications have been made for higher accuracy and sensitivity of coronavirus detection, including multiplex real-time and TaqMan-based real-time.[14] The reported sensitivity of RT-PCR for coronavirus detection is 71%–78% (Table 1)[15,16] and there are some reasons for the low efficiency of this method, like 1) low level of viral load in patients, 2) various detection rates of different manufacturers, 3) some errors in sampling, 4) variation of viral RNA sequence, and 5) limitation in the technology of nucleic acid detection.[2,15] The use of stable and protected RNA with a detection limit of 10 copies/microliter as an external positive control in real-time RT-PCR improves the accurate quantification of the coronavirus load in samples.[17] Given the high rate of genetic variations in coronavirus leading to possible false-negative results, multiplex real-time is required for sensitive detection of the virus.[14] Also, the use of degenerated primers designed for pan-coronavirus allows the detection of all known and unknown types of coronaviruses through RT-PCR and subsequently, sequencing.[6] TaqMan-based real-time can be performed for routine diagnosis of the human coronavirus.[14] Moreover, a quantitative real-time PCR (qPCR), which was designed by 2 TaqMan probes instead of 1 probe, has been valuable and highly sensitive for SARS coronavirus diagnosis, even in the presence of 1 copy of viral RNA.[18]

Envelope (E) gene, RNA-dependent RNA polymerase (RdRp) gene, nucleoprotein (N) gene and open reading frame-1b (ORF-1b) from genome of 2019-nCov are targets of primer design and real-time RT-PCR for diagnosis and confirmation of the virus infection.[5]

The Center for Disease Control (CDC) in the United States recommends to use the N gene for confirmation of COVID-19 and Lim et al in South Korea reported higher

**Table 1.** Sensitivity and Positivity Rate of Main Tests of 2019-nCov and Their Sampling Considerations

| Test | Sensitivity (%) | Positivity Rate (%)[23] | Timing of Sampling[37] | Sample Storage Temperature Until Test (°C)[37] | Sample Shipment Temperature (°C)[37] |
|---|---|---|---|---|---|
| RT-PCR | 71–78 | | | | |
| | Samples for RT-PCR | | Collect initialy on presentation. Repeat for monitoring of clearance | 2–8 | |
| | BAL | 93 | | | 2-8 if ≤2 days −70 (dry ice) if >2 days |
| | Sputum | 72 | | | |
| | Swab | 63 | | | 2-8 if ≤5 days −70 (dry ice) if >5 days |
| Serology | 89 | | In first week of illness. Second sample: 2-4 weeks later | 2–8 | 2-8 if ≤5 days −70 (dry ice) if >5 days |
| CT Scan | 97 | | | | |
| RT-PCR-CT Scan combination | 92 | | | | |

BAL, Bronchoalveolar lavage; RT-PCR, reverse-transcriptase polymerase chain reaction; CT, computed tomography.

reproducibility in real-time RT-PCR when targeting the N gene instead of RdRp. These findings ultimately led the World Health Organization (WHO) to announce the use of N gene in the confirmatory tests.[19] Considering the necessity of early viral infection detection, Chu et al[20] developed a 1-step quantitative real-time RT-PCR to detect two different regions of the 2019-nCov genome (N and ORF-1b). In addition to rapid virus detection, the authors also suggested that compared to ORF-1b, targeting the N gene led to 10-times higher sensitivity in positive samples.[20]

The criteria for discontinuation of hospital quarantine are (1) normal temperature for at least consecutive 3 days, (2) treated respiratory symptoms, (3) no feature lesions on computed tomography (CT) scan, and (4) two consecutive negative RT-PCR tests.[21] Lan et al reported that even though there were no clinical features of the disease or transmission of the virus to family members, the results of the RT-PCR test could be positive in patients recovering from COVID-19 for 5-13 days after hospital discharge, suggestive of the possibility of false-negative results of the previous tests.[21]

