Laboratory Detection of Covid19 Cases: A Systematic Review

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Abstract

Background: The world is struggling to contain the novel coronavirus (COVID-19) outbreak and the healthcare infrastructure and testing capacity have emerged as major issues. Adequate testing capacity for SARS-CoV-2 is lacking worldwide, preventing people from accessing care. It also means the community is relying on models and estimates to get an accurate picture of the outbreak and its evolution, even though this information is critical to inform public health measures that could stop or slow diseases transmission. Different countries have implemented different testing strategies, reflecting the availability of diagnostics and reagents and the needs of the individual health systems. **Objective:** The purpose of the present study was to see the different laboratory tests for detection of SARS Cov2 from Covid19 patients. **Methodology:** We searched electronic databases like MEDLINE, EMBASE, CINAHL, and Science Citation Index, checked documents and references, and contacted experts. We included WHO reported Corona diseases (COVID-19) situation reports from January 2020 to April 10, 2020 related to the diagnosis of COVID-19 diseases. Both reviewers independently screened titles and abstracts, assessed studies for inclusion, appraised quality, and extracted data. **Result:** Regular confirmation of COVID-19 was based on the detection of particular sequences of viral RNA by NAAT such as Polymerase chain reaction (PCR). Sensitivity was comparatively high in the first week. Serological assays are qualitative detection of IgM and IgG antibodies against SARS-CoV-2 in serum gave result within 2 to 10 minutes. The rapid antigen tests might be providing the advantage of fast time to results and low cost detection of human CoVs, however they were likely to suffer from reduced sensitivity based on the experience with this method for other respiratory viruses. **Conclusion:** Immunological assays are never goanna be better than molecular ones. May be molecular and immunological tests combined can be a good strategy. [*Bangladesh Journal of Infectious Diseases, April 2020;7(suppl_1):S11-S17*]

Keywords: COVID-19; PCR; WHO; SARS-CoV-2; antibodies; antigen; isolation of virus

Introduction

China has been shaken by several cases of COVID-19 at the end of 2019, which has now spread worldwide. Diagnostic testing for COVID-19 is critical to tracking the virus, understanding epidemiology, informing case management, and suppressing transmission. On 11-12 February 2020, WHO organized a forum[1] to identify research gaps and priorities for COVID-19, in collaboration with the GloPID-R. One of the eight immediate research needs[2] agreed upon as part of the forum was to "mobilize research on rapid point of care diagnostics for use at the community level". This was early recognition of the urgent need for access to accurate and standardized diagnostics for SARS-CoV-2 (the causative agent of COVID-19), which can be deployed in decentralized settings. An R&D Roadmap[3] for CoVID-19 was published in March 2020.This article describes the strategic use of diagnostic testing of COVID-19 outbreaks, including how testing might be rationalized when lack of reagents or testing capacity necessitates prioritization of certain populations or individuals for testing. The tests currently being used to identify coronavirus infection are known as PCR tests. PCR stands for 'polymerase chain reaction', and it's by no means a new testing method — PCR tests have been used since the 1980s and have a range of applications including the diagnosis of infectious diseases. The tests allow copying of a small amount of DNA millions of times over so that there is enough for detection and confirmation of infection.

Methodology

Types of Studies Included: We included any study related with different routes of transmission of COVID-191in a population-based or hospital-based sample. For pragmatic reasons, we also included different ways of transmission of SARS Coronavirus in poor tropical countries.

Identification and Selection of Studies: We had searched the electronic databases, MEDLINE, EMBASE, CINAHL, and Science Citation Index (up to 10 April 2020).

Data Extraction And Assessment of Methodological Quality: Authors abstracted data separately from the included studies in a predesigned proforma that included author, date of publications, country of study, study setting, population studied, type of study, source of samples, time and period of study. The proforma was pilot tested before extracting any study data following which data was abstracted separately for hospital-based and population-based studies. To discrepancies regarding the abstracted data, a consensus was made after discussion with the arbitor (MS).

Data Analysis: After data extraction, all the relevant data was entered into Microsoft excel. We combined the results from all the studies which were studied to find the most relevant and suitable diagnostic tests for COVID-19 and made an average conclusion about the presenting diagnostic tests for COVID-19.

