EVIDENCE SEARCH REPORT

RESEARCH QUESTION: What factors can be used to identify negative PCR tests that are ‘false negatives’?

UNIQUE IDENTIFIER: EOC052101-01 ESR

RESOURCES USED:
- bioRxiv
- CDC website & database
- CINAHL
- CMAJ
- CMPA
- Cochrane
- CPG Infobase
- ECDC
- ECR
- Essential Evidence Plus
- Evidence Aid
- Evidence Check
- FDA
- Google Advanced Search
- Google Scholar
- HSE
- IPAC
- JBI COVID-19 Special Collection
- LitCovid
- MEDLINE
- medRxiv
- NCCMT
- NHS
- NIPH
- TRIP Pro
- WHO website & database

LIMITS/EXCLUSIONS/INCLUSIONS: English

DATE: May 21, 2020

LIBRARIAN: Catherine Young, Michelle Dalidowicz, Lukas Miller

REQUESTOR: Bruce Reeder

TEAM: EOC

SEARCH ALERTS CREATED: N/A

CITE AS: Young, C; Dalidowicz, M; Miller, L. What factors can be used to identify negative PCR tests that are ‘false negatives’? 2020 May 21; Document no.: EOC052101-01 ESR. In: COVID-19 Rapid Evidence Reviews [Internet]. SK: SK COVID Evidence Support Team, c2020. 42 p. (CEST evidence search report)

LIBRARIAN NOTES/COMMENTS

We completed database searches and removed some of the irrelevant resources from the list of articles below. The database search strategies could be further refined for future searching or search alerts.

We completed grey literature searching using the resources listed above.

Please let us know if you have any follow-up questions or requests.

Kind regards,
Catherine Young, Michelle Dalidowicz & Lukas Miller

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SUMMARIES, GUIDELINES & OTHER RESOURCES


• Interpreting the results of Nucleic Acid Amplification testing (NAT; or PCR tests) for COVID-19 in the Respiratory Tract. April 30, 2020. [link]

BC College of Family Physicians. COVID-19 Rapid Review: What is the chance of obtaining an incorrect result with the polymerase chain reaction (PCR) test for COVID-19?. May 21, 2020. [link]

• What is the role of imaging and biomarkers within the current testing strategy for the diagnosis of Covid-19?. April 8, 2020. [link]


Librarian’s note: See section on “Laboratory testing for COVID-19 virus”

Other Journal Articles
ABSTRACT: The definite diagnosis of coronavirus disease 2019 (COVID-19) is based on the viral isolation or positive result of polymerase chain reaction (PCR) from sputum, or nasal swab, or throat swab. However, the sensitivity to detect COVID-19 of real time (RT)-PCR is reported to be lower than that of chest CT. We report a case of 34-year-old man who was diagnosed as negative for COVID-19 based on the four sequential RT-PCR tests of his pharyngeal swab. Chest CT showed patchy ground-glass opacity on admission, and it rapidly progressed to segmental mixed consolidation and ground-glass opacity 3 days after admission, and it resolved in left upper lobe, but showed multifocal ground-glass opacities 7 days after admission, and they resolved within 2 weeks. The fifth RT-PCR test finally revealed positive results at the fifth day after admission. It is difficult to distinguish COVID-19 pneumonia from other viral pneumonia on CT findings alone; however, we emphasize the utility of chest CT to detect early change of COVID-19 in cases which RT-PCR tests show negative results.

URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7136155/


ABSTRACT: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the etiologic agent associated with coronavirus disease, which emerged in late 2019. In response, we developed a diagnostic panel consisting of 3 real-time reverse transcription PCR assays targeting the nucleocapsid gene and evaluated use of these assays for detecting SARS-CoV-2 infection. All assays demonstrated a linear dynamic range of 8 orders of magnitude and an analytical limit of detection of 5 copies/reaction of quantified RNA transcripts and 1 x 10^{-1.5} 50% tissue culture infectious dose/mL of cell-cultured SARS-CoV-2. All assays performed comparably with nasopharyngeal and oropharyngeal secretions, serum, and fecal specimens spiked with cultured virus. We obtained no false-positive amplifications with other human coronaviruses or common respiratory pathogens. Results from all 3 assays were highly correlated during clinical specimen testing. On February 4, 2020, the Food and Drug Administration issued an Emergency Use Authorization to enable emergency use of this panel.

URL: https://wwwnc.cdc.gov/eid/article/26/8/20-1246_article


ABSTRACT: Due to the rapid spread and increasing number of coronavirus disease 19 (COVID-19) cases caused by a new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), rapid and accurate detection of virus and/or disease is increasingly vital to control the sources of infection and help patients to prevent the illness progression. Since December 2019, there has been considerable challenge regarding the use of nucleic acid test or clinical characteristics of infected patients as the reference standard to make a definitive diagnose of COVID-19 patients. As the early diagnosis of COVID-19 is critical for prevention and control of this pandemic, clinical characteristics cannot alone define the diagnosis of COVID-19, especially for patients presenting early-onset of symptoms.

URL: https://www.tandfonline.com/doi/full/10.1080/14737159.2020.1757437


ABSTRACT: We report co-infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza A virus in a patient with pneumonia in China. The case highlights possible co-detection of known respiratory viruses. We noted low sensitivity of upper respiratory specimens for SARS-CoV-2, which could further complicate recognition of the full extent of disease.

URL: https://wwwnc.cdc.gov/eid/article/26/6/20-0299_article
DOI: https://dx.doi.org/10.3201/eid2606.200299
URL: https://onlinelibrary.wiley.com/doi/10.1111/all.14316

ARTICLES FROM THE LIBRARY DATABASES

Note: References are sorted by year (newest to oldest)


ABSTRACT: Background The pandemic of coronavirus disease 2019 (COVID-19) has become the first concern in international affairs as the novel coronavirus (SARS-CoV-2) is spreading all over the world at a terrific speed. The accuracy of early diagnosis is critical in the control of the spread of the virus. Although the real-time RT-PCR detection of the virus nucleic acid is the current golden diagnostic standard, it has high false negative rate when only apply single test. Objective Summarize the baseline characteristics and laboratory examination results of hospitalized COVID-19 patients. Analyze the factors that could interfere with the early diagnosis quantitatively to support the timely confirmation of the disease. Methods All suspected patients with COVID-19 were included in our study until Feb 9th, 2020. The last day of follow-up was Mar 20th, 2020. Throat swab real-time RT-PCR test was used to confirm SARS-CoV-2 infection. The difference between the epidemiological profile and first laboratory examination results of COVID-19 patients and non-COVID-19 patients were compared and analyzed by multiple logistic regression. Receiver operating characteristic (ROC) curve and area under curve (AUC) were used to assess the potential diagnostic value in factors, which had statistical differences in regression analysis. Results In total, 315 hospitalized patients were included. Among them, 108 were confirmed as COVID-19 patients and 207 were non-COVID-19 patients. Two groups of patients have significance in comparing age, contact history, leukocyte count, lymphocyte count, C-reactive protein, erythrocyte sedimentation rate (p<0.10). Multiple logistic regression analysis showed age, contact history and decreasing lymphocyte count could be used as individual factor that has diagnostic value (p<0.05). The AUC of first RT-PCR test was 0.84 (95% CI 0.73-0.89), AUC of cumulative two times of RT-PCR tests was 0.92 (95% CI 0.88-0.96) and 0.96 (95% CI 0.93-0.99) for cumulative three times of RT-PCR tests. Ninety-six patients showed typical pneumonia radiological features in first CT scan, AUC was 0.74 (95% CI 0.60-0.73). The AUC of patients age, contact history with confirmed people and the decreased lymphocytes were 0.66 (95% CI 0.60-0.73), 0.67 (95% CI 0.61-0.73), 0.62 (95% CI 0.56-0.69), respectively. Taking chest CT scan diagnosis together with patients age and decreasing lymphocytes, AUC would be 0.86 (95% CI 0.82-0.90). The age threshold to predict COVID-19 was 41.5 years, with a diagnostic sensitivity of 0.70 (95% CI 0.61-0.79) and a specificity of 0.59 (95% CI 0.52-0.66). Positive and negative likelihood ratios were 1.71 and 0.50, respectively. Threshold of lymphocyte count to diagnose COVID-19 was 1.53x10^9/L, with a diagnostic sensitivity of 0.82 (95% CI 0.73-0.88) and a specificity of 0.50 (95% CI 0.43-0.57). Positive and negative likelihood ratios were 1.64 and 0.37, respectively. Conclusion Single RT-PCR test has relatively high false negative rate. When first RT-PCR test show negative result in suspected patients, the chest CT scan, contact history, age and lymphocyte count should be used combinedly to assess the possibility of SARS-CoV-2 infection.
URL: http://medrxiv.org/content/early/2020/04/14/2020.04.09.20059352.abstract
DOI: 10.1101/2020.04.09.20059352

DOI: 10.1101/2020.04.16.20066787
**ABSTRACT**: Background: Cases with negative reverse transcription-polymerase chain reaction (RT-PCR) results at initial testing for suspicion of SARS-CoV-2 infection, and found to be positive in a subsequent test, are considered as RT-PCR false-negative cases. False-negative cases have important implications for COVID-19 management, isolation, and risk of transmission. We aimed to review and critically appraise evidence about the proportion of RT-PCR false-negatives at initial testing for COVID-19. Methods: We performed a systematic review and critical appraisal of literature with high involvement of stakeholders in the review process. We searched on MEDLINE, EMBASE, LILACS, the WHO database of COVID-19 publications, the EPPI-Centre living systematic map of evidence about COVID-19, and the living systematic review developed by the University of Bern (ISPM). Two authors screened and selected studies according to the eligibility criteria and collected data of included studies (no-independent verification). Risk of bias was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. We calculated the false-negative proportion with the corresponding 95% CI using a multilevel mixed-effect logistic regression model using STATA 16. Certainty of the evidence about false-negative cases was rated using the GRADE approach for tests and strategies. The information is current up to 6 April 2020. Findings: Five studies enrolling 957 patients were included. All studies were affected by several biases and applicability concerns. Pooled estimation of false-negative proportion was 0.085 (95% CI= 0.034 to 0.196; tau-squared = 1.08; 95% CI= 0.27 to 8.28; p&l t;0.001); however, this estimation is highly affected by unexplained heterogeneity, and its interpretation should be avoided. The certainty of the evidence was judged as very low, due to the risk of bias, indirectness, and inconsistency issues. Conclusions: The collected evidence has several limitations, including risk of bias issues, high heterogeneity, and concerns about its applicability. Nonetheless, our findings reinforce the need for repeated testing in patients with suspicion of SARS-CoV-2 infection given that up to 29% of patients could have an initial RT-PCR false-negative result. Systematic review registration: Protocol available on OSF website: https://osf.io/gp38w/

**URL**: http://medrxiv.org/content/early/2020/04/21/2020.04.16.20066787.abstract

**DOI**: 10.1101/2020.04.16.20066787


**ABSTRACT**: Control of the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic requires accurate laboratory testing to identify infected individuals, while also clearing essential staff to continue work. At the current time a number of RT-PCR tests have been developed to identify SARS-CoV-2, targeting multiple regions in the viral genome. In comparison to other RNA viruses the mutation rate of SARS-CoV-2 is moderate, however given the large number of transmission chains it is prudent to monitor circulating viruses for mutations that might compromise these tests. Here we report the identification of a C-to-T transition at position 26340 of the SARS-CoV-2 genome which is associated with failure of the cobas® SARS-CoV-2 E-gene assay. This variant was detected in four health care workers from the same team. Whole genome sequencing of SARS-CoV-2 showed all four to carry genetically identical viruses. Examination of viral genomes deposited on GISAID showed this mutation has arisen independently on three occasions. This work highlights the necessity of monitoring SARS-CoV-2 for the emergence of SNPs which might adversely affect the RT-PCRs used in diagnostics. Additionally, it argues that two regions in the SARS-CoV-2 should be targeted in RT-PCRs to avoid false negatives.

**URL**: http://medrxiv.org/content/early/2020/05/03/2020.04.28.20083337.abstract

**DOI**: 10.1101/2020.04.28.20083337

ABSTRACT: COVID-19 threatens millions of lives especially elderly population and people with chronic diseases including diabetes, hypertension, cancer and cardiovascular diseases. Rapid and effective diagnosis are vital for the isolation of infected people and starting treatment immediately to stop the spread of COVID-19 virus. Bioinformatics techniques such as artificial intelligence should be used for collecting the hemogram and serum biochemistry data of all infected people by COVID-19 worldwide even they do not show severe symptoms. These data may be help to find a biomarker which can be used in combination with the CT results for rapid and accurate diagnosis of COVID-19.

DOI: 10.1002/bab.1936

ABSTRACT: COVID-19 has brought death and disease to large parts of the world. Governments must deploy strategies to screen the population and subsequently isolate the suspect cases. Diagnostic testing is critical for epidemiological surveillance, but the accuracy (sensitivity and specificity) and clinical utility (impact on health outcomes) of the current diagnostic methods used for SARS-CoV-2 detection are not known. I ran a quick search in PubMed/MEDLINE to find studies on laboratory diagnostic tests and rapid viral diagnosis. After running the search strategies, I found 47 eligible articles that I discuss in this review, commenting on test characteristics and limitations. I did not find any papers that report on the clinical utility of the tests currently used for COVID-19 detection, meaning that we are fighting a battle without proper knowledge of the proportion of false negatives that current testing is resulting in. This shortcoming should not be overlooked as it might hamper national efforts to contain the pandemic through testing community-based suspect cases.
URL: https://pubmed.ncbi.nlm.nih.gov/32353857/
DOI: 10.5867/medwave.2020.03.7891

ABSTRACT: Many countries are currently in a lockdown state due to the SARS-CoV-2 pandemic. One key aspect to transition safely out of lockdown is to continuously test the population for infected subjects. Currently, detection is performed at points of care using quantitative reverse-transcription PCR (RT-qPCR), and requires dedicated professionals and equipment. Here, we developed a protocol based on Reverse Transcribed Loop-Mediated Isothermal Amplification (RT-LAMP) for detection of SARS-CoV-2. This protocol is applied directly on SARS-CoV-2 nose and throat swabs, with no RNA purification step required. We tested this protocol on over 180 suspected patients, and compared its results to the standard method. We further succeeded to apply the protocol on self-sampled saliva from confirmed cases. Since the proposed protocol provides results on-the-spot, and can detect SARS-CoV-2 from saliva, it can allow simple and continuous surveillance of the community.
URL: http://medrxiv.org/content/early/2020/05/07/2020.04.22.20072389.abstract
DOI: 10.1101/2020.04.22.20072389

ABSTRACT: Nasopharyngeal (NP), nasal and throat swabs are the most practical specimen sources to test for upper respiratory pathogens. We compared the sensitivity of NP, nasal and throat swabs to detect SARS-CoV-2 in community patients. Using detection at any site as the standard, the sensitivities were 90%, 80% and 87% for NP, nasal and throat respectively (n=30 positive at any site). Throat swabs are likely a suitable alternative to NP swabs for the detection of COVID-19 infections.

**ABSTRACT:** Isothermal nucleic acid amplification tests (iNAT), such as loop-mediated isothermal amplification (LAMP), are good alternatives to polymerase chain reaction (PCR)-based amplification assays, especially for point-of-care and low resource use, in part because they can be carried out with relatively simple instrumentation. However, iNATs can generate spurious amplicons, especially in the absence of target sequences, resulting in false positive results. This is especially true if signals are based on non-sequence-specific probes, such as intercalating dyes or pH changes. In addition, pathogens often prove to be moving, evolving targets, and can accumulate mutations that will lead to inefficient primer binding and thus false negative results. Internally redundant assays targeting different regions of the target sequence can help to reduce such false negatives. Here we describe rapid conversion of three previously described SARS-CoV-2 LAMP assays that relied on non-sequence-specific readout into assays that can be visually read using sequence-specific fluorogenic oligonucleotide strand exchange (OSD) probes. We evaluate one-pot operation of both individual and multiplex LAMP-OSD assays and demonstrate detection of SARS-CoV-2 virions in crude human saliva.