The positive detection rate of 2019-nCov from a sample of sputum (as a sample from lower respiratory) by RT-PCR is significantly more than samples of throat swabs.[22] Overall, the samples with the highest to lowest positive detection rate are as follows (1) bronchoalveolar lavage (BAL) fluid sample, (2) sputum, (3) nasopharynx swabs, (4) fibrobronchoscope brush biopsy, (5) oropharynx swabs, (6) feces, and (7) blood (Table 1).[23] It is necessary to process the sample of sputum befor nucleic acid extraction due to the high viscosity, as presented by CDC.[24] Moreover, it has been reported that a positive nucleic acid test on fecal sample could be indicative of lack of gastrointestinal symptoms.[25]

## Isothermal Methods
Unlike real-time RT-PCR, there is no need for temperature control settings (e.g. thermocycler) for the isothermal molecular methods.[11] CRISPR-based method and loop-mediated isothermal amplification (LAMP) are two isothermal molecular techniques.

### CRISPR-Based Method
Although the main aim of CRISPR is gene editing, the cleavage activities of Cas nuclease in rule-less and irregular manner results in the use of CRISPR/Cas technique in nucleic acid detection.[11] Because of the global concern about the need for specific, sensitive and rapid diagnostic techniques, tremendous attention has been attracted by CRISPR-nCov, as this technique could detect as few as 10 RNA copies/µL of 2019-nCov just within 40 minutes.[11,26] The sensitivity and speed of the technique will be increased by applying machine learning designing.[26] Also, the technique should be considered for monitoring and

coping with genetic variations in 2019-nCov.[11]

### Loop-Mediated Isothermal Amplification
LAMP amplifies the DNA and RNA rapidly by at least 4 different primes without requiring thermocycler and expensive reagents. Moreover, its products could be detected by UV light or DNA stains.[14] Yu et al developed a LAMP-based method for detection of 2019-nCov using 6 primers targeting the ORF1b region and by comparing the sequence of 11 related viruses, they reported a high specificity for the method in detecting the novel virus. The sensitivity of the method is comparable to TaqMan qPCR with a detection limit of 10 copies of 2019-nCov RNA.[3]

The sensitivity and detection rate of LAMP for 2019-nCov are similar to conventional PCR.[14] Poon et al declared that although false-positive results were observed in neither LAMP nor highly sensitive qPCR,[27] the detection rate of qPCR was higher than LAMP within the first 3 days of disease onset.[27] However, due to its low cost, LAMP is applicable in areas where specialized technologies are not available.[3,14] In addition, LAMP as a point of care device could be utilized in outland regions like islands and cruise ship (e.g. Diamond Princess).[28] Also, by using double-strand DNA fluorescence dyes in the LAMP method, the real-time detection of amplicons can be feasible and through "sequence-specific LAMP-based method" specific signals could be separated from non-specific noise.[14]

### Chest Computed Tomography Scan
In Hubei, increased and improved diagnosis of COVID-19 was achieved with the introduction of chest CT scan parameters. Since many patients with respiratory disorders with pulmonary symptoms were waiting for the results of laboratory tests, CT scans became useful and applicable.[29] Chest CT scan has a critical role in COVID-19 diagnosis workup for suspected patients and can be used for screening patients with false-negative RT-PCR results.[1,29] At 9–13 days after the disease onset, the results of CT scan (multiple, bilateral lungs lesion with a peripheral and diffuse distribution like ground-glass opacity (GGO) or occasional consolidation with vascular enlargement and cobblestone/reticular pattern) would be intensified and would be indicative of the development of chest lesions.[1,30] It is worth mentioning that all of the indicated findings are not obvious in all patients.[1,29-31] In the early stages of infection, CT findings can be absent or mild, leading to misdiagnosis.[30] However, consolidation and vascular enlargement features, fibrosis, dilated bronchi with thickened wall, and the incidence of air-bronchogram are detected in all of the patients with progressed infection.[1] A high mortality rate is seen in those patients with a high rate of GGO and consolidation.[32]

