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Title: COVID-19 Testing: Challenges, Limitations and Suggestions for Improvement

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Abstract: Reliable methods to confirm the diagnosis of COVID-19 are essential to the successful management and containment of the virus. Current diagnostic options are limited in type, supply, and reliability. This article explores the controversial unreliability of existing diagnostic methods and maintains that more reliable diagnostic methods, combinations, and sequencing are necessary to effectively assist in reducing the occurrence of discharge of the patient on false negative test results. This reduction would in effect reduce transmission of the disease.

Keywords: COVID-19, Coronavirus, False-negative, Nucleic Acid Test, Screening, Diagnostic Accuracy, Missed Diagnosis, Epidemic, Infectious Disease

Introduction

Due to the sudden onset of the COVID-19 pandemic, healthcare providers worldwide have been left with a paucity of validated and reliable ways to confirm the diagnosis. Confirming who does and does not have COVID-19 is essential to the successful management and containment of the virus. Current diagnostic options are limited in type, supply, and reliability.

The four following scenarios have all been speculated as possible with COVID-19:

- discharge of patients after unreliable (false negative) test results without appropriate precautions,
- reinfection after symptomatic improvement with known COVID-19,
- relapse without full clearance of the initial infection, and
- mutation in the virus with new infection.

However, without reliable diagnostic testing, it is difficult to know the actual rates at which these scenarios occur, if they occur at all, and how this should alter the treatment protocol for future standard of care regarding diagnostics (1,2).

This article explores the controversial unreliability of existing diagnostic methods and maintains that more reliable diagnostic methods (or combinations and sequencing) are necessary to effectively assist in reducing the occurrence of the first scenario—discharge of the patient on false negative test results—while allowing for more accurate estimations of the rates of the remaining three: reinfection, relapse, and mutation in the virus.

Current Testing Options

Currently, there are no FDA-approved diagnostic tests for Covid-19, but more are becoming available by the day through the FDA's Expanded Access program. Expanded access, sometimes called "Compassionate Use," is the use of a drug, biologic, or medical device not yet approved by the FDA outside of a clinical trial (3). This method expedites the FDA review

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process to allow the use of an investigational device to save the life of a patient, or to treat a patient for which there are no clinical trials or alternative therapies available (4). A category of Expanded Access is the FDA's Emergency Use Authorization which allows the use of a test article on a human subject in a life-threatening situation in which no standard acceptable treatment is available, and in which there is insufficient time before the risk is likely realized to obtain FDA approval for the use. Although prior FDA approval for use is not required, all usage must be reported to the FDA within 5 days of use (5). At the time of this article, all diagnostic tests available for hospital use were made available using an Emergency Use Authorization (5 – 9).

Nucleic Acid Amplification Tests

Nucleic acid amplification tests (NAAT), such as reverse transcription polymerase chain reaction (RT PCR), are currently the most common diagnostic method used by hospitals worldwide to diagnose COVID-19; prior to March 23, 2020, they were the only chemical diagnostic test available in the United States (9,10). This technology is extremely reliable, but only *if* usability and reagent requirements are met. The greatest limitation of NAATs is the high incidence of false negative diagnoses seen specifically in COVID-19 diagnostic tests (1,2). The largest study on coronavirus testing to date estimates a rate of 41% false negatives on RT PCR diagnostic tests used in China (11). However, despite the high false-negative rate, at present, RT-PCR test remains the reference standard to make a definitive diagnose of COVID-19 infection (12).

Due to expedited development and rollout of these tests to far distant locations where staff had different levels of experience, the sensitivity and specificity of these assays remain unclear. It is likely that the performance of available detection kits may have been compromised by the urgency under which these assays had to be developed; kits could not be adequately validated for clinical or analytical accuracy prior to becoming available for use where needed. In the absence of sufficiently rigorous testing standards, the thresholds for a positive or negative diagnosis were make-do and questionably determined, as well as were the origin and fidelity of the initial calibration and validation data. Additionally, the many countries which developed and adopted the earliest tests vary in their regulation of diagnostic test development, further complicating the ability to assess the quality and sensitivity of detection kits and preservation solutions. The global device development community is lacking sufficiently harmonized device regulatory compliance environments for the stress of this pandemic.

The timeline of disease progression and sampling is another factor which contributes to diagnostic uncertainty. Given the long incubation time of the disease, a low viral load during the initial days of illness could produce a false negative diagnosis. In many reported false negative cases, patients did not carry enough viral load to be detected positive at the time of sampling (13). Moreover, the tests will only offer a positive result when the virus is still present—they cannot identify patients who were infected, recovered, and then cleared the virus from their bodies (9).