**URL:** [http://biorxiv.org/content/early/2020/04/14/2020.04.13.039941.abstract](http://biorxiv.org/content/early/2020/04/14/2020.04.13.039941.abstract)

**DOI:** 10.1101/2020.04.13.039941


**ABSTRACT:** Testing for the presence of coronavirus is an essential diagnostic tool for monitoring and managing the current COVID-19 pandemic. The only reliable test in current use for testing acute infection targets the genome of SARS-CoV-2, and the most widely used method is quantitative fluorescence-based reverse transcription polymerase chain reaction (RT-qPCR). Despite its ubiquity, there is a significant amount of uncertainty about how this test works, potential throughput and reliability. This has resulted in widespread misrepresentation of the problems faced using this test during the current COVID-19 epidemic. This primer provides simple, straightforward and impartial information about RT-qPCR.

**URL:** [https://www.mdpi.com/1422-0067/21/8/3004/pdf](https://www.mdpi.com/1422-0067/21/8/3004/pdf)

**DOI:** 10.3390/ijms21083004


**ABSTRACT:** The accuracy of commercially available tests for COVID-19 in Brazil remains unclear. We aimed to perform a meta-analysis to describe the accuracy of available tests to detect COVID-19 in Brazil. We searched at the Brazilian Health Regulatory Agency (ANVISA) online platform to describe the pooled sensitivity (Se), specificity (Sp), diagnostic odds ratio (DOR) and summary receiver operating characteristic curves (SROC) for detection of IgM/IgG antibodies and for tests using naso/oropharyngeal swabs in the random-effects models. We identified 16 tests registered, mostly rapid-tests. Pooled diagnostic accuracy measures [95%CI] were: (i) for IgM antibodies Se=82% [76-87]; Sp=97% [96-98]; DOR=168 [92-305] and SROC=0.98 [0.96-0.99]; (ii) for IgG antibodies Se=97% [90-99]; Sp=98% [97-99]; DOR=1994 [385-10334] and SROC=0.99 [0.98-1.00]; and (iii) for detection of SARS-CoV-2 by antigen or molecular assays in naso/oropharyngeal swabs Se=97% [85-99]; Sp=99% [77-100]; DOR=2649 [30-233056] and SROC=0.99 [0.98-1.00]. These tests can be helpful for emergency testing during the COVID-19 pandemic in Brazil. However, it is important to highlight the high rate of false negative results from
tests which detect SARS-CoV-2 IgM antibodies in the initial course of the disease and the scarce evidence-based validation results published in Brazil. Future studies addressing the diagnostic performance of tests for COVID-19 in the Brazilian population are urgently needed.

URL: http://www.sciencedirect.com/science/article/pii/S1413867020300295
DOI: https://doi.org/10.1016/j.bjid.2020.04.003


ABSTRACT: Background and Aims The COVID-19 pandemic has limited endoscopy utilization, causing significant health and economic losses. We aim to model the impact of PCR testing into resuming endoscopy practice. Methods We performed a retrospective review of endoscopy utilization during the COVID-19 pandemic for a baseline reference. A computer model compared 3 approaches—Strategy 1: endoscopy for urgent indications only; Strategy 2: testing for semi-urgent indications; and Strategy 3: testing all patients. Analysis was made under current COVID-19 prevalence, and projected prevalence of 5% and 10%. Primary outcomes were number of procedures performed/cancelled. Secondary outcomes were direct costs, reimbursement, personal protective equipment used and personnel infected. Disease prevalence, testing accuracy, and costs were obtained from literature. Results During the COVID-19 pandemic, endoscopy volume was 12.7% of expected. Strategy 2 and 3 were safe and effective interventions to resume endoscopy in semi-urgent and elective cases. Investing 22USD and 105USD in testing per patient allowed completing 19.4% and 95.3% of baseline endoscopies respectively. False negative results were seen after testing 4,700 patients (or 3 months applying Strategy 2 in our practice). Implementing PCR testing over 1 week in United States would require 13 and 64 million USD, with a return of 165 and 767 million USD to providers, leaving 65 and 325 health care workers infected. Conclusion PCR testing is an effective strategy to restart endoscopic practice in United States. PCR screening should be implemented during the second phase of the pandemic, once the healthcare system is able to test and isolate all suspected COVID-19 cases.

URL: http://www.sciencedirect.com/science/article/pii/S0016510720342486
DOI: https://doi.org/10.1016/j.gie.2020.04.049


ABSTRACT: Abstract: Background: The global pandemic of Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV2) has resulted in unprecedented challenges for healthcare systems. One barrier to widespread testing has been a paucity of traditional respiratory viral swab collection kits relative to the demand. Whether other sample collection kits, such as widely available MRSA nasal swabs can be used to detect SARS-CoV-2 is unknown. Methods: We compared simultaneous nasal MRSA swabs (COPAN ESwabs 480C flocked nasal swab in 1mL of liquid Amies medium) and virals wabs (BD H192(07) flexible mini-tip flocked nasopharyngeal swabs in 3mL Universal Transport Medium) for SARS-CoV-2 PCR testing using Simplexa COVID-19 Direct assay on patients over a 4-day period. When the results were discordant, the viral swab sample was run again on the Cepheid Xpert Xpress SARS-CoV-2 assay. Results: Of the 81 included samples, there were 19 positives and 62 negatives in viral media and 18 positives and 63 negative in the MRSA swabs. Amongst all included samples, there was concordance between the COPAN ESwabs 480C and the viral swabs in 78 (96.3%). Conclusion: We found a high rate of concordance in test results between COPAN ESwabs 480C in Amies solution and BD H192(07) nasopharyngeal swabs in in 3 mL of Universal Viral Transport medium viral media. Clinicians and laboratories should feel better informed and assured using COPAN ESwabs 480C to help in the diagnosis of COVID-19.

ABSTRACT: Objectives The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to date, the epidemic has gradually spread to 209 countries worldwide with more than 1.5 million infected people and 100,000 deaths. Amplification of viral RNA by rRT-PCR serves as the gold standard for confirmation of infection, yet it needs a long turnaround time (3-4 h to generate results) and shows false-negative rates as large as 15%-20%. In addition, the need of certified laboratories, expensive equipment and trained personnel led many countries to limit the rRT-PCR tests only to individuals with pronounced respiratory syndrome symptoms. Thus, there is a need for alternative, less expensive and more accessible tests. Methods We analyzed the plasma levels of white blood cells (WBCs), platelets, C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), alkaline phosphatase and lactate dehydrogenase (LDH) of 207 patients who, after being admitted to the emergency room of the San Raffaele Hospital (Milan, Italy) with COVID-19 symptoms, were rRT-PCR tested. Of them, 105 tested positive, whereas 102 tested negative. Results Statistically significant differences were observed for WBC, CRP, AST, ALT and LDH. Empirical thresholds for AST and LDH allowed the identification of 70% of either COVID-19-positive or -negative patients on the basis of routine blood test results. Conclusions Combining appropriate cutoffs for certain hematological parameters could help in identifying false-positive/negative rRT-PCR tests. Blood test analysis might be used as an alternative to rRT-PCR for identifying COVID-19-positive patients in those countries which suffer from a large shortage of rRT-PCR reagents and/or specialized laboratory.


ABSTRACT: Molecular testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the gold standard for diagnosis of coronavirus disease 2019 (COVID-19), but the test clinical performance is poorly understood. From 3/10/2020-5/1/2020 NewYork-Presbyterian laboratories performed 27,377 SARS-CoV-2 molecular assays from 22,338 patients. Repeat testing was performed in 3,432 patients, of which 2,413 had negative and 1,019 had positive first day results. Repeat-tested patients were more likely to be older, male, African-American or Hispanic, and to have severe disease. Among the patients with initially negative results, 18.6% became positive upon repeat-testing. Only 58.1% of any-time positive patients had a result of "detected" on the first test. The clinical sensitivity of COVID-19 molecular assays is estimated between 66.2% and 95.6%, depending on the unknown number of false negative results in single-tested patients. Conversion to a negative result is unlikely to occur before 15 to 20 days after initial testing or 20-30 days after the onset of symptoms, with 50% conversion occurring at 28 days after initial testing. Forty-nine initially-positive patients converted to negative and then back to positive in subsequent days. Conversion from first day negative to positive results increased linearly with each day of testing, reaching 25% probability in 20 days. In summary, our study provides estimates of the clinical performance of SARS-CoV-2 molecular assays and suggests time frames for appropriate repeat testing, namely 15 to 20 days after a positive test and the same or next 2 days after a negative test in a patient with high suspicion for COVID-19.

URL: http://medrxiv.org/content/early/2020/05/08/2020.05.06.20093575.abstract

DOI: 10.1101/2020.05.06.20093575

**ABSTRACT:** BACKGROUND: Emergence of coronavirus disease 2019 (COVID-19) is a major healthcare threat. Current method of detection involves qPCR-based technique, which identifies the viral nucleic acids when present in sufficient quantity. False negative results can be achieved and failure to quarantine the infected patient would be a major setback in containing the viral transmission. We here aim to describe the time kinetics of various antibodies produced against the 2019 novel coronavirus (SARS-CoV-2) and evaluate the potential of antibody testing to diagnose COVID-19.

**METHODS:** The host humoral response against SARS-CoV-2 including IgA, IgM and IgG response were examined by using an ELISA based assay on the recombinant viral nucleocapsid protein. Total 208 plasma samples were collected from 82 confirmed and 58 probable cases (qPCR negative but had typical manifestation). The diagnostic value of IgM was evaluated in this cohort.

**RESULTS:** The median duration of IgM and IgA antibody detection were 5 days (IQR 3-6), while IgG was detected on 14 days (IQR 10-18) after symptom onset, with a positive rate of 85.4%, 92.7% and 77.9% respectively. In confirmed and probable cases, the positive rates of IgM antibodies were 75.6% and 93.1%, respectively. The detection efficiency by IgM ELISA is higher than that of qPCR method after 5.5 days of symptom onset. The positive detection rate is significantly increased (98.6%) when combined IgM ELISA assay with PCR for each patient compare with a single qPCR test (51.9%).

**CONCLUSIONS:** Humoral response to SARS-CoV-2 can aid to the diagnosis of COVID-19, including subclinical cases.

**URL:** https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa310/5810754


**ABSTRACT:** Background: In December 2019, a novel coronavirus emerging in China and spread rapidly globally. Early identification and effective quarantine are essential to reduce the spread of the disease. However, the presence of false-negative makes the diagnosis difficult, especially in the early stages of the disease. Case presentation: A 34-year-old man who had an epidemiological link to Wuhan, presenting with intermittent fever and cough, with chest computed tomography showing ground-glass opacity, and repeated detection of negative 2019 novel coronavirus (2019-nCoV) nucleic acid by real-time reverse transcription-polymerase chain reaction assay, which was eventually diagnosed as coronavirus disease 2019 (COVID-19). Conclusions: This case highlights that a single negative result of the test, particularly if it is based on an upper respiratory tract specimen, in highly suspected cases, does not exclude COVID-19. Repeat and multiple-site sampling and testing in combination with dynamic imaging changes in the chest are strongly recommended in progressive disease.

**URL:** https://www.researchsquare.com/article/rs-17319/v1

**DOI:** 10.21203/rs.3.rs-17319/v1


**ABSTRACT:** Introduction: Chest CT is thought to be sensitive but less specific in diagnosing the 2019 coronavirus disease (COVID-19). The diagnostic value of CT is unclear. We aimed to compare the performance of CT and initial RT-PCR for clinically suspected COVID-19 patients outside the epicentre-Wuhan, China. **Material(s) and Method(s):** Patients clinically suspected of COVID-19 infection who underwent initial RT-PCR and chest CT at the same time were retrospectively enrolled. Two radiologists with specific training reviewed the CT images independently and final diagnoses of the presence or absence of COVID-19 was reached by consensus. With serial RT-PCR as reference standard, the performance of initial RT-PCR and chest CT was analysed. A strategy of combining initial RT-PCR and
chest CT was analysed to study the additional benefit. Result(s): 82 patients admitted to hospital between Jan 10, 2020 to Feb 28, 2020 were enrolled. 34 COVID-19 and 48 non-COVID-19 patients were identified by serial RT-PCR. The sensitivity, specificity was 79% (27/34) and 100% (48/48) for initial RT-PCR and 77% (26/34) and 96% (46/48) for chest CT. The image readers had a good interobserver agreement with Cohen’s kappa of 0.69. No statistical difference was found in the diagnostic performance between initial RT-PCR and chest CT. The comprehensive strategy had a higher sensitivity of 94% (32/34). Conclusion(s): Initial RT-PCR and chest CT had comparable diagnostic performance in identification of suspected COVID-19 patients outside the epidemic center. To compensate potential risk of false-negative PCR, chest CT should be applied for clinically suspected patients with negative initial RT-PCR.

**URL:** https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7172864/

**DOI:** http://dx.doi.org/10.1016/j.rmed.2020.105980


**ABSTRACT:** Background: Several point-of-care (POC) molecular tests have received emergency use authorization (EUA) from the Food and Drug Administration (FDA) for diagnosis of SARS-CoV-2. The test performance characteristics of the Accula (Mesa Biotech) SARS-CoV-2 POC test need to be evaluated to inform its optimal use. Objectives: The aim of this study was to assess test performance of the Accula SARS-CoV-2 test. Study design: The performance of the Accula test was assessed by comparing results of 100 nasopharyngeal swab samples previously characterized by the Stanford Health Care EUA laboratory-developed test (SHC-LDT) targeting the envelope (E) gene. Assay concordance was assessed by overall percent agreement, positive percent agreement (PPA), negative percent agreement (NPA), and Cohen's kappa coefficient. Results: Overall percent agreement between the assays was 84.0% (95% confidence interval [CI] 75.3 to 90.6%), PPA was 68.0% (95% CI 53.3 to 80.5%) and the kappa coefficient was 0.68 (95% CI 0.54 to 0.82). Sixteen specimens detected by the SHC-LDT were not detected by the Accula test, and showed low viral load burden with a median cycle threshold value of 37.7. NPA was 100% (95% CI 94.2 to 100%). Conclusion: Compared to the SHC-LDT, the Accula SARS-CoV-2 test showed excellent negative agreement. However, positive agreement was low for samples with low viral load. The false negative rate of the Accula POC test calls for a more thorough evaluation of POC test performance characteristics in clinical settings, and for confirmatory testing in individuals with moderate to high pre-test probability of SARS-CoV-2 who test negative on Accula.

**URL:** http://biorxiv.org/content/early/2020/05/13/2020.05.12.092379.abstract

**DOI:** 10.1101/2020.05.12.092379


**ABSTRACT:** Background: Emerging and reemerging infectious diseases such as the novel Coronavirus disease, COVID-19 and Ebola pose a significant threat to global society and test the public health community’s preparedness to rapidly respond to an outbreak with effective diagnostics and therapeutics. Recent advances in next generation sequencing technologies enable rapid generation of pathogen genome sequence data, within 24 hours of obtaining a sample in some instances. With these data, one can quickly evaluate the effectiveness of existing diagnostics and therapeutics using in silico approaches. The propensity of some viruses to rapidly accumulate mutations can lead to the failure of molecular detection assays creating the need for redesigned or newly designed assays. Results Here we describe a bioinformatics system named BioLaboro to identify signature regions in a given pathogen genome, design PCR assays targeting those regions, and then test the PCR assays in silico to determine
their sensitivity and specificity. We demonstrate BioLaboro with two use cases: Bombali Ebolavirus (BOMV) and the novel Coronavirus 2019 (SARS-CoV-2). For the BOMV, we analyzed 30 currently available real-time reverse transcription-PCR assays against the three available complete genome sequences of BOMV. Only two met our in silico criteria for successful detection and neither had perfect matches to the primer/probe sequences. We designed five new primer sets against BOMV signatures and all had true positive hits to the three BOMV genomes and no false positive hits to any other sequence. Four assays are closely clustered in the nucleoprotein gene and one is located in the glycoprotein gene. Similarly, for the SARS-CoV-2, we designed five highly specific primer sets that hit all 145 whole genomes (available as of February 28, 2020) and none of the near neighbors.