Time course is an important factor in CT findings and it is demonstrated that finding will become more frequent with passing time.[30,33] Additionally, the results of serial CT

scans showed that there is a progression in lung lesions and there will be a possibility for development of acute lung injury over time.[30]

There are some discrepancies between the CT scan and RT-PCR results. It is possible that patients with negative viral nucleic acid on two tests display significant changes on CT scan. On the contrary, even positive RT-PCR results could be accompanied by the normal CT findings.[1,29,33] Indeed, with greater spread of 2019-nCov, more results of RT-PCR analysis of infected persons would be negative.[1] Given these, the changes observed on CT scan, especially in asymptomatic patients, make the CT scan more sensitive approach compared to RT-PCR, although the specificity of RT-PCR is higher than CT.[1,29] In a patient with a history of travel to endemic areas and contact with infected patients, CT scan along with RT-PCR is considered to be an applicable screening method.[29] Therefore, CT scan findings have a critical role in the diagnosis of COVID-19 and should be considered to be the main method for the evaluation of the viral infection in endemic regions.[1,30]

### Combination of PCR and Chest CT Scan

The higher sensitivity of 2019-nCov is provided by the combination of RT-PCR and CT scan. Whereas the sensitivity of RT-PCR alone is 78%, a combination of the test with CT scan has a sensitivity of 92% (Table 1). The results of the two methods are in good agreement, except in mild viral infection.[16]

The low specificity of CT scan is troublesome in some cases, as it can not differentiate 2019-nCov from other pathogens. This lower sensitivity could be compensated for through combining CT scan with a higher specificity technique, such as RT-PCR.[16] The results of the meta-analysis conducted by Vaseghi G and colleagues showed that the sensitivity of CT scan combined with positive RT-PCR is 97% for the diagnosis of 2019-nCov.[34] Moreover,The use of CT scan is highly recommended as a screening tool in those patints who have clinical and epidemiologic features compatible with 2019-nCov infection, but whose results of RT-PCR analysis are negative.[15]

These results were further confirmed by a study performed on more than 1000 patients with the typical symptoms of 2019-nCov.[35] This study hightlighted that the total positive rate of RT-PCR at initial presentation was about 59%. In this study, 97% of RT-PCR positive results were also positive for chest CT, and about 81% of the patients with negative RT-PCR/positive chest CT scans were finally re-classified as likely or probable cases of COVID-19 infected patients. This study suggested the nesseccity and higher sensitivity of the combination of exposure history, clinical symptoms, typical CT imaging features, and dynamic changes for diagnosis of COVID-19 in patients with negative RT-PCR.[35]

### Serological Tests

It is well-established that a rapid and accurate diagnostic test for detection of COVID-19 could halt the progression of the disease, as well as preventing its transmission.[36] Additionally, retrospective assessment and evaluation of the ongoing outbreak, especialy in negative RT-PCR results, is made possible by epidemiological studies.[37] Serologic tests are quite appropriate for follow-up of individuals and identification of infection sources.[38]

Immunoglobulin M (IgM), as one of the first immune responses, and IgG, as a long-term adaptive and strong immune response, can be evaluated for detection of viral infection. Both IgM and IgG increase in response to the 2019-nCov infection and are detectable 3–6, and 8 days after virus onset, respectively. So, detection of IgM and IgG against 2019-nCov in human blood within 15 minutes can diagnose the infection in different stages with 89% and 91% sensitivity and specificity, respectively.[36] On average, 5.5 days after the onset of the symptoms, in which the results of the RT-PCR analysis could be negative, the detection efficiency of IgM is more than RT-PCR, suggesting that the combination of IgM enzyme-linked immunosorbent assay (ELISA) and RT-PCR results could be promising.[39] The results from both serum and plasma samples of venous blood and finger-stick blood, which are used for this test, are similar.[36]