Difficulty in obtaining a reliable biospecimen sample presents an additional challenge with NAATs. The current recommendation for this test is to obtain biospecimen samples from the

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oropharynx and the nasopharynx. Other sources of biospecimen such as feces, blood, cheek, and urine have lowest meaningful detection rates so low – often with false negative rates greater than 70% - that they greatly increase the risk of a false negative diagnosis (8, 14 – 17). However, there are some challenges with nasal and throat swabs, including potential patient discomfort and even bleeding, the need for an experienced healthcare provider to administer, the risk of droplet exposure to the healthcare provider, and inability to obtain specimens from the lower respiratory tract (18).

The further development of point-of-care NAATs will allow hospitals to move towards a diagnostic method in which biospecimen processing is not dependent on sample storage or transport conditions, and minimal user controls are required to ensure proper sample processing. An additional benefit of point-of-care testing is that it could allow patients to be tested at screening centers, reducing the burden on hospitals. However, as promising as this technology is, it does not reduce the many ongoing challenges in reagent supply, storage, and handling (5,11).

CT Scan

Current research indicates that time sequencing CT scans may be the most sensitive diagnostic testing method available for the coronavirus and could be capable of yielding an accurate diagnosis faster than RT-PCR by an average of 3 days (12,19). However, there is still a false negative rate of ≥12% reported with attempted CT diagnoses of COVID-19 (14). Mass CT scans are also not a practical solution as many countries do not have enough CT machines, technicians, and radiologists to support pandemic testing rates. Most hospitals in the United States have 1 or 2 CT machines and it usually takes doctors between five and 15 minutes to analyze one CT scan and give an interpretation (20,21). A significant limitation is also the time it takes to appropriately disinfect a CT scanner in between patients being studied, which can take up to an hour depending on the scanner and associated ventilation system of the area that houses the scanner. Additionally, to obtain maximum diagnostic accuracy, multiple scans from different time frames should be reviewed (22), and previous studies have indicated that performing 2 scans, 6 days apart, would lead to increased diagnostic accuracy (11,14).

AI algorithms capable of detecting coronavirus in CT scans have been developed in China to reduce clinical diagnosis times while increasing diagnostic accuracy (23). However, while these algorithms reduce the number of scans required to produce diagnoses, patients will still need to undergo CT and the bottleneck of one or two CT machines per hospital will still remain.

Serological Tests/Immunoassays

Serological tests (ST) use a surface protein or an array of virus peptides to capture antibodies specific to the virus in patients' blood. As this test measures the body's immune response to the virus, these tests confirm whether someone was infected even after their immune system has cleared the virus; moreover, these tests can help track who has been exposed to the virus but remain asymptomatic (23). At the time of this article, these assays were still under development in the United States, with one having just received approval to begin emergency use, but STs have recently been developed and used without prior validation in China (9,24). Initial findings in

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China have reported a detection accuracy of up to 93% when tests were conducted under the proper conditions (25).

Current limitations of STs include the fact that new tests need to be validated using the antibodies from the blood of infected people, which increases the development timeline. Another limitation is that the tests cannot provide a diagnosis within the first week of infection; a significant lag period exists as virus-targeting antibodies normally appear between 7-14 days after the onset of the illness (26). The test is the most accurate starting 2 weeks after the onset of the illness – with the CDC recommending a wait of 3 weeks after a person becomes ill to let antibody levels build – which may be an unreasonable amount of time to wait for a diagnosis, given the infectiousness of this disease (27).

Lab Developed Tests

Lab Developed Tests (LDT), including Lab Developed Home Tests, may contain the same or similar components as IVD tests; however, they must be developed and used within the same facility. At the time of this article, many LDTs were in development with only one available for hospital use in the United States (8).

The limitation of these tests is that while these products are relatively quick to market, regulatory review is not required of their clinical or analytical validity or marketing claims before entering the market. Although LDTs may contain the same or similar components as IVD tests, they are not subject to the same efficacy standards. Another challenge is that these tests also must be processed and analyzed at the manufacturer and not at the hospital or sample collection site, creating potential modes of failure during specimen storage and transport.