Conclusions
Here we applied BioLaboro in two real-world use cases to demonstrate its capability; 1) to identify signature regions, 2) to assess the efficacy of existing PCR assays to detect pathogens as they evolve over time, and 3) to design new assays with perfect in silico detection accuracy, all within hours, for further development and deployment. BioLaboro is designed with a user-friendly graphical user interface for biologists with limited bioinformatics experience.

URL: http://biorxiv.org/content/early/2020/04/10/2020.04.08.031963.abstract
DOI: 10.1101/2020.04.08.031963

ABSTRACT: Rapid testing of appropriate specimens from patients suspected for a disease during an epidemic, such as the current Coronavirus outbreak, is of a great importance for the disease management and control. We propose a method to enhance processing large amounts of collected samples. The method is based on mixing samples in testing tubes in a specific configuration, as opposed to testing single samples in each tube, and accounting for natural virus amounts in infected patients from variation of positiveness in test tubes. To illustrate the efficiency of the suggested method we carry out numerical tests for actual scenarios under various tests. Applying the proposed method enhances the number of tests by order of magnitudes, where all positives are identified with no false negatives, and the effective testing time can be reduced drastically even when the uncertainty in the test is relatively high.

URL: http://medrxiv.org/content/early/2020/04/29/2020.04.24.20078147.abstract
DOI: 10.1101/2020.04.24.20078147

ABSTRACT: Improper nasopharyngeal swab collection could contribute to false-negative COVID-19 results. In support of this, specimens from confirmed or suspected COVID-19 cases that tested negative or indeterminate (i.e. suspected false-negatives) contained less human DNA (a stable molecular marker of sampling quality) compared to a representative pool of specimens submitted for testing.

URL: http://medrxiv.org/content/early/2020/05/08/2020.05.05.20091728.abstract
DOI: 10.1101/2020.05.05.20091728

ABSTRACT: While counting cases of disease appears straightforward, there are issues to consider when enumerating disease counts during an epidemic. For example, for Coronavirus Disease-2019 (COVID-19), how is a case defined? Hubei province in China changed its case definition twice in a fortnight—from laboratory-confirmed cases to clinically-confirmed cases without laboratory tests, and back to laboratory-confirmed cases. This caused confusion in the reported number of cases. If a confirmed case
requires laboratory testing, what is the population who are laboratory-tested? Due to limited laboratory testing capacity in the early phase of an emerging epidemic, only "suspected cases" are laboratory-tested in most countries. This will result in underdiagnosis of confirmed cases and also raises the question: how is a "suspect case" defined? With the passage of time and increased capability to perform laboratory tests, more people can be screened and the number of confirmed cases will increase. What are the technical considerations of laboratory testing? This includes specimen collection (variable collection methods), samples collected (upper or lower respiratory tract biospecimens), time of collection in relation to course of disease, different laboratory test methods and kits (not all of which may be standardised or approved by authorities such as the Food and Drug Administration). Are approved laboratory facilities and trained manpower available, and how are test results interpreted and false-negatives excluded? These issues will affect the accuracy of disease counts, which in turn will have implications on how we mount an appropriate response to the outbreak.

URL: https://pubmed.ncbi.nlm.nih.gov/32301478/


ABSTRACT: Background: Currently, there is a pandemic caused by the 2019 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes Covid-19. We wanted to compare specimen types and collection methods to explore if a simpler to collect specimen type could expand access to testing. Methods: We recruited individuals recently tested for SARS-CoV-2 infection through a testing program (drive-through). In homes of participants, we assessed the performance of self-collected oral fluid swab specimens with and without clinician supervision, clinician-supervised self-collected mid-turbinate (nasal) swab specimens, and clinician-collected nasopharyngeal swab specimens. We tested specimens with a validated reverse transcription-quantitative polymerase chain reaction assay for the detection of SARS-CoV-2 and measured cycle threshold values. Symptom status and date of onset of symptoms was also recorded for each participant. Results: We recruited 45 participants. The median age of study participant was 42 years old (Interquartile range, 31 to 52 years). Of the participants, 29 had at least one specimen test positive for SARS-CoV-2. Of those, 21 (73%) of 29 reported active symptoms. By specimen type and home-based collection method, clinician-supervised self-collected oral fluid swab specimens detected 26 (90%) of 29 infected individuals, clinician-supervised self-collected nasal swab specimens detected 23 (85%) of 27, clinician-collected posterior nasopharyngeal swab specimens detected 23 (79%) of 29, and unmonitored self-collected oral fluid swab specimens detected 19 (66%) of 29. Despite nasopharyngeal swabs being considered the gold standard, 4 participants tested negative by clinician-collected nasopharyngeal swab and positive by the 3 other specimen types. Additionally, false negative results by each sample type were seen to generally not overlap. Conclusions: Supervised self-collected oral fluid and nasal swab specimens performed similarly to, if not better than clinician-collected nasopharyngeal swab specimens for the detection of SARS-CoV-2 infection. No sample type captured all SARS-CoV-2 infections, suggesting potential heterogeneity in the distribution of viral load in different parts of the respiratory tract between individuals. Supervised self-collection performed comparably to clinician collection and would allow for rapid expansion of testing capacity in the United States by reducing the need for trained healthcare workers, reducing exposure of healthcare workers, and reducing the amount of PPE (personal protective equipment) being used for testing during a critical shortage.

URL: http://medrxiv.org/content/early/2020/04/15/2020.04.11.20062372.abstract
DOI: 10.1101/2020.04.11.20062372

ABSTRACT: SARS-CoV-2 RT-PCR based tests are being used to rule out infection among high-risk individuals such as exposed inpatients and healthcare workers. It is critical to understand how the predictive value of the test varies with time from exposure and symptom onset in order to avoid being falsely reassured by negative tests. As such, the goal of our study was to estimate the false negative rate by day since infection. We used previously published data on RT-PCR sensitivity on samples derived from nasal swabs by day since symptom onset (n=633) and fit a cubic polynomial spline to calculate the false negative rate by day since exposure and symptom onset. Over the four days of infection prior to the typical time of symptom onset (day 5) the probability of a false negative test in an infected individual falls from 100% on day one (95% CI 69-100%) to 61% on day four (95% CI 18-98%), though there is considerable uncertainty in these numbers. On the day of symptom onset, the median false negative rate was 39% (95% CI 16-77%). This decreased to 26% (95% CI 18-34%) on day 8 (3 days after symptom onset), then began to rise again, from 27% (95% CI 20-34%) on day 9 to 61% (95% CI 54-67%) on day 21. Care must be taken when interpreting RT-PCR tests for SARS-CoV-2 infection, particularly if performed early in the course of infection, when using these results as a basis for removing precautions intended to prevent onward transmission. If there is high clinical suspicion, patients should not be ruled out on the basis of RT-PCR alone, and the clinical and epidemiologic situation should be carefully considered.

URL: http://medrxiv.org/content/early/2020/04/10/2020.04.07.20051474.abstract
DOI: 10.1101/2020.04.07.20051474


ABSTRACT: BACKGROUND: Tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on reverse transcriptase polymerase chain reaction (RT-PCR) are being used to "rule out" infection among high-risk persons, such as exposed inpatients and healthcare workers. It is critical to understand how the predictive value of the test varies with time from exposure and symptom onset to avoid being falsely reassured by negative test results. OBJECTIVE: To estimate the false-negative rate by day since infection. DESIGN: Literature review and pooled analysis. SETTING: 7 previously published studies providing data on RT-PCR performance by time since symptom onset or SARS-CoV-2 exposure using samples from the upper respiratory tract (n = 1330). PATIENTS: A mix of inpatients and outpatients with SARS-CoV-2 infection. MEASUREMENTS: A Bayesian hierarchical model was fitted to estimate the false-negative rate by day since exposure and symptom onset. RESULTS: Over the 4 days of infection before the typical time of symptom onset (day 5), the probability of a false-negative result in an infected person decreases from 100% (95% CI, 100% to 100%) on day 1 to 67% (CI, 27% to 94%) on day 4. On the day of symptom onset, the median false-negative rate was 38% (CI, 18% to 65%). This decreased to 20% (CI, 12% to 30%) on day 8 (3 days after symptom onset) then began to increase again, from 21% (CI, 13% to 31%) on day 9 to 66% (CI, 54% to 77%) on day 21. LIMITATION: Imprecise estimates due to heterogeneity in the design of studies on which results were based. CONCLUSION: Care must be taken in interpreting RT-PCR tests for SARS-CoV-2 infection-particularly early in the course of infection-when using these results as a basis for removing precautions intended to prevent onward transmission. If clinical suspicion is high, infection should not be ruled out on the basis of RT-PCR alone, and the clinical and epidemiologic situation should be carefully considered. PRIMARY FUNDING SOURCE: National Institute of Allergy and Infectious Diseases, Johns Hopkins Health System, and U.S. Centers for Disease Control and Prevention.

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**ABSTRACT:** Accurate designing of polymerase chain reaction (PCR) primers targeting conserved segments in viral genomes is desirable for preventing false negative results and decreasing the need for standardization across different PCR protocols. In this work, we designed and described a set of primers and probes targeting conserved regions identified from a multiple sequence alignment of 2341 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) genomes from the Global Initiative on Sharing All Influenza Data (GISAID). Those primers and probes were subsequently validated in 3067 SARS-CoV-2 whole-genome sequences. From these analyses, we obtained nine systems (forward primer + reverse primer + probe) that potentially anneal to highly conserved regions of the virus genome. In silico predictions also demonstrated that those primers do not bind to nonspecific targets for human, bacterial, fungal, or apicomplexan sequences. The availability of these primer and probe sequences will make it possible to accelerate the beginning of in vitro tests in order to validate more efficient protocols for the identification of SARS-CoV-2.

**URL:** [https://www.researchsquare.com/article/rs-26306/v1](https://www.researchsquare.com/article/rs-26306/v1)


**ABSTRACT:** Since December 2019, the novel coronavirus, SARS-CoV-2, has garnered global attention due to its rapid transmission, which has infected more than two million people worldwide. Early detection of SARS-CoV-2 is one of the crucial interventions to control virus spread and dissemination. Molecular assays have been the gold standard to directly detect for the presence of viral genetic material in infected individuals. However, insufficient viral RNA at the point of detection may lead to false negative results. As such, it is important to also employ immune-based assays to determine one's exposure to SARS-CoV-2, as well as to assist in the surveillance of individuals with prior exposure to SARS-CoV-2. Within a span of 4 months, extensive studies have been done to develop serological systems to characterize the antibody profiles, as well as to identify and generate potentially neutralizing antibodies during SARS-CoV-2 infection. The vast diversity of novel findings has added value to coronavirus research, and a strategic consolidation is crucial to encompass the latest advances and developments. This review aims to provide a concise yet extensive collation of current immunoassays for SARS-CoV-2, while discussing the strengths, limitations and applications of antibody detection in SARS-CoV-2 research and control.

**URL:** [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194125/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194125/)

**DOI:** [https://dx.doi.org/10.3389/fimmu.2020.00879](https://dx.doi.org/10.3389/fimmu.2020.00879)


**ABSTRACT:** Abstract An ongoing outbreak of viral pneumonia was caused by a novel coronavirus in China in 2019. By March 19, over 200 thousand confirmed cases of SARS-CoV-2 infection and over 9000 deaths have been reported throughout the world. For this infectious disease, nucleic acid detection is still the gold standard for pathogenic detection. However, nucleic acid detection takes a long time and has relatively high 'false negative'; therefore, we need urgently a convenient and accurate detection method to make up for this deficiency. In this article, we will show such technical characteristics of IgM/IgG serum antibody detection, compared with nucleic acid detection.


**DOI:** [https://doi.org/10.1111/jcmm.15275](https://doi.org/10.1111/jcmm.15275)

**ABSTRACT:** OBJECTIVES: To investigate the role of chest CT for the diagnostic work-up for patients with suspected infection of coronavirus disease 2019 (COVID-19). METHODS: The clinical data and imaging findings of the first nucleic acid-negative COVID-19 patients were analyzed and compared with the first nucleic acid-positive patients. RESULTS: Compared with the first nucleic acid-positive patients, the onset time of the first nucleic acid-negative patients was shorter [(3.58+/−2.94) d], but the diagnosis was longer [(3.92+/−3.66) d]. There were no significant differences in the characteristics of the clinical data and radiological findings between the 2 groups (P>0.05). CONCLUSION: Chest CT examination is important to avoid COVID-19 missed diagnosis due to false negative nucleic acid.

URL: https://pubmed.ncbi.nlm.nih.gov/32386016/
DOI: 10.11817/j.issn.1672-7347.2020.200141


**ABSTRACT:** In this study, we collected a total of 610 hospitalized patients from Wuhan between Feb 2, 2020, and Feb 17, 2020. We reported a potentially high false negative rate of RT-PCR testing for SARS-CoV-2 in the 610 hospitalized patients clinically diagnosed with COVID-19 during the 2019 outbreak. We also found that the RT-PCR results from several tests at different points were variable from the same patients during the course of diagnosis and treatment of these patients. Our results indicate that in addition to the emphasis on RT-PCR testing, clinical indicators such as CT images should also be used not only for diagnosis and treatment but also for isolation, recovery/discharge and transferring for hospitalized patients clinically diagnosed with COVID-19 during the current epidemic. These results suggested the urgent needs for standard of procedures(SOP) of sampling from different anatomic sites, sample transportation, optimization of RT-PCR, serology diagnosis/screening for SARS-CoV-2 infection, and distinct diagnosis from other respiratory diseases such as flu infections as well. This article is protected by copyright. All rights reserved.

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DOI: 10.1002/jmv.25786


**ABSTRACT:** The outbreak of the novel coronavirus disease (COVID-19) quickly spread all over China and to more than 20 other countries. Although the virus (SARS-Cov-2) nucleic acid RT-PCR test has become the standard method for diagnosis of SARS-CoV-2 infection, these real-time PCR test kits have many limitations. In addition, high false negative rates were reported. There is an urgent need for an accurate and rapid test method to quickly identify large number of infected patients and asymptomatic carriers to prevent virus transmission and assure timely treatment of patients. We have developed a rapid and simple point-of-care lateral flow immunoassay which can detect IgM and IgG antibodies simultaneously against SARS-CoV-2 virus in human blood within 15 minutes which can detect patients at different infection stages. With this test kit, we carried out clinical studies to validate its clinical efficacy uses. The clinical detection sensitivity and specificity of this test were measured using blood samples collected from 397 PCR confirmed COVID-19 patients and 128 negative patients at 8 different clinical sites. The overall testing sensitivity was 88.66% and specificity was 90.63%. In addition, we evaluated clinical diagnosis results obtained from different types of venous and fingerstick blood samples. The results indicated great detection consistency among samples from fingerstick blood, serum and plasma of
venous blood. The IgM-IgG combined assay has better utility and sensitivity compared with a single IgM or IgG test. It can be used for the rapid screening of SARS-CoV-2 carriers, symptomatic or asymptomatic, in hospitals, clinics, and test laboratories. This article is protected by copyright. All rights reserved.