### Other Suggested Methods

1. Fluorescence immunochromatographic assay (FICA) for detection of 2019-nCov antigens might be a valuable and simple method for rapid and accurate diagnosis. The results of measurement of the coronavirus nucleocapsid protein by FICA from nasopharyngeal swab and urine samples are in 100% accordance with RT-PCR.[40]

2. A combination of CT and fluorodeoxyglucose-positron emission tomography (FDG-PET) can be used in the differential diagnosis of complex cases, but not as a routine test.[41]

3. Mass spectrometry (MS) as the complement of next generation sequencing (NGS) is applicable for large scale screening evaluations of COVID-19. MS is a relatively simple method to perform with acceptable sensitivity.[42]

4. Deep machine learning models and artificial intelligence methods have been developed based on the results of CT scans, and recommended to be used for rapid and accurate identification of infection through specific and sensitive extracted radiological features.[43,44]

### Other Laboratory Findings

There are some laboratory findings that could differentiate between COVID-19 and non-COVID-19 pneumonia, which are valuable for evaluating the patients' prognosis

and outcome. Based on the complete blood count results, lymphopenia and thrombopenia are two common findings in both severe and non-severe COVID-19 patients.[45,46] The lymphopenia in COVID-19 patients was further evaluated by flowcytometry and significant low counts of CD4- and CD8-positive T-cells as well as CD19-positive B-cells were reported in COVID-19 patients.[46] Significantly higher levels of C-reactive protein, erythrocyte sedimentation rate and serum ferritin were also observed in COVID-19 in comparison with non-COVID-19.[45,46] Furthermore, eosinophil count, which is decreased in COVID-19, could differentiate this disease from other viral infections with higher sensitivity and specificity in comparison to lymphocyte count.[47] The laboratory features affecting the survival of the patients are shown in Table 2.[32] The elevation in serum levels of creatine phosphokinase (CPK), lactate dehydrogenase (LDH), D-dimer, creatinine, prothrombin time (PT) and aspartate aminotransferase/alanine aminotransferase (AST/ALT) are the parameters that could be associated with severe disease.[4]

### Clinical Findings
The most common clinical findings of COVID-19 are fever, cough, fatigue, shortness of breath, muscle pain, diarrhea, expectoration, and anorexia.[2,45] As shown in Table 2, some of the underlying diseases, such as hypertension and diabetes could increase the mortality rate of COVID-19.[32]

### Concluding Remarks
Decision to perform the diagnostic methods are based on the epidemiological findings, such as contact with a patient, and the clinical features. Nucleic acid detection tests (RT-PCR) confirm COVID-19.[37] Sensitive RT-PCR in combination with chest CT scan are the main applicable

and available methods for diagnosis of the disease. In the case of a negative RT-PCR result, repeating the test and considering other laboratory findings should be considered for accurate diagnosis. Detection of infectious clusters and following up the individuals can be the target of serologic tests and rapid methods like LAMP, especially in regions with lack of specific equipment.

### Conclusion
While the progress of 2019-nCov infection is shown a slowing trend in the mainland of China, new cases of COVID-19 are increasing in other countries. According to the WHO recommendations, all suspected cases should be tested in the countries dealing with clusters of infection. Early and accurate diagnosis of the viral disease not only cuts the transmission chain, but also reduces the epidemic. Given the fact that there is little knowledge about the possible factors affecting the proper diagnosis of COVID-19, it is not surprising if tremendous attempts are needed to reduce the limitations leading to false-negative results of RT-PCR. Moreover, it seems that the increase in the basic knowledge about immune response dynamic, optimum sampling time, the correlation between disease severity and viral load and the virus mutation monitoring could be indispensable for confronting this disease.

**Authors' Contribution**
MR, FP, and VA contributed to idea generation, literature review, and data collection. SRP and SS contributed to article drafting and editing.