Methods to Increase Diagnostic Accuracy on the Operator Side

Given the very high infection rate for this virus, it is important to have accurate diagnostic data as soon as possible, as false negatives have proven to have an especially deleterious epidemiological effect (28,29). Reducing the number of false negative diagnoses is critical when determining isolation precautions and cohorting for hospitalized patients - with so many asymptomatic carriers, it's very possible that some patients admitted to hospitals for other conditions or trauma may be unknowingly carrying COVID-19. Knowing a patient has cleared COVID-19 and has antibodies to it, or knowing patients are silent carriers would allow hospitals to prioritize whom to isolate and help immensely to decrease hospital-based transmissions. Moreover this data would be extremely beneficial to healthcare providers as the rates of asymptomatic carriers among hospital workers is very high (17). Knowing if providers have developed immunity, or are silent carriers, would allow hospitals to better determine when to keep providers at home, as well as how to risk-stratify providers in high risk professions or procedures.

Under the current circumstances, the following actions may be taken on the operator side to increase the diagnostic efficiency of the tools currently available.

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Selecting optimal sources for biospecimen is paramount when conducting NAATs. Initial findings indicate that for NAATs, the throat and nasal cavity are the most accurate swab sites, although studies differ on which one is the most accurate (1,2,28,29). A previous study has found that detection strengths of using nasopharyngeal (nasal) or oropharyngeal (throat) swabs differ for different pathogens infecting the respiratory tract, and that one is not superior to the other for all cases (30). Initial rapid guidelines from China only indicate the usage of throat swabs (31). Similarly, the CDC recommends a nasal swab for COVID-19 diagnostic testing using RT-PCR and, in the absence of a proper nasal swab, a throat swab in place of or in addition to, the nasal swab (32).

Having a quantitative test of viral load could help direct therapy or detect which patients are likely to decompensate, however, this would take weeks to months of research after release of a test.

Additionally, performing just one type of test to diagnose COVID-19 could proliferate false-negative results or misdiagnoses due to the complex medical conditions of the patients. Therefore, a multi-prong approach in which multiple diagnostic tools are used to confirm results should be employed. This approach should also include diagnostic testing at multiple timepoints throughout the course of infection. The implementation of a combined diagnostic workflow of integrated serological testing with nucleic acid detection would provide a high-quality, multi-dimensional, and cost-effective diagnostic solution that could meet the detection needs for early disease prevention and control, differential diagnosis, and epidemiological investigations. Ideally, patients admitted to the hospital should be tested upon admission, and at weekly intervals to determine COVID-19 diagnosis, and every provider tested weekly to account for the long incubation time of the virus (17).

References:

1. Zhuang GH, Shen MW, Zeng LX, Mi BB, Chen FY, Liu WJ, Pei LL, Qi X, Li C. Potential false-positive rate among the 'asymptomatic infected individuals' in close contacts of COVID-19 patients. Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi. 2020 Mar 5;41(4):485.
2. Qinjian Hao, Hongmei Wu, Qiang Wang et al. Difficulties in False Negative Diagnosis of Coronavirus Disease 2019: A Case Report, 13 March 2020, PREPRINT (Version 1) available at Research Square [+https://doi.org/10.21203/rs.3.rs-17319/v1+].
3. Expanded Access for Medical Devices. [Cited 2 April 2020]. Available from: https://www.fda.gov/medical-devices/investigational-device-exemption-ide/expanded-access-medical-devices#:~:text=
4. Emergency Use and Compassionate Use of Experimental Drugs and Device. [Cited 2 April 2020]. Available from: https://irb.ucsf.edu/emergency-use-and-compassionate-use-experimental-drugs-and-devices#:~:text=
5. Xpert® Xpress SARS-CoV-2 has received FDA Emergency Use Authorization. [Cited 2 April 2020]. Available from: https://www.cepheid.com/coronavirus
6. FDA authorizes emergency use of hand-held COVID-19 PCR diagnostic test. [Cited 2 April 2020]. Available from: https://www.fiercebiotech.com/medtech/fda-authorizes-hand-held-covid-19-diagnostic-test