URL: https://pubmed.ncbi.nlm.nih.gov/32104917
DOI: 10.1002/jmv.25727

ABSTRACT: Background: The outbreak of coronavirus disease 2019 (COVID-19) has become a worldwide emergency. Fangcang shelter hospitals have been applied in COVID-19 to e

DOI: 10.1101/2020.03.28.20045765
ABSTRACT: Background: As the increasing number of Corona Virus Disease 2019 (COVID-19) patients caused by the severe acute respiratory coronavirus 2 (SARS-CoV-2), which caused an outbreak initiated from Wuhan, China in December, 2019, the clinical features and treatment of COVID-19 patients have been understood. However, it is urgent to need the rapid and accurate detection for SARS-CoV-2 infection diagnosis. We aimed to evaluate the antibodies-based and nucleic acid-based tests (NAT) for SARS-CoV-2-infected patients. Method: We retrospectively and observationally studied 133 patients diagnosed with SARS-CoV-2 and admitted in Renmin Hospital of Wuhan University, China, from Feb 17 to Mar 1, 2020. Demographic data, symptoms, clinical examination, laboratory tests, and clinical outcomes were collected. Data were compared between IgM-IgG antibody test and real-time RT-PCR detection for COVID-19 patients. Results: Of 133 patients with SARS-CoV-2 infection, there were 44 moderate cases, 52 severe cases, and 37 critical cases with no significant difference of gender and age among three subgroups. Overall, the positive ratio in IgM antibody test was higher than in RT-PCR detection. In RT-PCR detection, the positive ratio was 65.91%, 71.15%, and 67.57% in moderate, severe, and critical cases, respectively. Whereas, the positive ratio of IgM/IgG antibody detection in patients was 79.55%/93.18%, 82.69%/100%, and 72.97%/97.30% in moderate, severe, and critical cases, respectively. Moreover, the concentrations of antibodies were also measured in three subgroups. Conclusion: The IgM-IgG antibodies-based test exhibited a comparative superiority to the NAT for COVID-19 diagnosis, which provides an effective complement to the false negative results from NAT for SARS-CoV-2 infection diagnosis.
URL: http://medrxiv.org/content/early/2020/03/30/2020.03.28.20045765.abstract
DOI: 10.1101/2020.03.28.20045765

ABSTRACT: Background: A novel coronavirus disease 2019 (COVID-19) broke out in Wuhan of Hubei province and had spread throughout the world since December 2019. Because the clinically diagnosed cases in Hubei province were reported for the first time on February 13, 2020, a very high peak of new cases in China was observed. The reason why so many clinically diagnosed cases appeared was not clear. Methods: All data of new cases in China were acquired from WHO situation reports. Linear fitting was used to infer the ability to detect COVID-19 infections. Primer-BLAST and nucleotide blast were applied to check the specificity of primers. Expression data of human mRNA in different tissues was obtained
from Human Protein Atlas. Finding: Based on the data and analysis of changes of new laboratory-confirmed cases and new clinically diagnosed cases, it was inferred that there were many false-negative results in all clinically diagnosed cases in Hubei province. There were eight non-specific primers in dozens of primers used in clinical or research detection of COVID-19. Among them, a pair of primer for the ORF1ab regions of SARS-CoV-2 genome, which widely applied to detect SARS-CoV-2 virus in China, well matched some human mRNAs such as Cathepsin C transcripts. Compared to other transcripts, Cathepsin C mRNA had a high abundance in tonsil, lung and small intestine. Interpretation: Some non-specific RT-PCR primers could cause the serious interference during RT-PCR amplification so as to increase the risk of false-negative diagnoses for COVID-19 infections.

**URL**: [http://medrxiv.org/content/early/2020/04/20/2020.04.07.20056804.abstract](http://medrxiv.org/content/early/2020/04/20/2020.04.07.20056804.abstract)

**DOI**: 10.1101/2020.04.07.20056804


**ABSTRACT**: Purpose: To evaluate the diagnostic value of computed tomography (CT) and real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) for COVID-19 pneumonia. Methods: This retrospective study included all patients with COVID-19 pneumonia suspicion, who were examined by both CT and rRT-PCR at initial presentation. The sensitivities of both tests were then compared. For patients with a final confirmed diagnosis, clinical and laboratory data, in addition to CT imaging findings were evaluated. Results: A total of 36 patients were finally diagnosed with COVID-19 pneumonia. Thirty-five patients had abnormal CT findings at presentation, whereas one patient had a normal CT. Using rRT-PCR, 30 patients were tested positive, with 6 cases initially missed. Amongst these 6 patients, 3 became positive in the second rRT-PCR assay (after 2 days, 2 days and 3 days respectively), and the other 3 became positive only in the third round of rRT-PCR tests (after 5 days, 6 days and 8 days respectively). At presentation, CT sensitivity was therefore 97.2%, whereas the sensitivity of initial rRT-PCR was only 83.3%. Conclusion: rRT-PCR may produce initial false negative results. We suggest that patients with typical CT findings but negative rRT-PCR results should be isolated, and rRT-PCR should be repeated to avoid misdiagnosis.

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**DOI**: 10.1016/j.ejrad.2020.108961


**ABSTRACT**: Background: SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) testing remains the cornerstone of laboratory-based identification of patients with COVID-19. As the availability and speed of SARS-CoV-2 testing platforms improve, results are increasingly relied upon to inform critical decisions related to therapy, use of personal protective equipment, and workforce readiness. However, early reports of RT-PCR test performance have left clinicians and the public with concerns regarding the reliability of this predominant testing modality and the interpretation of negative results. In this work, two independent research teams report the frequency of discordant SARS-CoV-2 test results among initially negative, repeatedly tested patients in regions of the United States with early community transmission and access to testing. Methods: All patients at the University of Washington (UW) and Stanford Health Care undergoing initial testing by nasopharyngeal (NP) swab between March 2nd and April 7th, 2020 were included. SARS-CoV-2 RT-PCR was performed targeting the N, RdRp, S, and E genes and ORF1ab, using a combination of Emergency Use Authorization laboratory-developed tests and commercial assays. Results through April 14th were extracted to allow for a complete 7-day observation period and an additional day for reporting. Results: A total of 23,126 SARS-CoV-2 RT-PCR tests (10,583 UW, 12,543 Stanford) were performed in 20,912 eligible patients (8,977 UW, 11,935
Stanford) undergoing initial testing by NP swab; 626 initially test-negative patients were re-tested within 7 days. Among this group, repeat testing within 7 days yielded a positive result in 3.5% (4.3% UW, 2.8% Stanford) of cases, suggesting an initial false negative RT-PCR result; the majority (96.5%) of patients with an initial negative result who warranted reevaluation for any reason remained negative on all subsequent tests performed within this window. Conclusions: Two independent research teams report the similar finding that, among initially negative patients subjected to repeat SARS-CoV-2 RT-PCR testing, the occurrence of a newly positive result within 7 days is uncommon. These observations suggest that false negative results at the time of initial presentation do occur, but potentially at a lower frequency than is currently believed. Although it is not possible to infer the clinical sensitivity of NP SARS-CoV-2 RT-PCR testing using these data, they may be used in combination with other reports to guide the use and interpretation of this common testing modality.

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DOI: 10.1101/2020.05.03.20089151


ABSTRACT: Background: SARS-CoV-2 nucleic acid detection by RT-PCR is one of the criteria approved by China FDA for diagnosis of COVID-19. However, inaccurate test results (for example, high false negative rate and some false positive rate) were reported in both China and US CDC using the RT-PCR method. Inaccurate results are caused by inadequate detection sensitivity of RT-PCR, low viral load in some patients, difficulty to collect samples from COVID-19 patients, insufficient sample loading during RT-PCR tests, and RNA degradation during sample handling process. False negative detection could subject patients to multiple tests before diagnosis can be made, which burdens health care system. Delayed diagnosis could cause infected patients to miss the best treatment time window. False negative detection could also lead to prematurely releasing infected patients who still carry residual SARS-CoV-2 virus. In this case, these patients could infect many others. A high sensitivity RNA detection method to resolve the existing issues of RT-PCR is in need for more accurate COVID-19 diagnosis. Methods: Digital PCR (dPCR) instrument DropX-2000 and assay kits were used to detect SARS-CoV-2 from 108 clinical specimens from 36 patients including pharyngeal swab, stool and blood from different days during hospitalization. Double-blinded experiment data of 108 clinical specimens by dPCR methods were compared with results from officially approved RT-PCR assay. A total of 109 samples including 108 clinical specimens and 1 negative control sample were tested in this study. All of 109 samples, 26 were from 21patients reported as positive by officially approved clinical RT-PCR detection in local CDC and then hospitalized in Nantong Third Hospital. Among the 109 samples, dPCR detected 30 positive samples on ORFA1ab gene, 47 samples with N gene positive, and 30 samples with double positive on ORFA1ab and N genes. Results: The lower limit of detection of the optimize dPCR is at least 10-fold lower than that of RT-PCR. The overall accuracy of dPCR for clinical detection is 96.3%. 4 out 4 of (100 %) negative pharyngeal swab samples checked by RT-PCR were positive judged by dPCR based on the follow-up investigation. 2 of 2 samples in the RT-PCR grey area (Ct value > 37) were confirmed by dPCR with positive results. 1 patient being tested positive by RT-PCR was confirmed to be negative by dPCR. The dPCR results show clear viral loading decrease in 12 patients as treatment proceed, which can be a useful tool for monitoring COVID-19 treatment. Conclusions: Digital PCR shows improved lower limit of detection, sensitivity and accuracy, enabling COVID-19 detection with less false negative and false positive results comparing with RT-PCR, especially for the tests with low viral load specimens. We showed evidences that dPCR is powerful in detecting asymptomatic patients and suspected patients. Digital PCR is capable of checking the negative results caused by insufficient sample loading by quantifying internal reference gene from human RNA in the PCR reactions. Multi-channel fluorescence dPCR system (FAM/HEX/CY5/ROX) is able to detect more target genes in a single multiplex assay,
providing quantitative count of viral load in specimens, which is a powerful tool for monitoring COVID-19 treatment.

**URL**: [http://medrxiv.org/content/early/2020/03/30/2020.03.24.20042689.abstract](http://medrxiv.org/content/early/2020/03/30/2020.03.24.20042689.abstract)

**DOI**: 10.1101/2020.03.24.20042689

38. MacGowan SA, Barton GJ. Missense variants in ACE2 are predicted to encourage and inhibit interaction with SARS-CoV-2 Spike and contribute to genetic risk in COVID-19. bioRxiv. 2020:2020.05.03.074781. DOI: 10.1101/2020.05.03.074781

**ABSTRACT**: SARS-CoV-2 invades host cells via an endocytic pathway that begins with the interaction of the SARS-CoV-2 Spike glycoprotein (S-protein) and human Angiotensin-converting enzyme 2 (ACE2). Genetic variability in ACE2 may be one factor that mediates the broad-spectrum severity of SARS-CoV-2 infection and COVID-19 outcomes. We investigated the capacity of ACE2 variation to influence SARS-CoV-2 infection with a focus on predicting the effect of missense variants on the ACE2 SARS-CoV-2 S-protein interaction. We validated the mCSM-PPI2 variant effect prediction algorithm with 26 published ACE2 mutant SARS-CoV S-protein binding assays and found it performed well in this closely related system (True Positive Rate = 0.7, True Negative Rate = 1). Application of mCSM-PPI2 to ACE2 missense variants from the Genome Aggregation Consortium Database (gnomAD) identified three that are predicted to strongly inhibit or abolish the S-protein ACE2 interaction altogether (p.Glu37Lys, p.Gly352Val and p.Asp355Asn) and one that is predicted to promote the interaction (p.Gly326Glu). The S-protein ACE2 inhibitory variants are expected to confer a high degree of resistance to SARS-CoV-2 infection whilst the S-protein ACE2 affinity enhancing variant may lead to additional susceptibility and severity. We also performed in silico saturation mutagenesis of the S-protein ACE2 interface and identified a further 38 potential missense mutations that could strongly inhibit binding and one more that is likely to enhance binding (Thr27Arg). A conservative estimate places the prevalence of the strongly protective variants between 12-70 per 100,000 population but there is the possibility of higher prevalence in local populations or those underrepresented in gnomAD. The probable interplay between these ACE2 affinity variants and ACE2 expression polymorphisms is highlighted as well as gender differences in penetrance arising from ACE2's situation on the X-chromosome. It is also described how our data can help power future genetic association studies of COVID-19 phenotypes and how the saturation mutant predictions can help design a mutant ACE2 with tailored S-protein affinity, which may be an improvement over a current recombinant ACE2 that is undergoing clinical trial.

**URL**: [http://biorxiv.org/content/early/2020/05/04/2020.05.03.074781.abstract](http://biorxiv.org/content/early/2020/05/04/2020.05.03.074781.abstract)

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**ABSTRACT**: COVID-19 antibody tests have imperfect accuracy. There has been lack of clarity on the meaning of reported rates of false positives and false negatives. For risk assessment and clinical decision making, the rates of interest are the positive and negative predictive values of a test. Positive predictive value (PPV) is the chance that a person who tests positive has been infected. Negative predictive value (NPV) is the chance that someone who tests negative has not been infected. The medical literature regularly reports different statistics, sensitivity and specificity. Sensitivity is the chance that an infected person receives a positive test result. Specificity is the chance that a non-infected person receives a negative result. Knowledge of sensitivity and specificity permits one to predict the test result given a person's true infection status. These predictions are not directly relevant to risk assessment or clinical decisions, where one knows a test result and wants to predict whether a person has been infected. Given estimates of sensitivity and specificity, PPV and NPV can be derived if one knows the prevalence of the disease, the rate of illness in the population. There is considerable uncertainty about the prevalence of COVID-19. This paper addresses the problem of inference on the PPV and NPV of COVID-
19 antibody tests given estimates of sensitivity and specificity and credible bounds on prevalence. I explain the methodological problem, show how to estimate bounds on PPV and NPV, and apply the findings to some tests authorized by the FDA.

URL: http://medrxiv.org/content/early/2020/05/18/2020.05.14.20102061.abstract
DOI: 10.1101/2020.05.14.20102061


ABSTRACT: The coronavirus disease 2019 (COVID-19) outbreak that originated in Wuhan, China has rapidly propagated due to widespread person-to-person transmission and has resulted in over 1,133,758 cases in 197 countries with a total of 62,784 deaths as of April 5, 2020. Laboratory confirmation of SARS-CoV-2 is performed with a virus-specific reverse transcriptase polymerase chain reaction (RT-PCR) test. This test can take up to two days to complete, and, due to the possibility of false negatives, serial testing may be required to reliably exclude infection. A current supply shortage of RT-PCR test kits compounds the shortcomings of entrusting diagnosis to the PCR test alone and underscores the urgent need to provide alternative methods for the rapid and accurate diagnosis of SARS-CoV-2 patients. Chest computed tomography (CT) is a valuable component in the evaluation of patients with suspected SARS-CoV-2 infection. Nevertheless, CT alone may have limited negative predictive value to fully exclude infection, because of the normal radiologic findings in some early disease patients. In this study, we use artificial intelligence (AI) algorithms to integrate chest CT findings with clinical symptoms, exposure history, and/or laboratory testing to more accurately and rapidly diagnose SARS-CoV-2 (+) patients. We included 905 RT-PCR confirmed patients. 419 (46.2%) tested positive for SARS-CoV-2 by laboratory-confirmed real-time RT-PCR assay and next-generation sequencing, while 486 patients (53.8%) tested negative (confirmed by at least two additional negative RT-PCR tests and clinical observation). The proposed AI system achieved an AUC of 0.92 and performed equally well in sensitivity compared to a senior thoracic radiologist on a testing set of 279 cases. The AI system also improved the detection of RT-PCR positive SARS-CoV-2 patients who presented with normal CTs, correctly identifying 17/25 (68%) patients, whereas all 25 RT-PCR SARS-CoV-2-positive CT-normal patients were classified as SARS-CoV-2 negative by radiologists.

URL: http://medrxiv.org/content/early/2020/04/17/2020.04.12.20062661.abstract
DOI: 10.1101/2020.04.12.20062661


ABSTRACT: For diagnosis of coronavirus disease 2019 (COVID-19), a SARS-CoV-2 virus-specific reverse transcriptase polymerase chain reaction (RT-PCR) test is routinely used. However, this test can take up to 2 d to complete, serial testing may be required to rule out the possibility of false negative results and there is currently a shortage of RT-PCR test kits, underscoring the urgent need for alternative methods for rapid and accurate diagnosis of patients with COVID-19. Chest computed tomography (CT) is a valuable component in the evaluation of patients with suspected SARS-CoV-2 infection. Nevertheless, CT alone may have limited negative predictive value for ruling out SARS-CoV-2 infection, as some patients may have normal radiological findings at early stages of the disease. In this study, we used artificial intelligence (AI) algorithms to integrate chest CT findings with clinical symptoms, exposure history and laboratory testing to rapidly diagnose patients who are positive for COVID-19. Among a total of 905 patients tested by real-time RT-PCR assay and next-generation sequencing RT-PCR, 419 (46.3%) tested positive for SARS-CoV-2. In a test set of 279 patients, the AI system achieved an area under the curve of 0.92 and had equal sensitivity as compared to a senior thoracic radiologist. The AI system also improved the detection of patients who were positive for COVID-19 via RT-PCR who presented with normal CT scans, correctly identifying 17 of 25 (68%) patients, whereas radiologists classified all of these patients...
as COVID-19 negative. When CT scans and associated clinical history are available, the proposed AI system can help to rapidly diagnose COVID-19 patients.