**Table 2.** Laboratory, CT Scan and Clinical Characteristics That Influence the Mortality of COVID-19*

| Characteristics | Parameter | Death by COVID-19 | Survivors of COVID-19 |
|---|---|---|---|
| Laboratory | WBC count | High | Normal/low |
| | Lymphocyte count | Low | Normal/high |
| | D-dimer | High | Normal |
| | Serum ferritin | High | Normal |
| | Interleukin-6 | High | Normal |
| CT scan | Consolidation | High stage | Low stage/normal |
| | GGO | High stage | Low stage/normal |
| Clinical | Blood pressure | High | Normal |
| | Diabetes | Seen | Not seen |
| | Coronary heart disease | Seen | Not seen |
| | SOFA score | High | Low |

SOFA, Sequential Organ Failure Assessment; CT, computed tomography; GGO, ground-glass opacity.
* These comparisons between death and survival are relative to each other.

### References
1. Xu Z, Wu W, Jin Y, Pan A. Key Points of Clinical and CT Imaging Features of 2019 Novel Coronavirus (2019-nCoV) Imported Pneumonia Based On 21 Cases Analysis. Available at SSRN 3543610. 2020. Available from: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3543610
2. Wang Y, Kang H, Liu X, Tong Z. Combination of RT-qPCR Testing and Clinical Features For Diagnosis of COVID-19 facilitates management of SARS-CoV-2 Outbreak. J Med Virol. 2020;92(6):538-539. doi: 10.1002/jmv.25721.
3. Yu L, Wu S, Hao X, Li X, Liu X, Ye S, et al. Rapid colorimetric detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic plat-form: iLACO. Clin Chem. 2020;66(7):975-977. doi: 10.1093/clinchem/hvaa102.
4. Singhal T. A review of coronavirus disease-2019 (COVID-19). Indian J Pediatr. 2020;87(4):281-286. doi: 10.1007/s12098-020-03263-6.
5. Yu F, Du L, Ojcius DM, Pan C, Jiang S. Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhan, China. Microbes Infect. 2020;22(2):74-79. doi: 10.1016/j.micinf.2020.01.003.
6. Vijgen L, Moës E, Keyaerts E, Li S, Van Ranst M. A pancorona-virus RT-PCR assay for detection of all known coronaviruses. SARS-and Other Coronaviruses. Totowa, NJ. Springer; 2008:3-

12. Available from: https://experiments.springernature.com/articles/10.1007/978-1-59745-181-9_1

7. Hadjinicolaou AV, Farcas GA, Demetriou VL, Mazzulli T, Poutanen SM, Willey BM, et al. Development of a molecular-beacon-based multi-allelic real-time RT-PCR assay for the detection of human coronavirus causing severe acute respiratory syndrome (SARS-CoV): a general methodology for detecting rapidly mutating viruses. Arch Virol. 2011;156(4):671-80. doi: 10.1007/s00705-010-0906-7.

8. van Elden LJ, Anton M AM, van Alphen F, Hendriksen KA, Hoepelman AI, van Kraaij MG, et al. Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. J Infect Dis. 2004;189(4):652-7. doi: 10.1086/381207.

9. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-574. doi: 10.1016/S0140-6736(20)30251-8.

10. Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chin Med J (Engl). 2020;133(9):1015-1024. doi: 10.1097/CM9.0000000000000722.

11. Hou T, Zeng W, Yang M, Chen W, Ren L, Ai J, et al. Development and Evaluation of A CRISPR-based Diagnostic For 2019-novel Coronavirus. PLoS Pathog. 2020;16(8):e1008705. doi: 10.1371/journal.ppat.1008705.

12. Kuang J, Yan X, Genders AJ, Granata C, Bishop DJ. An overview of technical considerations when using quantitative real-time PCR analysis of gene expression in human exercise research. PLoS One. 2018;13(5):e0196438. doi: 10.1371/journal.pone.0196438.

13. Osawa Y, Ikebukuro K, Motoki H, Matsuo T, Horiuchi M, Sode K. The simple and rapid detection of specific PCR products from bacterial genomes using Zn finger proteins. Nucleic Acids Res. 2008;36(11):e68. doi: 10.1093/nar/gkn274.