How Current Tests Work
• A swab is taken of the inside of a patient's nose or the back of their throat. This sample is then sent to a lab to test.
• The RNA of the virus is extracted and purified. An enzyme, reverse transcriptase, converts the RNA to DNA.
• The DNA is mixed with primers, sections of DNA designed to bind to characteristic parts of the virus DNA. Repeatedly heating then cooling DNA with these primers and a DNA-building enzyme makes millions of copies of virus DNA.
• Fluorescent dye molecules bind to the virus DNA as it is copied. Binding makes them give off more light, which is used to confirm the presence of the virus in the sample.

Positive and negative tests: The fluorescence increases as more copies of the virus DNA are produced. If it crosses a certain threshold, the test is positive. If the virus isn't present, no DNA copies are made and the threshold isn't reached. In this case, the test is negative.

Issues with Testing

• **Reagent Issues:** High demand and issues with reagents have delayed testing in some countries.
• **Time-consuming:** It takes a few hours to get results from the test, limiting how many tests can be done.
• **False positives and negatives:** In some cases sample degradation or contamination can affect the results.

Future Tests: The current tests are good for diagnosing an infection – but they can't tell us if

someone has had it and then recovered. Tests that look for antibodies against the virus can do this. ANTIBODIES Produced by the immune system remain in the blood for some time after infection. Tests that look for proteins on the surface of the virus are also in development. These tests are faster, but less accurate.

Results

Twenty-eight articles, among these articles, seventeen articles were identified for inclusion depended on proper diagnostic criteria (S1 Table) in final review. Eleven articles were excluded due to alternative or unclear diagnosis, the full text could not be obtained as proper scientific article, the tests were not done on preferred sample (S2 Table). The sensitivity of the different diagnostic methods with the day following symptoms showed in Table 3.

Table 1: Current testing situation worldwide

	Molecular test	**Immunoassay**		**Non-disease specific tests**
		Antibody based	**Antigen based**	
How does it work?	Detects the presence of viral genetic material in a sample	Detects the presence of anti-viral antibodies in a sample	Detects the presence of viral proteins (antigens) in a sample	Detects signs and symptoms of disease
What technique is used?	Usually based on a technique called polymerase chain reaction (PCR), which makes millions of copies of a specific section of the viral genome, amplifying small amounts to detectable levels	Usually based on a technique called enzyme-linked immunosorbent assay (ELISA), in which molecules attach to the antibodies or antigen in the sample and produce a detectable signal		Techniques include thermal scanning to identify people with a fever (higher than normal temperature) and computed tomography (CT) chest scans to distinguish from other chest infections
Where does testing take place?	Usually performed in a laboratory due to equipment requirements	May be laboratory based or performed at point of care, depending on test design		Usually performed outside of the laboratory, in clinic or at point of care, depending on equipment needs
What is the most common use?	Testing people suspected of having COVID-19	Assessing overall infection and immunity rates in a community	Testing people suspected of having COVID-19 or screening/triage to identify candidates for further testing (depending on test design)	Screening/triage to identify candidates for further testing
A Positive result	Confirms a current SARS-CoV-2 infection	Indicates a recent or past infection, and could be used to screen for current infection (tests may not be reliable in early phase of infection)	Confirms a current SARS-CoV-2 infection or suggests a potential infection (depending on test design)	Suggests a potential infection and indicates that further testing is needed

Table 2: Supplementary Table -S1

Sample types	• Nasopharyngeal swabs are commonly taken for COVID-19 diagnostic testing • Other sample types that may be tested include: - Sputum (if you are coughing it up) - Blood - Stool and/or urine - Bronchoalveolar lavage (fluid that has been used to wash the lungs)

Testing procedure	• Samples may be taken:
	   - At home, by a visiting healthcare professional
	   - At a drive-thru centre (where a nasopharyngeal swab is taken through your car window) - At a hospital or clinic
	• Samples are then sent to a laboratory for testing
	• It will usually take around 72 hours to receive a result