URL: https://www.nature.com/articles/s41591-020-0931-3
DOI: https://doi.org/10.1038/s41591-020-0931-3

ABSTRACT: *ID NOW EUA SARS-CoV-2 assay had an overall agreement of 78.7% when compared to the standard of care reference methods.*ID NOW had a sensitivity of 71.7% and specificity of 100%.*All the false-negative results occurred with weakly positive samples, with reference method CT values >/=35.
URL: https://www.sciencedirect.com/science/article/pii/S1386653220301712
DOI: 10.1016/j.jcv.2020.104429

43. Mohamed E-T, Haim H B, Jinzhao S. A Single and Two-Stage, Closed-Tube, Molecular Test for the 2019 Novel Coronavirus (COVID-19) at Home, Clinic, and Points of Entry. chemRxiv. 2020. DOI: 10.26434/chemrxiv.11860137.v1
ABSTRACT: p The 2019 novel coronavirus (COVID-19) is a newly emerged strain that has never been found in humans before. At present, the laboratory-based reverse transcription-polymerase chain reaction (RT-PCR) is the main method to confirm COVID-19 infection. The intensification of the COVID-19 epidemic overwhelms limited clinical resources in particular, but not only, in developing countries, resulting in many patients not being tested for the infection and in large queues of potentially infected individuals waiting to be tested while providing a breeding ground for the disease. We describe here a rapid, highly sensitive, point-of-care, molecular test amenable for use at home, in the clinic, and at points of entry by minimally trained individuals and with minimal instrumentation. Our test is based on loop mediated isothermal amplification (COVID-19 LAMP) and for higher sensitivity on nested nucleic acid, two stage isothermal amplification (COVID-19 Penn-RAMP). Both tests can be carried out in closed tubes with either fluorescence or colorimetric detection. COVID-19 LAMP performs on par with COVID-19 RT-PCR. COVID-19 RAMP has 10 fold better sensitivity than COVID-19 LAMP and COVID-19 RT-PCR when testing purified targets and 100 times better sensitivity than COVID-19 LAMP and COVID-19 RT-PCR when testing rapidly prepared sample mimics. Due to fortunate scarcity of COVID-19 infections in the USA, we were not able to test our assays and methods with patient samples. We hope that such tests will be carried out by colleagues in impacted countries. Our Closed-Tube Penn-RAMP has the potential to significantly reduce false negatives while being amenable to use with minimal instrumentation and training. /p
URL: https://chemrxiv.org/articles/A_Single_and_Two-Stage_Closed-Tube_Molecular_Test_for_the_2019_Novel_Coronavirus_COVID-19_at_Home_Clinic_and_Points_of_Entry/11860137
DOI: 10.26434/chemrxiv.11860137.v1

ABSTRACT: We describe a method for rapid in silico selection of diagnostic peptides from newly described viral pathogens and applied this approach to SARS-CoV-2/COVID-19. This approach is multi-tiered, beginning with compiling the theoretical protein sequences from genomic derived data. In the case of SARS-CoV-2 we begin with 496 peptides that would be produced by proteolytic digestion of the viral proteins. To eliminate peptides that would cause cross-reactivity and false positives we remove peptides from consideration that have sequence homology or similar chemical characteristics using a progressively larger database of background peptides. Using this pipeline, we can remove 47 peptides
from consideration as diagnostic due to the presence of peptides derived from the human proteome. To address the complexity of the human microbiome, we describe a method to create a database of all proteins of relevant abundance in the saliva microbiome. By utilizing a protein-based approach to the microbiome we can more accurately identify peptides that will be problematic in COVID-19 studies which removes 12 peptides from consideration. To identify diagnostic peptides, another 7 peptides are flagged for removal following comparison to the proteome backgrounds of viral and bacterial pathogens of similar clinical presentation. By aligning the protein sequences of SARS-CoV-2 field isolates deposited to date we can identify peptides for removal due to their presence in highly variable regions that may lead to false negatives as the pathogen evolves. We provide maps of these regions and highlight 3 peptides that should be avoided as potential diagnostic or vaccine targets. Finally, we leverage publicly deposited proteomics data from human cells infected with SARS-CoV-2, as well as a second study with the closely related MERS-CoV to identify the two proteins of highest abundance in human infections. The resulting final list contains the 24 peptides most unique and diagnostic of SARS-CoV-2 infections. These peptides represent the best targets for the development of antibodies or clinical diagnostics. To demonstrate one application of this we model peptide fragmentation using a deep learning tool to rapidly generate targeted LCMS assays and data processing method for detecting CoVID-19 infected patient samples.

**URL:** [http://biorxiv.org/content/early/2020/04/10/2020.03.08.980383.abstract](http://biorxiv.org/content/early/2020/04/10/2020.03.08.980383.abstract)  
**DOI:** 10.1101/2020.03.08.980383

**ABSTRACT:** The novel coronavirus SARS-CoV-2 (Covid-19), spreading from Wuhan, China, is one of the causes of respiratory infections that can spread to other people through respiratory particles, and can cause symptoms such as fever, dry cough, shortness of breath, anorexia, fatigue and sore throat in infected patients. This review summarizes current strategies on the diagnosis. Additionally, treatments, infection prevention and control of the SARS-CoV-2 are addressed. In addition to the respiratory system, this virus can infect the digestive system, the urinary system and the haematological system, which causes to observe the virus in the stool, urine and blood samples in addition to throat sample. The SARS-CoV-2 causes changes in blood cells and factors and makes lung abnormalities in patients, which can be detected by serological, molecular, and radiological techniques by detecting these changes and injuries. Radiological and serological methods are the most preferred among the other methods and the radiological method is the most preferred one which can diagnose the infection quickly and accurately with fewer false-negatives, that can be effective in protecting the patient’s life by initiating treatment and preventing the transmission of infection to other people.  

**ABSTRACT:** Background. Real-time reverse transcription polymerase chain reaction (RT-PCR) targeting select genes of the SARS-CoV-2 RNA has been the main diagnostic tool in the global response to the COVID-19 pandemic. However, the diagnostic accuracy of the test has not been studied systematically outside of the laboratory setting. The aim of this study is to provide estimates of the diagnostic sensitivity and specificity of the RT-PCR test developed by China CDC. Methods. The study design is a secondary analysis of published findings on 1014 patients in Wuhan, China, of whom 601 tested positive and 413 were negative for COVID-19. Sensitivity and specificity were reconstructed using a Bayesian approach from probabilistic knowledge of the diagnostic errors. Predictive values of the test were calculated, resulting in estimates for the number of confirmatory tests that are needed for establishing the presence or absence of COVID-19, depending on the prior probability of a patient having the
disease. Results. The sensitivity of the RT-PCR diagnostic test was estimated to be 0.777 (95% CI: 0.715, 0.849), while the specificity was 0.988 (95% CI: 0.933, 1.000). The confidence intervals include sampling error in addition to the error due to probabilistic knowledge of the data. Discussion. The Chinese version of the RT-PCR test had a conspicuous rate of false negative results, likely missing between 15% and 29% of patients with COVID-19. For a patient with a prior probability of COVID-19 greater than 18%, at least two negative test results would be needed to lower the chances of COVID-19 below 5%. Caution is advised in generalizing these findings to other versions of the RT-PCR test that are being used in diverse geographic regions.

URL: http://medrxiv.org/content/early/2020/04/29/2020.04.24.20078949.abstract
DOI: 10.1101/2020.04.24.20078949

ABSTRACT: An outbreak of new coronavirus SARS-CoV-2 was occurred in Wuhan, China and rapidly spread to other cities and nations. The standard diagnostic approach that widely adopted in the clinic is nuclear acid detection by real-time RT-PCR. However, the false-negative rate of the technique is unneglectable and serological methods are urgently warranted. Here, we presented the colloidal gold-based immunochromatographic (ICG) strip targeting viral IgM or IgG antibody and compared it with real-time RT-PCR. The sensitivity of ICG assay with IgM and IgG combinatorial detection in nuclear acid confirmed cases were 11.1%, 92.9% and 96.8% at the early stage (1-7 days after onset), intermediate stage (8-14 days after onset), and late-stage (more than 15 days), respectively. The ICG detection capacity in nuclear acid-negative suspected cases was 43.6%. In addition, the consistencies of whole blood samples with plasma were 100% and 97.1% in IgM and IgG strips, respectively. In conclusion, serological ICG strip assay in detecting SARS-CoV-2 infection is both sensitive and consistent, which is considered as an excellent supplementary approach in clinical application.
URL: http://medrxiv.org/content/early/2020/03/17/2020.03.13.20035428.abstract
DOI: 10.1101/2020.03.13.20035428

ABSTRACT: BACKGROUND: Corona Virus Disease-2019 (COVID-19) has spread widely throughout the world since the end of 2019. Nucleic acid testing (NAT) has played an important role in patient diagnosis and management of COVID-19. In some circumstances, thermal inactivation at 56°C has been recommended to inactivate Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) before NAT. However, this procedure could theoretically disrupt nucleic acid integrity of this single-stranded RNA virus and cause false negatives in real-time polymerase chain reaction (RT-PCR) tests. METHODS: We investigated whether thermal inactivation could affect the results of viral NAT. We examined the effects of thermal inactivation on the quantitative RT-PCR results of SARS-CoV-2 particularly with regard to the rates of false-negative results for specimens carrying low viral loads. We additionally investigated the effects of different specimen types, sample preservation times and a chemical inactivation approach on NAT. RESULTS: Our work showed increased Ct values in specimens from diagnosed COVID-19 patients in RT-PCR tests after thermal incubation. Moreover, about half of the weak-positive samples (7 of 15 samples, 46.7%) were RT-PCR negative after heat inactivation in at least one parallel testing. The use of guanidinium-based lysis for preservation of these specimens had a smaller impact on RT-PCR results with fewer false negatives (2 of 15 samples, 13.3%) and significantly less increase in Ct values than heat inactivation. CONCLUSION: Thermal inactivation adversely affected the efficiency of RT-PCR for SARS-CoV-2 detection. Given the limited applicability associated with chemical inactivators, other approaches to ensure the overall protection of laboratory personnel need consideration.

ABSTRACT: BACKGROUND: We retrospectively analysed 26 persistently asymptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) carriers. METHODS: Epidemiological and clinical characteristics from the 26 asymptomatic patients with positive results for SARS-CoV-2 RNA testing were obtained. RESULTS: Twenty-two patients (84.6%) correlated with clustering occurrence. The median period from contact to diagnosis and the last positive nucleic acid test was 19 (8-24 days) and 21.5 days (10-36 days), respectively. The median period from diagnosis to negative nucleic acid test was significantly different between patients with normal or atypical chest computed tomography (CT) findings (n=16, 61.5%; 7.5 days [2-20 days]) and patients with typical ground-glass or patchy opacities on CT(n=10, 38.5%; 12.5 days [8-22 days]; P<0.01). Seven patients (70.0%) with initial positive nucleic acid test results had a negative result simultaneously with improved CT findings. Obvious improvement in CT findings was observed in three patients (30.0%) despite positive nucleic acid test results. CONCLUSION: In asymptomatic patients, changes in biochemical and inflammatory variables are small and changes on chest CT can occur. It is worth noting the long existence of SARS-CoV-2 in some asymptomatic patients and false-negative results need to be considered in SARS-CoV-2 nucleic acid test.


ABSTRACT: Acute respiratory tract infections (ARTI), including the common cold, pharyngitis, sinusitis, otitis media, bronchiolitis and pneumonia are the most common diagnoses among patients seeking medical care in western countries, and account for most antibiotic prescriptions. While a confirmed and fast ARTI diagnosis is key for antibiotic prescribing, empiric antimicrobial treatment remains common, because viral symptoms are often clinically similar and difficult to distinguish from those caused by bacteria. As a result, inappropriate antibiotic prescriptions are high and in certain settings likely higher than the commonly estimated 30%. The QIAstat Respiratory Panel R assay (QIAstat RP) is a multiplexed in vitro diagnostics test for the rapid simultaneous detection of 21 pathogens directly from respiratory samples, including human mastadenovirus A-G, primate bocaparvovirus 1+2, human coronavirus (HKU1, NL63, OC43, 229E), human metapneumovirus A/B, rhinovirus/enterovirus, influenza A virus (no subtype, subtype H1, H1N1/2009, H3), influenza B virus, human respirovirus 1+3, human orthopneumovirus, Bordetella pertussis, Chlamydia pneumoniae, Mycoplasma pneumoniae and Legionella pneumophila. We describe the first multicenter study of 445 respiratory samples, collected through the 2016-2017 and 2018 respiratory seasons, with performance compared against BioFire FilmArray RP v1.7 and discrepancy testing by Seegene Allplex RP. The QIAstat RP demonstrated a positive percentage of agreement of 98.0% (95% CI: 96.0-99.1%) and a negative percentage agreement of 99.8% (95% CI: 99.6-99.9%). With use of this comprehensive and rapid test, improved patient outcomes and antimicrobial stewardship may potentially be achieved.

ABSTRACT: BACKGROUND: The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread globally. The laboratory diagnosis of SARS-CoV-2 infection has relied on nucleic acid tests. However, there are many limitations of nucleic acid tests, including low throughput and high rates of false negatives. More sensitive and accurate tests to effectively identify infected patients are needed. METHODS: This study has developed fully automated chemiluminescent immunoassays (CLIA) to determine IgM and IgG antibodies to SARS-CoV-2 in human serum. The assay performance has been evaluated at 10 hospitals. Clinical specificity was evaluated by measuring 972 hospitalized patients with diseases other than COVID-19, and 586 donors of a normal population. Clinical sensitivity was assessed on 503 confirmed cases of SARS-CoV-2 by RT-PCR and 52 suspected cases. RESULTS: The assays demonstrated satisfied assay precision with coefficient of variation (CV) of less than 4.45%. Inactivation of specimen does not affect assay measurement. SARS-CoV-2 IgM shows clinical specificity of 97.33% and 99.49% for hospitalized patients and normal population respectively. SARS-CoV-2 IgG shows clinical specificity of 97.43% and 99.15% for the hospitalized patients and the normal population respectively. SARS-CoV-2 IgM and IgG show clinical sensitivity of 85.88% and 96.62% respectively for confirmed SARS-Cov-2 infection with RT-PCR, of 73.08% and 86.54% respectively for suspected cases. CONCLUSIONS: we have developed fully automated immunoassays for detecting SARS-CoV-2 IgM and IgG antibodies in human serum. The assays demonstrated high clinical specificity and sensitivity, and add great value to nucleic acid testing in fighting against the global pandemic of the SARS-CoV-2 infection.

URL: http://medrxiv.org/content/early/2020/04/21/2020.04.16.20067231.abstract
DOI: 10.1101/2020.04.16.20067231


ABSTRACT: •Most of the COVID-19 cases in Nepal are in the Southern districts of Nepal bordering India and many cases have travel history to India. •Very few positive cases of COVID-19 are detected in Nepal which could either be due to early national lockdown or inefficiency of PCR methods. •Whole genomes of 93 clinical samples from COVID-19 patients were analysed to find the primer and probe binding sites. •Mutations in probe binding sites were found and this could significantly impact PCR efficiency resulting in false negative results.

URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7233248/
DOI: 10.1016/j.mcp.2020.101599

53. Rosebrock AP. Patient DNA cross-reactivity of CDC SARS-nCoV2 extraction control leads to potential false negative results. bioRxiv. 2020:2020.05.13.094839. DOI: 10.1101/2020.05.13.094839

ABSTRACT: Detecting RNA viruses such as SARS-nCoV2 requires careful handling of inherently labile RNA. Molecular tests incorporate controls to verify the presence of intact RNA and ensure that all steps of an assay have succeeded. The Centers for Disease Control and Prevention (CDC)-specified extraction control recognizes both reverse-transcribed RNA and human genomic DNA. Human DNA co-purified from nasopharyngeal swabs by multiple clinically-used RNA extraction approaches, is sufficient for a strong "extraction control positive" signal using the CDC design, creating the potential for false-negative results. Moreover, DNA cross-reactivity precludes control of RNA integrity in real-world samples. A properly designed control that monitors sample collection, extraction, reverse transcription, and qPCR processes is essential and demonstrated here. This control can be immediately implemented in the CDC testing workflow. Competing Interest Statement The authors have declared no competing interest.