14. Shen M, Zhou Y, Ye J, AL-maskri AAA, Kang Y, Zeng S, et al. Recent advances and perspectives of nucleic acid detection for coronavirus. J Pharm Anal. 2020;10(2):97-101. doi: 10.1016/j.jpha.2020.02.010.

15. Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, et al. Sensitivity of chest CT for COVID-19: comparison to RT-PCR. Radiology. 2020;296(2):E115-E117. doi: 10.1148/radiol.2020200432.

16. Jiang G, Ren X, Liu Y, Chen H, Liu W, Guo Z, et al. Application and optimization of RT-PCR in diagnosis of SARS-CoV-2 infection. medRxiv. 2020.

17. Yu XF, Pan JC, Ye R, Xiang HQ, Kou Y, Huang ZC. Preparation of armored RNA as a control for multiplex real-time reverse transcription-PCR detection of influenza virus and severe acute respiratory syndrome coronavirus. J Clin Microbiol. 2008;46(3):837-41. doi: 10.1128/JCM.01904-07.

18. Yip SP, To SST, Leung PH, Cheung TS, Cheng PK, Lim WW. Use of dual TaqMan probes to increase the sensitivity of 1-step quantitative reverse transcription-PCR: application to the detection of SARS coronavirus. Clin Chem. 2005;51(10):1885-8. doi: 10.1373/clinchem.2005.054106.

19. Kim S, Kim DM, Lee B. Insufficient Sensitivity of RNA Dependent RNA Polymerase Gene of SARS-CoV-2 Viral Genome as Confirmatory Test using Korean COVID-19 Cases. Preprints 2020. doi: 10.20944/preprints202002.0424.v1.

20. Chu DK, Pan Y, Cheng S, Hui KP, Krishnan P, Liu Y, et al. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clin Chem. 2020;66(4):549-555. doi: 10.1093/clinchem/hvaa029.

21. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR Test Results in Patients Recovered From COVID-19. JAMA. 2020;323(15):1502-1503. doi: 10.1001/jama.2020.2783.

22. Lin C, Xiang J, Yan M, Li H, Huang S, Shen C. Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-Cov-2) infected pneumonia (COVID-19). Clin Chem Lab Med. 2020;58(7):1089-1094. doi: 10.1515/cclm-2020-0187.

23. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. 2020;323(18):1843-1844. doi: 10.1001/jama.2020.3786.

24. Processing of Sputum Specimens for Nucleic Acid Extraction. 2020. Available from: https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf.

25. Zhang J, Wang S, Xue Y. Fecal specimen diagnosis 2019 novel coronavirus–infected pneumonia. J Med Virol. 2020 Jun;92(6):680-682. doi: 10.1002/jmv.25742.

26. Metsky HC, Freije CA, Kosoko-Thoroddsen TSF, Sabeti PC, Myhrvold C. CRISPR-based surveillance for COVID-19 using genomically-comprehensive machine learning design. bioRxiv. 2020. Available from: https://www.biorxiv.org/content/10.1101/2020.02.26.967026v2.abstract.

27. Poon LL, Wong BW, Chan KH, Ng SS, Yuen KY, Guan Y, et al. Evaluation of real-time reverse transcriptase PCR and real-time loop-mediated amplification assays for severe acute respiratory syndrome coronavirus detection. J Clin Microbiol. 2005;43(7):3457-9. doi: 10.1128/JCM.43.7.3457-3459.2005.

28. Nguyen T, Duong Bang D, Wolff A. 2019 Novel coronavirus disease (COVID-19): paving the road for rapid detection and point-of-care diagnostics. Micromachines (Basel). 2020;11(3):306. doi: 10.3390/mi11030306.

29. Lee EY, Ng MY, Khong PL. COVID-19 pneumonia: what has CT taught us? Lancet Infect Dis. 2020;20(4):384-385. doi: 10.1016/S1473-3099(20)30134-1.