7. Abbott Launches Molecular Point-of-Care Test to Detect Novel Coronavirus in as Little as Five Minutes. [Cited 2 April 2020]. Available from: https://abbott.mediaroom.com/2020-03-27-Abbott-Launches-Molecular-Point-of-Care-Test-to-Detect-Novel-Coronavirus-in-as-Little-as-Five-Minutes
8. Our commitment to fighting COVID-19. [Cited 2 April 2020]. Available from: https://www.everlywell.com/blog/news-and-info/our-commitment-to-fighting-covid-19/
9. Normile D. Singapore claims first use of antibody test to track coronavirus infections. Science. 2020:02-27.
10. At-Home Tests for COVID-19. [Cited 2 April 2020]. Available from: https://health.usnews.com/conditions/articles/at-home-tests-for-covid-19
11. Wang M, Wu Q, Xu W, Qiao B, Wang J, Zheng H, Jiang S, Mei J, Wu Z, Deng Y, Zhou F. Clinical diagnosis of 8274 samples with 2019-novel coronavirus in Wuhan. medRxiv. 2020 Jan 1.
12. Yang W, Yan F. Patients with RT-PCR confirmed COVID-19 and normal chest CT. Radiology. 2020 Mar 6:200702.
13. Yang L. Clinical Reports on Early Diagnosis of Novel Coronavirus (2019-nCoV) Pneumonia in Stealth Infected Patients.
14. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, Tao Q, Sun Z, Xia L. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. 2020 Feb 26:200642.
15. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. Journal of travel medicine. 2020 Mar 13.
16. Jung SM, Akhmetzhanov AR, Hayashi K, Linton NM, Yang Y, Yuan B, Kobayashi T, Kinoshita R, Nishiura H. Real-time estimation of the risk of death from novel coronavirus (COVID-19) infection: Inference using exported cases. Journal of clinical medicine. 2020 Feb;9(2):523.
17. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Eurosurveillance. 2020 Mar 12;25(10).
18. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. Jama. 2020 Mar 11.
19. Li Y, Xia L. Coronavirus Disease 2019 (COVID-19): Role of chest CT in diagnosis and management. American Journal of Roentgenology. 2020 Feb 21:1-7.
20. M. Castillo. The Industry of CT Scanning. American Journal of Neuroradiology Apr 2012, 33 (4) 583-585;
21. 50 things to know about the hospital industry | 2017. [Cited 2 April 2020]. Available from: https://www.beckershospitalreview.com/hospital-management-administration/50-things-to-know-about-the-hospital-industry-2017.html#:~:text=
22. Salehi S, Abedi A, Balakrishnan S, Gholamrezanezhad A. Coronavirus Disease 2019 (COVID-19): A Systematic Review of Imaging Findings in 919 Patients. American Journal of Roentgenology. 2020 Feb 29:1-7.
23. Song Y, Zheng S, Li L, Zhang X, Zhang X, Huang Z, Chen J, Zhao H, Jie Y, Wang R, Chong Y. Deep learning Enables Accurate Diagnosis of Novel Coronavirus (COVID-19) with CT images. medRxiv. 2020 Jan 1.
24. qSARS-CoV-2 IgG/IgM Rapid Test - Letter of Authorization. [Cited 2 April 2020]. Available from: https://www.fda.gov/media/136622/download
25. Pan Y, Li X, Yang G, Fan J, Tang Y, Zhao J, Long X, Guo S, Zhao Z, Liu Y, Hu H. Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. medRxiv. 2020 Jan 1.
26. Wei Zhang, Rong-Hui Du, Bei Li, Xiao-Shuang Zheng, Xing-Lou Yang, Ben Hu, Yan-Yi Wang, Geng-Fu Xiao, Bing Yan, Zheng-Li Shi & Peng Zhou (2020) Molecular and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 April 2020 doi:10.20944/preprints202004.0155.v1

serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes, Emerging Microbes & Infections, 9:1, 386-389, DOI: 10.1080/22221751.2020.1729071
27. Cohen J, Kupferschmidt K. Labs scramble to produce new coronavirus diagnostics.
28. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. Journal of travel medicine. 2020 Mar 13.
29. Jung SM, Akhmetzhanov AR, Hayashi K, Linton NM, Yang Y, Yuan B, Kobayashi T, Kinoshita R, Nishiura H. Real-time estimation of the risk of death from novel coronavirus (COVID-19) infection: Inference using exported cases. Journal of clinical medicine. 2020 Feb;9(2):523.
30. Kim C, Ahmed JA, Eidex RB, Nyoka R, Waiboci LW, Erdman D, Tepo A, Mahamud AS, Kabura W, Nguhi M, Muthoka P. Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. PloS one. 2011;6(6):e21610-.
31. Yap JC, Ang IY, Tan SH, Jacinta I, Pei CH, Lewis RF, Qian YA, Yap RK, Ng BX, Tan HY. COVID-19 Science Report: Diagnostics.
32. Guidelines for Clinical Specimens. [Cited 2 April 2020]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html