URL: http://biorxiv.org/content/early/2020/05/14/2020.05.13.094839.abstract
DOI: 10.1101/2020.05.13.094839

ABSTRACT: Background: In the pandemic, testing for SARS-CoV-2 by RT-PCR in one of the pillars on which countermeasures are based. Factors limiting the output of laboratories interfere with the effectiveness of public health measures. Conserving reagents by pooling samples in low-probability settings is proposed, but may cause dilution and loss of sensitivity. Methods: We tested an alternate approach (FACT) by simultaneously incubating multiple respiratory swabs in a single tube. This protocol was evaluated by serial incubation of a respiratory swab in up to 10 tubes. The analytics validity of this concept was demonstrated in a five-sample mini pool set-up. It was consequently applied in the testing of 50 symptomatic patients (five-sample pools) as well as 100 asymptomatic residents of a nursing home (ten-sample pools). Results: Serial incubation of a respiratory swab in up to 10 tubes did not lead to a significant decline in viral concentration. The novel FACT-protocol did not cause a false negative result in a five-sample mini-pool setup, with non-significantly differing Ct values between single sample and mini-pool NAT. In two routine applications, all mini pools containing positive patient samples were correctly identified. Conclusions: Our proposed FACT-protocol did not cause a significant loss in analytic or diagnostic sensitivity compared to single sample testing in multiple setups. It reduced the amount of reagents needed by up to 40%, and also reduced hands-on time. This method could enhance testing efficiency, especially in groups with a low pretest-probability, such as systemically relevant professional groups.

URL: http://medrxiv.org/content/early/2020/05/01/2020.04.28.20074187.abstract
DOI: 10.1101/2020.04.28.20074187


ABSTRACT: The COVID-19 pandemic is rapidly spreading throughout the world. Recent reports suggest that 10-30% of SARS-CoV-2 infected patients are asymptomatic. Other studies report that some subjects have significant viral shedding prior to symptom onset. Since both asymptomatic and pre-symptomatic subjects can spread the disease, identifying such individuals is critical for effective control of the SARS-CoV-2 pandemic. Therefore, there is an urgent need to increase diagnostic testing capabilities in order to also screen asymptomatic carriers. In fact, such tests will be routinely required until a vaccine is developed. Yet, a major bottleneck of managing the COVID-19 pandemic in many countries is diagnostic testing, due to limited laboratory capabilities as well as limited access to genome-extraction and Polymerase Chain Reaction (PCR) reagents. We developed P-BEST - a method for Pooling-Based Efficient SARS-CoV-2 Testing, using a non-adaptive group-testing approach, which significantly reduces the number of tests required to identify all positive subjects within a large set of samples. Instead of testing each sample separately, samples are pooled into groups and each pool is tested for SARS-CoV-2 using the standard clinically approved PCR-based diagnostic assay. Each sample is part of multiple pools, using a combinatorial pooling strategy based on compressed sensing designed for maximizing the ability to identify all positive individuals. We evaluated P-BEST using leftover samples that were previously clinically tested for COVID-19. In our current proof-of-concept study we pooled 384 patient samples into 48 pools providing an 8-fold increase in testing efficiency. Five sets of 384 samples, containing 1-5 positive carriers were screened and all positive carriers in each set were correctly identified. P-BEST provides an efficient and easy-to-implement solution for increasing testing capacity that will work with any clinically approved genome-extraction and PCR-based diagnostic methodologies.

URL: http://medrxiv.org/content/early/2020/04/20/2020.04.14.20064618.abstract

ABSTRACT: Background: The SARS-CoV-2 virus responsible for COVID-19 poses a significant challenge to healthcare systems worldwide. Despite governmental initiatives aimed at containing the spread of the disease, several countries are experiencing unmanageable increases in the demand for ICU beds, medical equipment, and larger testing capacity. Efficient COVID-19 diagnosis enables healthcare systems to provide better care for patients while protecting caregivers from the disease. However, many countries are constrained by the limited amount of test kits available, the lack of equipment and trained professionals. In the case of patients visiting emergency rooms (ERs) with a suspect of COVID-19, a prompt diagnosis can improve the outcome and even provide information for efficient hospital management. In this context, a quick, inexpensive and readily available test to perform an initial triage at ER could help to smooth patient flow, provide better patient care, and reduce the backlog of exams.

Methods: In this Case-control quantitative study, we developed a strategy backed by artificial intelligence to perform an initial screening of suspect COVID-19 cases. We developed a machine learning classifier that takes widely available simple blood exams as input and predicts if that suspect case is likely to be positive (having SARS-CoV-2) or negative(not having SARS-CoV-2). Based on this initial classification, positive cases can be referred for further highly sensitive testing (e.g. CT scan, or specific antibodies). We used publicly available data from the Albert Einstein Hospital in Brazil from 5,644 patients. Focussing on using simple blood exams, a sample of 599 subjects that had the fewest missing values for 16 common exams were selected. From these 599 patients, only 81 were positive for SARS-CoV-2 (determined by RT-PCR). Based on this data, we built an artificial intelligence classification framework, ER-CoV, aiming at determining which patients were more likely to be negative for SARS-CoV-2 when visiting an ER and that were categorized as a suspect case by medical professionals. The primary goal of this investigation is to develop a classifier with high specificity and high negative predictive values, with reasonable sensitivity. Findings: We identified that our framework achieved an average specificity of 92.16% [95% CI 91.73 - 92.59] and negative predictive value (NPV) of 95.29% [95% CI 94.65 - 95.90%]. Those values are completely aligned with our goal of providing an effective low-cost system to triage suspected patients at ERs. As for sensitivity, our model achieved an average of 63.98% [95% CI 59.82% - 67.50%] and positive predictive value (PPV) of 48.00% [95% CI 44.88% - 51.56%]. An error analysis identified that, on average, 45% of the false negative results would have been hospitalized anyway, thus the model is making mistakes for severe cases that would not be overlooked, partially mitigating the fact that the test is not high-sensitive. All code for our AI model, called ER-CoV is publicly available at https://github.com/soares-f/ER-CoV. Interpretation: Based on the capacity of our model to accurately predict which cases are negative from suspected patients arriving at emergency rooms, we envision that this framework can play an important role in patient triage. Probably the most important outcome is related to testing availability, which at this point is extremely low in many countries. Considering the achieved specificity, we would reduce by at least 90% the number of SARS-CoV-2 tests performed at emergency rooms, with the chance of getting a false negative at around 5%. The second important outcome is related to patient management in hospitals. Patients predicted as positive by our framework could be immediately separated from the other patients while waiting for the results of confirmatory tests. This could reduce the spread rate inside hospitals since in many hospitals all suspected cases are kept in the same ward. In Brazil, where the data was collected, rate infection is starting to quickly spread, the lead time of a SARS-CoV-2 can be up to 2 weeks.

URL: http://medrxiv.org/content/early/2020/04/14/2020.04.10.20061036.abstract

DOI: 10.1101/2020.04.10.20061036

Song L, He M, Jia X. A case of SARS-CoV-2 carrier for 32 days with several times false negative nucleic acid tests. medRxiv. 2020:2020.03.31.20045401. DOI: 10.1101/2020.03.31.20045401
ABSTRACT: In 2019, a novel coronavirus (SARS-CoV-2) was first discovered in Wuhan, Hubei, China, causing severe respiratory disease in humans, and has been identified as a public health emergency of international concern. With the spread of the virus, there are more and more false negative cases of RT-PCR nucleic acid detection in the early stage of potential infection. In this paper, we collected the epidemiological history, clinical manifestations, outcomes, laboratory results and images of a SARS-CoV-2 carrier with no significant past medical history. The patient was quarantined because of her colleague had been diagnosed. After the onset of clinical symptoms, chest CT results showed patchy ground-glass opacity (GGO) in her lungs, but it took a total of nine nucleic acid tests to confirm the diagnosis, among which the first eight RT-PCR results were negative or single-target positive. In addition to coughing up phlegm during her stay in the hospital, she did not develop chills, fever, abdominal pain, diarrhea and other clinical symptoms. Since initial antiviral treatment, the lung lesions were absorbed. But the sputum nucleic acid test was still positive. In combination with antiviral and immune therapy, the patient tested negative for the virus. Notably, SARS-CoV-2 was detected only in the lower respiratory tract samples (sputum) throughout the diagnosis and treatment period. This is a confirmed case of SARS-CoV-2 infection with common symptoms, and her diagnosis has undergone multiple false negatives, suggesting that it is difficult to identify certain carriers of the virus and that such patients may also increase the spread of the SARS-CoV-2.

URL: http://medrxiv.org/content/early/2020/04/06/2020.03.31.20045401.abstract
DOI: 10.1101/2020.03.31.20045401


ABSTRACT: Background: Real-Time PCR (RT-PCR) is widely used as the gold standard for clinical detection of SARS-CoV-2. However, due to the low viral load in patient throat and the limitation of RT-PCR, significant numbers of false negative reports are inevitable, which should not be ignored. Methods: We explored the feasibility of droplet digital PCR (ddPCR) to detect SARS-CoV-2 from 57 clinical pharyngeal swab samples and compared with RT-PCR in terms of the sensitivity and accuracy. Among 57 samples, all of which were reported as negative nucleic acid by officially approved clinical RT-PCR detection, 43 samples were collected from suspected patients with fever in clinic, and 14 were from supposed convalescents who were about to discharge after treatment. The experiment was double-blind. Results: The lower limit of detection of the optimized ddPCR is at least 500 times lower than that of RT-PCR. The overall accuracy of ddPCR for clinical detection is 94.3 %. 33 out of 35 negative pharyngeal swab samples checked by RT-PCR were correctly judged by ddPCR based on the follow-up investigation. In addition, 9 out of 14 (64.2 %) supposed convalescents with negative nucleic acid test twice by RT-PCR were positive by ddPCR detection. Conclusions: ddPCR shows superiority for clinical detection of SARS-CoV-2 to reduce the false negatives, which could be a powerful complement to the current standard RT-PCR. Before the ddPCR to be approved for diagnosis, the current clinical practice that the convalescent continues to be quarantined for 2 weeks is reasonable and necessary.

URL: http://medrxiv.org/content/early/2020/03/06/2020.02.29.20029439.abstract
DOI: 10.1101/2020.02.29.20029439


ABSTRACT: We here report a case of coronavirus disease-19 (COVID-19) in Japan in which the initial throat swab polymerase chain reaction result was negative. The possibility of false-negative results in the early phase of disease suggest reconsideration of the feasibility of a community or national infection control framework to prevent transmission. We recommend establishing an alternative feasible system, such as self-isolation by contact history in non-endemic community and by symptoms in endemic
community, not relying on the PCR examination, to minimize this ongoing COVID-19 outbreak. Further rapid accumulation of knowledge including incubation period, clinical course and types of transmission is warranted to control this outbreak.

URL: [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7205727/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7205727/)
DOI: 10.1016/j.jiac.2020.05.002

DOI: 10.1080/14737159.2020.1757437

ABSTRACT: We propose an additional intervention that would contribute to the control of the COVID-19 pandemic, offer more protection for people working in essential jobs, and help guide an eventual reopening of society. The intervention is based on: (1) testing every individual (2) repeatedly, and (3) self-quarantine of infected individuals. Using a standard epidemiological model (SIR), we show here that by identification and isolation of the majority of infectious individuals, including those who may be asymptomatic, the reproduction number R0 of SARS-CoV-2 would be reduced well below 1.0, and the epidemic would collapse. We replicate these observations in a more complex stochastic dynamic model on a social network graph. We also find that the testing regime would be additive to other interventions, and be effective at any level of prevalence. If adopted as a policy, any industrial society could sustain the regime for as long as it takes to find a safe and effective cure or vaccine. Our model also indicates that unlike sampling-based tests, population-scale testing does not need to be very accurate: false negative rates up to 15% could be tolerated if 80% comply with testing every ten days, and false positives can be almost arbitrarily high when a high fraction of the population is already effectively quarantined. Testing at the required scale would be feasible if existing qPCR-based methods are scaled up and multiplexed. A mass produced, low throughput field test kit could also be carried out at home. Economic analysis also supports the feasibility of the approach: current reagent costs for tests are in the range of a dollar or less, and the estimated benefits for population-scale testing are so large that the policy would be cost-effective even if the costs were larger by more than two orders of magnitude. To identify both active and previous infections, both viral RNA and antibodies could be tested. All technologies to build such test kits, and to produce them in the scale required to test the entire world’s population exist already. Integrating them, scaling up production, and implementing the testing regime will require resources and planning, but at a scale that is very small compared to the effort that every nation would devote to defending itself against a more traditional foe.
URL: [http://medrxiv.org/content/early/2020/05/01/2020.04.27.20078329.abstract](http://medrxiv.org/content/early/2020/05/01/2020.04.27.20078329.abstract)
DOI: 10.1101/2020.04.27.20078329

ABSTRACT: Background: Since the outbreak of coronavirus disease (COVID-19) in Wuhan in December 2019, by March 10, 2020, a total of 80,932 confirmed cases have been reported in China. Two consecutively negative RT-PCR test results in respiratory tract specimens is required for the evaluation of discharge from hospital, and oropharyngeal swabs were the most common sample. However, false negative results occurred in the late stage of hospitalization, and avoiding false negative result is critical essential. Method(s): We reviewed the medical record of 353 patients who received tests with both specimens simultaneously, and compared the performance between nasopharyngeal and oropharyngeal
swabs. Result(s): Of the 353 patients (outpatients, 192; inpatients, 161) studied, the median age was 54 years, and 177 (50.1%) were women. Higher positive rate (positive tests/total tests) was observed in nasopharyngeal swabs than oropharyngeal swabs, especially in inpatients. Nasopharyngeal swabs from inpatients showed higher positive rate than outpatients. Nasopharyngeal swabs from male showed higher positive rate than female, especially in outpatients. Detection with both specimens slightly increased the positive rate than nasopharyngeal swab only. Moreover, the consistency between from nasopharyngeal and oropharyngeal swabs were poor (Kappa = 0.308). Conclusion(s): In conclusion, our study suggests that nasopharyngeal swabs may be more suitable than oropharyngeal swab at this stage of COVID-19 outbreak.

URL: https://www.ijidonline.com/article/S1201-9712(20)30235-6/pdf
DOI: http://dx.doi.org/10.1016/j.ijid.2020.04.023


ABSTRACT: Background The recent emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a rapid proliferation of serologic assays. However, little is known about their clinical performance. Here, we compared two commercial SARS-CoV-2 IgG assays. Methods 103 specimens from 48 patients with PCR confirmed SARS-CoV-2 infections and 153 control specimens were analyzed using SARS-CoV-2 serologic assays by Abbott and EUROIMMUN (EI). Duration from symptom onset was determined by medical record review. Diagnostic sensitivity, specificity, and concordance were calculated. Results The Abbott SARS-CoV-2 assay had a diagnostic specificity of 99.4% (95% CI; 96.41-99.98%), and sensitivity of 0.0% (95% CI; 0.00-26.47%) at <3 days post symptom onset, 30.0% (95% CI; 11.89-54.28) at 3-7d, 47.8% (95% CI; 26.82-69.41) at 8-13d and 93.8% (95% CI; 82.80-98.69) at ≥14d. Diagnostic specificity on the EI assay was 94.8% (95% CI; 89.96-97.72) if borderline results were considered positive and 96.7% (95% CI; 92.54-98.93) if borderline results were considered negative. The diagnostic sensitivity was 0.0% (95% CI; 0.00-26.47%) at <3d, 25.0% (95% CI; 8.66-49.10) at 3-7d, 56.5% (95% CI; 34.49-76.81) at 3-7d and 85.4% (95% CI; 72.24-93.93) at ≥14d if borderline results were considered positive. The qualitative concordance between the assays was 0.83 (95% CI; 0.75-0.91). Conclusions The Abbott SARS-CoV-2 assay had fewer false positive and false negative results than the EI assay. However, diagnostic sensitivity was poor in both assays during the first 14 days of symptoms.