30. Kanne JP, Little BP, Chung JH, Elicker BM, Ketai LH. Essentials for radiologists on COVID-19: an update—Radiology Scientific Expert Panel. Radiology. 2020;296(2):E113-E114. doi: 10.1148/radiol.2020200527.

31. Ng MY, Lee EY, Yang J, Yang F, Li X, Wang H, et al. Imaging profile of the COVID-19 infection: radiologic findings and literature review. Radiology: Cardiothoracic Imaging. 2020;2(1):e200034. doi: 10.1148/ryct.2020200034.

32. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3.

33. Bernheim A, Mei X, Huang M, Yang Y, Fayad ZA, Zhang N, et al. Chest CT Findings in Coronavirus Disease-19 (COVID-19): Relationship to Duration of Infection. Radiology. 2020;295(3):200463. doi: 10.1148/radiol.2020200463.

34. L Fu, B Wang, T Yuan, X Chen, Y Ao, T Fitzpatrick, P Li, et al. Clinical characterization and chest CT findings in laboratory-confirmed COVID-19 in China: a systematic review and meta-analysis. medRxiv. 2020. Available from: https://www.sciencedirect.com/science/article/pii/S0163445320301705.

35. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. 2020;296(2):E32-E40. doi: 10.1148/radiol.2020200642.

36. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. J Med Virol. 2020:10.1002/jmv.25727. doi: 10.1002/jmv.25727.

37. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. 2020. Available from: https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117.

38. Okba N, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome

Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients. Emerg Infect Dis. 2020;26(7):1478-1488. doi: 10.3201/eid2607.200841.

39. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis. 2020;71(15):778-785. doi: 10.1093/cid/ciaa310.

40. Diao B, Wen K, Chen J, Liu Y, Yuan Z, Han C, et al. Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. medRxiv. 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.03.07.20032524v2.abstract.

41. Qin C, Liu F, Yen TC, Lan X. 18 F-FDG PET/CT findings of COVID-19: a series of four highly suspected cases. Eur J Nucl Med Mol Imaging. 2020;47(5):1281-1286. doi: 10.1007/s00259-020-04734-w.

42. Xiu L, Zhang C, Wu Z, Peng J. Establishment and application of a universal coronavirus screening method using MALDI-TOF mass spectrometry. Front Microbiol. 2017;8:1510. doi: 10.3389/fmicb.2017.01510.

43. Song Y, Zheng S, Li L, Zhang X, Zhang X, Huang Z, et al. Deep learning Enables Accurate Diagnosis of Novel Coronavirus (COVID-19) with CT images. medRxiv. 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.02.23.20026930v1.abstract

44. Wang S, Kang B, Ma J, Zeng X, Xiao M, Guo J, et al. A deep learning algorithm using CT images to screen for Corona Virus Disease (COVID-19). medRxiv. 2020. Available from: https://www.medrxiv.org/content/medrxiv/early/2020/02/17/2020.02.14.20023028.full.pdf.

45. Ai J, Chen J, Wang Y, Liu X, Fan W, Qu G, et al. The cross-sectional study of hospitalized coronavirus disease 2019 patients in Xiangyang, Hubei province. medRxiv. 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.02.19.20025023v1.abstract.

46. Zheng Y, Huang Z, Ying G, Zhang X, Ye W, Hu Z, et al. Comparative study of the lymphocyte change between COVID-19 and non-COVID-19 pneumonia cases suggesting uncontrolled inflammation might not be the main reason of tissue injury. medRxiv. 2020. Available from: https://www.medrxiv.org/content/medrxiv/early/2020/03/27/2020.02.19.20024885.full.pdf.

47. Li Q, Ding X, Xia G, Geng Z, Chen F, Wang L, et al. A simple laboratory parameter facilitates early identification of COVID-19 patients. medRxiv. 2020. Available from: http://www.mlpla.org.cn/dzfw/yhjy/xgzl/202002/P020200219702253465022.pdf.