DOI: 10.1093/clinchem/hva120


ABSTRACT: The recent spread of severe acute respiratory syndrome coronavirus (SARS-CoV-2) exemplifies the critical need for accurate and rapid diagnostic assays to prompt public health actions. Currently, several quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assays are being used by clinical, research, and public health laboratories for rapid detection of the virus. However, it is currently unclear if results from different tests are comparable. Our goal was to evaluate the primer-probe sets used in four common diagnostic assays available on the World Health Organization (WHO) website. To facilitate this effort, we generated RNA transcripts to create standards and distributed them to other laboratories for internal validation. We then used these RNA transcript standards, full-length SARS-CoV-2 RNA, and RNA-spiked mock samples to determine analytical efficiency and sensitivity of nine primer-probe sets. We show that all primer-probe sets can be used to detect SARS-CoV-2, but there are clear differences in the ability to differentiate between true negatives and positives with low amounts of virus. Adding to this, many primer-probe sets, including the "N2" and "N3" sets issued by the US Centers for Disease Control and Prevention, have background amplification with SARS-CoV-2-negative nasopharyngeal swabs, which may lead to inconclusive results. Our findings characterize the
limitations of commonly used primer-probe sets and can assist other laboratories in selecting appropriate assays for the detection of SARS-CoV-2.

URL: http://medrxiv.org/content/early/2020/04/01/2020.03.30.20048108.abstract
DOI: 10.1101/2020.03.30.20048108


ABSTRACT: ABSTRACT In the age of a pandemic, such as the ongoing one caused by SARS-CoV-2, the world faces a limited supply of tests, personal protective equipment, and factories and supply chains are struggling to meet the growing demands. This study aimed to evaluate the efficacy of specimen pooling for testing of SARS-CoV-2 virus, to determine whether costs and resource savings could be achieved without impacting the sensitivity of the testing. Ten previously tested nasopharyngeal and throat swab specimens by real-time PCR, were pooled for testing, containing either one or two known positive specimens of varying viral concentrations. Specimen pooling did not affect the sensitivity of detecting SARS-CoV-2 when the PCR cycle threshold (Ct) of original specimen was lower than 35. In specimens with low viral load (Ct>35), 2 out of 15 pools (13.3%) were false negative. Pooling specimens to test for COVID-19 infection in low prevalence (≤1%) areas or in low risk populations can dramatically decrease the resource burden on laboratory operations by up to 80%. This paves the way for large-scale population screening, allowing for assured policy decisions by governmental bodies to ease lockdown restrictions in areas with a low incidence of infection, or with lower risk populations. This article is protected by copyright. All rights reserved.

DOI: https://doi.org/10.1002/jmv.26005


ABSTRACT: In December 2019, a cluster of acute respiratory illness occurred in Wuhan, Hubei Province, China. This disease is now officially known as 2019 novel coronavirus disease (COVID-19) from WHO, novel coronavirus pneumonia

URL: https://pubmed.ncbi.nlm.nih.gov/32096564
DOI: 10.1002/jmv.25721


ABSTRACT: This manuscript is based on the a simple but robust model we developed urgently to accurately monitor and predict viral dynamics for each SARS-CoV-2-infected patient, given the limited number of RT-PCR tests and the complexity of each individual's physical health situation. In this study, we used the mathematical model to monitor and predict the changes of viral loads from different nasal and throat swab of clinical specimens collected from diagnosed patients. We also tested our real time model by using the data from the SARS-CoV-2-infected patients with different severity. By using this personal model, we can predict the viral dynamics of patients, minimize false-negative test results, and screen the patients who are at risk of testing positive again after recovery. We sincerely thank those who are on the front lines battling SARS-CoV-2 virus. We hope this model will be useful for SARS-CoV-2-infected patients.

URL: http://medrxiv.org/content/early/2020/04/17/2020.04.14.20060491.abstract
ABSTRACT: As health care systems around the world attempt to cope with the coronavirus disease 2019 (COVID19) "tsunami," concerns about ongoing spread of disease from individuals who are infected without symptoms have been raised. Efforts to develop and implement testing protocols are underway, and expanded testing for COVID-19 is a necessary immediate step toward understanding and resolving this crisis. As tests become more available, observing principles of evidence-based clinical reasoning concerning the meaning of diagnostic test results is essential. For negative test results in particular, failure to do so has direct implications for the safety of the public and health care workers and for the success of efforts to curb the pandemic. Specifically, anticipation of a less-visible second wave of infection from individuals with false-negative test results is needed.
URL: https://www.mayoclinicproceedings.org/article/S0025-6196(20)30365-7/pdf
DOI: 10.1016/j.mayocp.2020.04.004

ABSTRACT: Reverse transcription-polymerase chain reaction (RT-PCR) assays are used to test patients and key workers for infection with the causative SARS-CoV-2 virus. RT-PCR tests are highly specific and the probability of false positives is low, but false negatives can occur if the sample contains insufficient quantities of the virus to be successfully amplified and detected. The amount of virus in a swab is likely to vary between patients, sample location (nasal, throat or sputum) and through time as infection progresses. Here, we analyse publicly available data from patients who received multiple RT-PCR tests and were identified as SARS-CoV-2 positive at least once. We identify that the probability of a positive test decreases with time after symptom onset, with throat samples less likely to yield a positive result relative to nasal samples. Empirically derived distributions of the time between symptom onset and hospitalisation allowed us to comment on the likely false negative rates in cohorts of patients who present for testing at different clinical stages. We further estimate the expected numbers of false negative tests in a group of tested individuals and show how this is affected by the timing of the tests. Finally, we assessed the robustness of these estimates of false negative rates to the probability of false positive tests. This work has implications both for the identification of infected patients and for the discharge of convalescing patients who are potentially still infectious.
URL: http://medrxiv.org/content/early/2020/04/07/2020.04.05.20053355.abstract
DOI: 10.1101/2020.04.05.20053355

URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7180262/
DOI: https://dx.doi.org/10.1128/JCM.00297-20

ABSTRACT: COVID-19 (corona virus disease 2019) is a kind of acute severe pneumonia caused by 2019-nCoV (2019-nCoV) infection. Since December 2019, it has been found in Wuhan, Hubei Province, and then spread to the whole country. Some parts of the world also showed an outbreak trend [1–3]. Real-time fluorescence quantitative reverse transcriptase polymerase chain reaction (reverse transcriptase-polymerase chain reaction,RT-PCR) and viral gene sequencing are the gold standard for the diagnosis of COVID-19. At present, upper respiratory tract nasopharyngeal swabs are mostly used as nucleic acid

ABSTRACT: Background: With the spread of Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection, its effect on society is amplified. We aimed to describe the viral detection results across different timepoints throughout the disease course. Method(s): A retrospective study of 301 confirmed COVID-19 patients hospitalized at Tongji Hospital in Wuhan, China, were included. Demographic characteristics of the patients were collected. Upper respiratory specimens (throat and/or nasal swabs) were obtained and analyzed by real-time RT-PCR for SARS-CoV-2 infection. Period of viral infection and the contagious stage were analyzed. Result(s): Of 301 hospitalized COVID-19 patients, the median age was 58 years and 51.2 % were male. The median period between symptoms presence and positive SARS-CoV-2 RT-PCR results was 16 days (IQR, 10-23, N = 301). The median period between symptoms presence and an effective negative SARS-CoV-2 RT-PCR result was 20 days (IQR, 17-24; N = 216). Infected patient >=65 years old stayed contagious longer (22 days vs 19 days, p = 0.015). Although two consecutive negative results were confirmed in 70 patients, 30 % of them had positive viral test results for the third time. Using specimens from nasal swabs to run the RT-
PCR test showed a higher positive rate than using specimens from throat swabs. Conclusion(s): This large-scale investigation with 1113 RT-PCR test results from 301 COVID-19 patients showed that the average contagious period of SARS-CoV-2 infected patients was 20 days. Longer observation period and more than 2 series of negative viral test are necessary for patients >=65 years.

**ABSTRACT:** A novel coronavirus (COVID-19) pandemic cause by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) threatens the world. We read with interest the recent report by Li et al. that included 610 patients with Coronavirus Disease 2019 (COVID-19). They reported a high false-negative rate of real-time reverse transcription polymerase chain reaction (RT-PCR) results for SARS-CoV-2 detection. In addition, recent report regarding SARS-CoV-2 "turn positive" in recovered cases with COVID-19 were published. Here, we studied the characteristics of nucleic acid conversion for SARS-CoV-2 from 70 COVID-19 patients. We found that 15 (21.4%) patients experienced a "turn positive" of nucleic acid detection by RT-PCR test for SARS-CoV-2 after two consecutive negative results, which may be related to the false negative of RT-PCR test and prolonged nucleic acid conversion.

**ABSTRACT:** An ongoing outbreak of severe respiratory pneumonia associated with the 2019 novel coronavirus has recently emerged in China. Here we report the epidemiological, clinical, laboratory and radiological characteristics of 19 suspect cases. We compared the positive ratio of 2019-nCoV nucleic acid amplification test results from different samples including oropharyngeal swab, blood, urine and stool with 3 different fluorescent RT-PCR kits. Nine out of the 19 patients had 2019-nCoV infection detected using oropharyngeal swab samples, and the virus nucleic acid was also detected in eight of these nine patients using stool samples. None of positive results was identified in the blood and urine samples. These three different kits got the same result for each sample and the positive ratio of nucleic acid detection for 2019-nCoV was only 47.4% in the suspect patients. Therefore, it is possible that infected patients have been missed by using nucleic acid detection only. It might be better to make a diagnosis combining the computed tomography scans and nucleic acid detection.

**ABSTRACT:** Objective: To construct and evaluate a diagnosis process for 2019-Coronavirus Disease (COVID-19). Methods: A continuous cohort of adults and adolescent (â‰¥12 years) who screened
COVID-19 was included in Xiangya Hospital of Central South University from January 23 to February 3, 2020 in which cases were divided into the test library and the verification library. Their gender, age, onset time were recorded. Take epidemiological history, fever, and the blood lymphocytes decline as clinical indicators, used CT to evaluate the possibility of COVID-19 and range of lung involvement. According to the current national standards, throat swabs of suspected cases were collected and the nucleic acid of COVID-19 was detected by reverse transcription-polymerase chain reaction (RT-PCR). The Xiangya process was constructed according to multi-index, compared with clinical indicators, CT results and national standards, the efficiency of detecting confirmed cases were verified in the test and verification library. Results: A continuous cohort of 382 adults who screened COVID-19 was included in which 261 cases were in the test library and 121 cases were in the verification library. In the 382 cases, 192 were males (50.3%) and 190 were females (49.7%), with a median age of 35 years (range: 15-92 years). There were 183 cases (47.9%) with epidemiological history, 275 cases (72.0%) with fever, 212 cases (55.5%) with decreased hemolytic lymphocytes, CT positive 114 cases (29.8%), 43 cases (11.3%) with positive CT-COVID-19, and 30 cases (7.9%) with positive throat swab nucleic acid. Compared with clinical indicators, the sensitivity and specificity of CT were 0.950 and 0.704, respectively. The accuracy of CT to make a definite diagnosis was higher than that of epidemiological history, fever, and blood lymph count decline (0.809 vs 0.660, 0.532, 0.596, P=0.001, 0.002, 0.003, respectively). The sensitivity of this process and the program recommended by the Health Commission all were high (all were 1.000), and the specificity and accuracy of the process were higher than the program recommended by the Health Commission (0.872 vs 0.765, 0.778 vs 0.592, both P<0.001). The CT-COVID-19 would have reduced the missed diagnosis rate caused by false negative of nucleic acid test (31 vs 64, difference rate 51.6%), the positive rate of nucleic acid test was 64.5% (20/31). In validation library, the specificity and accuracy of the Xiangya process was 0.967, the positive rate of nucleic acid test was 76.9% (10/13). Conclusions: The Xiangya process can predict the nucleic acid test results of COVID-19 well, and can be applied as a reliable basis for confirmed cases detection in adults and adolescent (≥12 years) in areas other than Hubei during the epidemic period of COVID-19. The cohort size needs to be increased for further validation.

URL: https://pubmed.ncbi.nlm.nih.gov/32157849
DOI: 10.3760/cma.j.cn112137-20200228-00499
false negatives, and were found to extend the time window for testing positive by 23% and 41%, respectively. Age and gender also affected the significantly. Limitation: Data were generated in a large single-center study. Conclusion: Testing outcome and positive window of SARS-CoV-2 detection for COVID-19 patients were associated with CT imaging results, blood routine tests, and clinical symptoms. Taking into account relevant information in CT imaging reports, blood routine tests, and clinical symptoms helped reduce a false negative testing outcome. The predictive AFT model, what we believe to be one of the first statistical models for predicting time window of SARS-CoV-2 detection, could help clinicians improve the accuracy and efficiency of the diagnosis, and hence, optimizes the timing of nucleic acid detection and alleviates the shortage of nucleic acid detection kits around the world.

Primary Funding Source: None.

URL: [http://medrxiv.org/content/early/2020/03/30/2020.03.26.20043042.abstract](http://medrxiv.org/content/early/2020/03/30/2020.03.26.20043042.abstract)

DOI: 10.1101/2020.03.26.20043042


**ABSTRACT:** Emerging and reemerging infectious diseases are global public concerns. With the outbreak of unknown pneumonia in Wuhan, China in December 2019, a new coronavirus, SARS-CoV-2 has been attracting tremendous attention. Rapid and accurate laboratory testing of SARS-CoV-2 is essential for early discovery, early reporting, early quarantine, early treatment, and cutting off epidemic transmission. The genome structure, transmission, and pathogenesis of SARS-CoV-2 are basically similar to SARS-CoV and MERS-CoV, the other two beta-CoVs of medical importance. During the SARS-CoV and MERS-CoV epidemics, a variety of molecular and serological diagnostic assays were established and should be referred to for SARS-CoV-2. In this review, by summarizing the articles and guidelines about specimen collection, nucleic acid tests (NAT) and serological tests for SARS-CoV, MERS-CoV, and SARS-CoV-2, several suggestions are put forward to improve the laboratory testing of SARS-CoV-2. In summary, for NAT: collecting stool and blood samples at later periods of illness to improve the positive rate if lower respiratory tract specimens are unavailable; increasing template volume to raise the sensitivity of detection; putting samples in reagents containing guanidine salt to inactivate virus as well as protect RNA; setting proper positive, negative and inhibition controls to ensure high-quality results; simultaneously amplifying human RNase P gene to avoid false-negative results. For antibody test, diverse assays targeting different antigens, and collecting paired samples are needed.


DOI: [https://dx.doi.org/10.1002/rmv.2106](https://dx.doi.org/10.1002/rmv.2106)


**ABSTRACT:** Background The recent emergence of SARS-CoV-2 lead to a current pandemic of unprecedented scale. Though diagnostic tests are fundamental to the ability to detect and respond, overwhelmed healthcare systems are already experiencing shortages of reagents associated with this test, calling for a lean immediately-applicable protocol. Methods RNA extracts of positive samples were tested for the presence of SARS-CoV-2 using RT-qPCR, alone or in pools of different sizes (2-, 4-, 8-, 16-, 32- and 64-sample pools) with negative samples. Transport media of additional 3 positive samples were also tested when mixed with transport media of negative samples in pools of 8. Results A single positive sample can be detected in pools of up to 32 samples, using the standard kits and protocols, with an estimated false negative rate of 10%. Detection of positive samples diluted in even up to 64 samples may also be attainable, though may require additional amplification cycles. Single positive samples can be detected when pooling either after or prior to RNA extraction. Conclusions As it uses the standard protocols, reagents and equipment, this pooling method can be applied immediately in current clinical
testing laboratories. We hope that such implementation of a pool test for COVID-19 would allow expanding current screening capacities thereby enabling the expansion of detection in the community, as well as in close organic groups, such as hospital departments, army units, or factory shifts.


**DOI:** 10.1093/cid/ciaa531


**ABSTRACT:** Background: COVID-19 RNA detected by RT-PCR is not sensitive enough to diagnose COVID-19. The purpose of this study was to identify cost-effective biomarkers for predicting COVID-19 pneumonia.

**Methods:** This retrospective study examined the records of 28 COVID-19 cases. Apriori algorithm of association rules was employed to identify laboratory indexes related to COVID-19 pneumonia.

**Finding:** The symptom of COVID-19 was 64.29% fever, 46.43% expectoration, 32.14% dry cough, 14.29% fatigue, 14.29% pharyngalgia, 3.57% myalgia, 3.57% dyspnea. In the first stages of hospitalization, the level of neutrophil, monocyte, MPV, MCHC, NMR, AST, ALT and DBIL were significantly increased, while lymphocyte, eosinophils, basophilic granulocyte, platelet, PDW, MPV, MCV, NLR, total protein (TP), albumin, globulin and uric acid were significantly elevated in COVID-19 pneumonia. Moreover, TP, DBIL, M% and albumin were strongly associated with COVID-19 pneumonia, and TP was identified as an independent risk factor for it. The area under ROC curve of TP was 0.844 and the optimal clinical cutoff level was 72.8g/L, which provided 78.6% sensitivity and 79.3% specificity.

Interestingly, TP combined with COVID-19 RNA exhibited 96.4% sensitivity and 0.05 negative likelihood ratios. Importantly, the false negative rate of COVID-19 RNA was 39.3%, and 90.91% of them can be detected by TP. In addition, TP was markedly reduced in patients with COVID-19 pneumonia compared with no-COVID-19 pneumonia and healthy control, and it was significantly decreased in severe and critical of COVID-19 pneumonia than the common stage and mild stage.

**Interpretation:** Our study suggested that TP may be a biomarker for predicting COVID-19 pneumonia.


**ABSTRACT:** Objective: On the premise of ensuring the sensitivity and specificity of nucleic acid detection, explore the choice and number of gene targets on the nucleic acid detection reagent of severe acute respiratory syndrome virus 2 (SARS-CoV-2), to reduce national medical burden.

**Methods:** From February 25, 2020 to March 11, 2020, a quality control material (each including 5 samples) were distributed to 50 participating laboratories by Fujian Clinical Laboratory Center, and each participating laboratory used different reagent kits to detect the nucleic acid gene of SARS-CoV-2. As of March 11, 2020, we received a total of 48 research data. This paper adopts the method of descriptive statistics.

**Results:** The research results of 48 participating laboratories were collected, of which 1 laboratory used reagent kit to detect one target gene open reading frame 1ab (ORF 1ab)], 28 laboratories used reagent kit to detect two target genes ORF 1ab and nucleocapsid protein gene (N)], and 19 laboratories use reagent kit to detect three target genes ORF 1ab, N and envelope protein gene (E)]. None of the 48 participating laboratories testing the ORF1ab gene and 19 participating laboratories testing the E gene showed false negatives and false positives, and the coincidence rate was 100%. Among the 47 laboratories that tested the N gene, four false negatives appeared, with a compliance rate of 98.3%. All four false negatives occurred in kits that tested three gene targets.

**Conclusion:** Selecting two target genes and optimizing the composition and reaction system of the reagent can improve the quality of the kit, reduce false negatives and the cost of detection.

**URL:** [https://ssrn.com/abstract=3558020](https://ssrn.com/abstract=3558020)

**ABSTRACT:** Background: The colloidal gold immunochromatography assay (GICA) is a rapid diagnostic tool for novel coronavirus disease 2019 (COVID-19) infections. However, with significant numbers of false negatives, improvements to GICA are needed. Methods: Six recombinant HCoV-19 nucleocapsid and spike proteins were prepared and evaluated. The optimal proteins were employed to develop a sandwich-format GICA strip to detect total antibodies (IgM and IgG) against HCoV-19. GICA performance was assessed with comparison of viral RNA detection. Results: Recombinant HCoV-19 proteins were obtained, including three prokaryotically expressed rN, rN1, rN2 nucleocapsid proteins, and three eukaryotically expressed rS1, rS-RBD, rS-RBD-mFc spike proteins. The recombinant proteins with the highest ELISA titers (rS1 and rS-RBD-mFc) against coronavirus-specific IgM and IgG were chosen for GICA development. The GICA has a sensitivity and specificity of 86.89% (106/122) and 99.39% (656/660), respectively. Furthermore, 65.63% (21/32) of the clinically confirmed but RT-PCR negative samples were GICA positive. Conclusions: The eukaryotically expressed spike proteins (rS1 and rS-RBD-mFc) are more suitable than the prokaryotically expressed nucleocapsid proteins for HCoV-19 serological diagnosis. The GICA sandwich used to detect total antibodies is a powerful complement to the current standard RNA-based tests.

**URL:** [http://medrxiv.org/content/early/2020/03/20/2020.03.17.20036954.abstract](http://medrxiv.org/content/early/2020/03/20/2020.03.17.20036954.abstract)

**DOI:** 10.1101/2020.03.17.20036954


**DOI:** 10.3760/cma.j.issn.0376-2491.2020.0008


**ABSTRACT:** Rapid and accurate tests that detect IgM and IgG antibodies to SARS-CoV-2 proteins are essential in slowing the spread of COVID-19 by identifying patients who are infected with COVID-19. Using a SARS-CoV-2 proteome microarray developed in our lab, we comprehensively profiled both IgM and IgG antibodies in forty patients with early-stage COVID-19, influenza, or non-influenza who had similar symptoms. The results revealed that the SARS-CoV-2 N protein is not an ideal biomarker for COVID-19 diagnosis because of its low immunogenicity, thus tests that rely on this marker alone will have a high false negative rate. Our data further suggest that the S protein subunit 1 receptor binding domain (S1-RBD) might be the optimal antigen for IgM antibody detection, while the S protein extracellular domain (S1+S2ECD) would be the optimal antigen for both IgM and IgG antibody detection. Notably, the combination of all IgM and IgG biomarkers can identify 87% and 73.3% COVID-19 patients, respectively. Finally, the COVID-19-specific antibodies are significantly correlated with the clinical indices of viral infection and acute myocardial injury (p≤0.05). Our data may help understand the function of anti-SARS-CoV-2 antibodies and improve serology tests for rapid COVID-19 screening.


**DOI:** 10.1101/2020.04.14.20064535


**ABSTRACT:** Background: An outbreak of a novel coronavirus (SARS-CoV-2) that began in Wuhan, has spread rapidly to more than 26 countries. False negative diagnosis of respiratory samples,
predominantly nasal or throat swabs, has made diagnoses and control difficult. Methods: To identify the detection rates in different respiratory sample types, we recruited 65 patients with confirmed SARS-CoV-2 infection and sequentially collected throat swabs, nasal swabs and saliva or sputum, followed by a comparative analysis of quantitative RT-PCR and viral loads. Results: The median age of COVID-19 patients recruited for the study was 52.5 years (IQR 39.3-61). Fever (83.3%), cough (54.2%) and expectoration (25.0%) were the most common clinical manifestations. The detection rates of sputum (95.65%, 22/23) and saliva (88.09%, 37/42) were significantly higher than in throat swabs (41.54%, 27/65) and nasal swabs (72.31%, 47/65) (P <0.001). Further, the Ct Value from sputum, saliva and nasal swabs were significantly higher than in throat swabs, whereas no significant difference was observed between sputum and saliva samples. Conclusions: The detection rate of SARS-CoV-2 is higher in saliva than other respiratory samples. Our results show that saliva and sputum are reliable sample types that can be used to detect SARS-CoV-2, and worthy of clinical promotion. The convenience in sampling saliva may also significantly reduce the risk of infection of medical staff.

URL: https://ssrn.com/abstract=3543605


ABSTRACT: An ongoing outbreak of pneumonia associated with SARS-CoV-2 has now been confirmed globally. In absence of effective vaccines, infection prevention and control through diagnostic testing and quarantine is critical. Early detection and differential diagnosis of respiratory infections increases the chances for successful control of COVID-19 disease. The nucleic acid RT-PCR test is regarded as the current standard for molecular diagnosis with high sensitivity. However, the highest specificity confirmation target ORF1ab gene is considered to be less sensitive than other targets in clinical application. In addition, a large amount of recent evidence indicates that the initial missed diagnosis of asymptomatic patients with SARS-CoV-2 and discharged patients with "re-examination positive" may be due to low viral load, and the ability of rapid mutation of coronavirus also increases the rate of false negative results. We aimed to evaluate the sensitivity of different nucleic acid detection kits so as to make recommendations for the selection of validation kit, and amplify the suspicious result to be reportable positive by means of simple continuous amplification, which is of great significance for the prevention and control of the current epidemic and the discharge criteria of low viral load patients.

URL: http://medrxiv.org/content/early/2020/05/05/2020.04.28.20083956.abstract

DOI: 10.1101/2020.04.28.20083956

SEARCH STRATEGIES

CINAHL
Date Run: May 21, 2020 13:20
S16 S13 OR S15 3
S15 S10 AND S14 2
S14 TX ("polymerase chain reaction" or PCR or qPCR or rPCR or RT-PCR) N5 (false negative* or true negative*) 110
S13 S10 AND S11 AND S12 3
S12 (MH "False Negative Results") OR TI (false negative* or true negative*) OR AB (false negative* or true negative*) 7,471
S11 (MH "Polymerase Chain Reaction+") OR TI ("polymerase chain reaction" or PCR or qPCR or rPCR or RT-PCR) OR AB ("polymerase chain reaction" or PCR or qPCR or rPCR or RT-PCR) 74,762
S10  S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 Limiters - Published Date: 20191201-20201231; English Language 3,491
S9  S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 15,397
S8  TX ("severe acute respiratory syndrome**") 3,735
S7  TX (outbreak* or wildlife* or pandemic* or epidemic*) N1 (Wuhan* or Hubei or China* or Chinese* or Huanan*) 684
S6  TX ("seafood market**" or "food market**" or pneumonia*) N10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*) 467
S5  TX (respiratory* N2 (symptom* or disease* or illness* or condition*) N10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*)) 1,254
S4  TX ("2019-nCoV" or 2019nCoV or nCoV2019 or "nCoV-2019" or "COVID-19" or COVID19 or "CORVID-19" or CORVID19 or "WN-CoV" or WNCoV or "HCoV-19" or HCoV19 or "2019 novel*" or Ncov or "n-cov" or "SARS-CoV-2" or "SARSCoV-2" or "SARSCoV2" or SARS-CoV2 or SARSCov19 or "SARS-CoV-19" or "SARSCoV-19" or "SARS-CoV-2" or SARSCov19 or Ncovor or NcovWuhan* or NcovHubei* or NcovChina* or NcovChinese* or SAR2 or "SARS-2" or SARScoronavirus2 or "SARS-coronavirus-2" or "SARScoronavirus 2" or "SARS coronavirus2" or SARScoronavirus2 or "SARS-coronavirus-2" or "SARS-coronavirus 2" or "SARS coronavirus 2") 3,103
S3  TX (coronavirus* or coronavirus* or coronavirinae* or CoV or HCoV*) 8,770
S2  TX ((corona* or corono*) adj1 (virus* or viral* or virinae*)) 0
S1  (MH "Coronavirus Infections+") OR (MH "Coronavirus+") OR (MH "COVID-19") 5,392

Embase <1974 to 2020 May 20>
Date Run: May 21, 2020 12:30
1  exp Coronavirinae/ or exp Coronavirus infection/ (21866)
2  (coronavirus disease 2019 or severe acute respiratory syndrome coronavirus 2).sh,dj. (8002)
3  ((corona* or corono*) adj1 (virus* or viral* or virinae*)).ti,ab,kw. (770)
4  (coronavirus* or coronavirus* or coronavirinae* or CoV).ti,ab,kw. (20985)
5  ("2019-nCoV" or 2019nCoV or nCoV2019 or "nCoV-2019" or "COVID-19" or COVID19 or "CORVID-19" or CORVID19 or "WN-CoV" or WNCoV or "HCoV-19" or HCoV19 or "2019 novel*" or Ncov or "n-cov" or "SARS-CoV-2" or "SARSCoV-2" or "SARSCoV2" or SARS-CoV2 or SARSCov19 or "SARS-CoV-19" or "SARSCoV-19" or "SARS-CoV-2" or SARSCov19 or Ncovor or NcovWuhan* or NcovHubei* or NcovChina* or NcovChinese* or SAR2 or "SARS-2" or SARScoronavirus2 or "SARS-coronavirus-2" or "SARScoronavirus 2" or "SARS coronavirus2" or SARScoronavirus2 or "SARS-coronavirus-2" or "SARS-coronavirus 2" or "SARS coronavirus 2")
6  (respiratory* adj2 (symptom* or disease* or illness* or condition*) adj10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*)).ti,ab,kw. (560)
7  ("seafood market**" or "food market**" or pneumonia*) adj10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*).ti,ab,kw. (108)
8  (outbreak* or wildlife* or pandemic* or epidemic*) adj1 (Wuhan* or Hubei* or China* or Chinese* or Huanan*).ti,ab,kw. (6233)
9  "severe acute respiratory syndrome**".ti,ab,kw. (38850)
10 10 and 9 (38850)
11 10 and 20191201:20201231.(dc). (13654)
12 11 and 11 to english language (12911)
13  exp polymerase chain reaction/ or exp PCR assay kit/ or ("polymerase chain reaction" or PCR or qPCR or rPCR or RT-PCR).ti,ab,kw. (1187547)
14  false negative result/ or (false negative* or true negative*).ti,ab,kw. (56355)
15 12 and 13 and 14 (34)
16  ("polymerase chain reaction" or PCR or qPCR or rPCR or RT-PCR) adj5 (false negative* or true negative*).af. (1325)
Google Scholar
(coronavirus|covid-19 PCR|RT-PCR false negative positive biomarkers testing)

Ovid MEDLINE(R) ALL <1946 to May 20, 2020>
Date Run: May 21, 2020 13:05

1 exp coronavirus/ or exp Coronavirus Infections/ (18136)
2 ((corona* or corono*) adj1 (virus* or viral* or virinae*)).ti,ab,kw,kf. (1047)
3 (coronavirus* or coronavirus* or coronavirinae* or CoV).ti,ab,kw,kf. (20485)
4 ("2019-nCoV" or 2019nCoV or nCoV2019 or "nCoV-2019" or "COVID-19" or COVID19 or "CORVID-19" or CORVID19 or "WN-CoV" or WNCoV or "HCoV-19" or HCoV19 or "2019 novel*" or Ncov or "n-cov" or "SARS-CoV-2" or "SARSCoV2" or "SARS-CoV2" or SARSCov19 or "SARS-Cov19" or "SARSCov-19" or "SARS-Cov-19" or Ncovor or Ncorona* or Ncorono* or NcovWuhan* or NcovHubei* or NcovChina* or NcovChinese* or SARS2 or "SARS-2" or SARScoronavirus2 or "SARS-coronavirus-2" or "SARScoronavirus2" or SARScoronavirus2 or "SARS-coronavirus-2" or "SARScoronavirus 2" or "SARS coronavirus2" or SARScoronavirus2 or "SARS-coronavirus-2" or "SARS coronavirus2".ti,ab,kw,kf. (14538)
5 (respiratory* adj2 (symptom* or disease* or illness* or condition*) adj10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*)).ti,ab,kw,kf. (457)
6 ("seafood market*" or "food market*" or pneumonia*) adj10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*).ti,ab,kw,kf. (1236)
7 (outbreak* or wildlife* or pandemic* or epidemic*) adj1 (Wuhan* or Hubei* or China* or Chinese* or Huanan*).ti,ab,kw,kf. (233)
8 "severe acute respiratory syndrome*".ti,ab,kw,kf. (6199)
9 or/1-8 (37068)
10 9 and 20191201:20201231.(dt). (16303)
11 limit 10 to english language (15453)
12 exp Polymerase Chain Reaction/ or ("polymerase chain reaction" or PCR or qPCR or rPCR or RT-PCR).ti,ab,kw,kf. (842155)
13 false negative reactions/ or (false negative* or true negative*).ti,ab,kw,kf. (45543)
14 11 and 12 and 13 (34)
15 ("polymerase chain reaction" or PCR or qPCR or rPCR or RT-PCR) adj5 (false negative* or true negative*).af. (965)
16 11 and 15 (11)
17 14 or 16 (34)