SARS-CoV-2 versus other Coronaviruses	• SARS-CoV-2 is part of the coronavirus family • Molecular tests for COVID-19 are based on genetic sequences from the SARS-CoV-2 viral genome • Tests can use sequences that are unique to SARS-CoV-2 to distinguish from infections with other human coronaviruses • There are currently two known strains of SARS-CoV-2, which are thought to have different infection rates and severity of disease - Tests can use sequences common to both strains to ensure that they can both be detected
Molecular tests	• Molecular tests are diagnostics that detect viral genetic material, usually performed in a laboratory • A molecular test requires a number of basic ingredients: - The enzymes and short DNA sequences (known as primers) that copy the genetic material - The building blocks of DNA (nucleotides) - A buffer solution - The viral genetic material (if present), extracted from the sample using a separate kit • The tests are run in a machine that uses repeated cycles of heating and cooling to drive the amplification of the viral genetic material until it reaches detectable levels
'Point of care' tests	• Point of care tests are diagnostics that can be performed outside of the laboratory • They are required for wide-scale global testing - Some companies are attempting to develop 'rapid diagnostic tests', which are quick, inexpensive and easy to perform without laboratory facilities 4 Rapid diagnostic tests are often based on immunoassays - Some companies are adapting molecular tests for use in mobile laboratories
COVID-19 diagnostic test quality	• Diagnostic tests are evaluated in validation studies to ensure that they are accurate and reliable • Validation studies assess: - Sensitivity (ability to detect SARS-CoV-2 in samples known to be positive) - Specificity (ability to avoid falsely detecting SARS-CoV-2 in samples known to be negative) • FIND is conducting independent evaluations of COVID-19 molecular tests and immunoassays, in collaboration with WHO and other partners • Results from these studies will help governments and health authorities decide which tests are most suitable for use in their populations

Table 2: Supplementary Table -S2

Study ID	Diagnostic method	Reasons for inclusion
COVID-19 testing – B Bibel, The Bumbling Biochemist	All preferred methods	Filled up the purpose of this article.
Everything you need to know about coronavirus testing – M Molteni & A Rogers	All preferred methods	Filled up the purpose of this article
Real-time PCR in virology – I Mackay, K Arden, & A Nitsche	PCR	Filled up the purpose of this article
Beginner's guide to real-time PCR – Primer Design	PCR	Filled up the purpose of this article
COVID-19 information for laboratories – Centers for Disease Control & Prevention	PCR	Filled up the purpose of this article
Coronavirus testing shouldn't be this complicated – N Wetsman	All preferred methods	Filled up the purpose of this article
Shortage of RNA extraction kits hampers efforts to ramp up COVID-19 coronavirus testing – M Satyanarayana	PCR	Filled up the purpose of this article
How many tests for COVID-19 are being performed around the world? – Our World in Data	All preferred methods	Filled up the purpose of this article

Zhao, Quan Yuan, Haiyan Wang, *et al;* Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, Clinical Infectious Diseases, , ciaa344, https://doi.org/10.1093/cid/ciaa344	All preferred methods	Filled up the purpose of this article
Sample collection and Diagnosis of COVID-19 (SARS-CoV-2), Sagar Aryal, https://microbenotes.com/diagnosis-of-covid-19/	All preferred methods	Filled up the purpose of this article
Laboratory testing for coronavirus disease (COVID-19) in suspected human cases, interim guidance. March 19. World Health Organization. 2020. https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117	All preferred methods	Filled up the purpose of this article
Diagnosis of Novel Coronavirus, SARS-CoV-2 (COVID-19), Sushmita Soni https://thebiologynotes.com/diagnosis-of-novel-coronavirus-2019-ncov/	All preferred methods	Filled up the purpose of this article
What test methods to doctors use to detect and diagnose COVID-19 (SARS-CoV-2)?, https://www.medicinenet.com/how_do_the_covid-19_coronavirus_tests_work/article.htm	All preferred methods	Filled up the purpose of this article
Serology-based tests for COVID-19, JOHNS HOPKINS CENTER FOR HEALTH SECURITY http://www.centerforhealthsecurity.org/resources/COVID-19/Serology-based-tests-for-COVID-19.html	Serology-based tests	Filled up the purpose of this article
World Health Organization. (2020). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. World Health Organization. https://apps.who.int/iris/handle/10665/331329	All preferred methods	Filled up the purpose of this article
A coordinated global research roadmap: 2019 novel coronavirus	All preferred methods	Filled up the purpose of this article
COVID-19 diagnosis: Abbott RealTime SARS-CoV-2 assay, April 10, 2020 by Faith Mokobi Zablon, https://thebiologynotes.com/abbott-realtime-sars-cov-2-assay/	PCR	Filled up the purpose of this article

Table 2: Supplementary Table -S2

Study ID	Reasons for exclusion
World experts and funders set priorities for COVID-19 research- WHO News release, 12 February 2020	Not a scientific article
Guo Y, Cae Q, Hong Z, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. Millitary Medical Research. (2020) 7:11. https://doi.org/10.1186/s40779-020-00240-0	Unclear diagnosis
Adhikari et al. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. Infectious Diseases of Poverty. (2020) 9:29. https://doi.org/10.1186/s40249-020-00646-x	Unclear diagnosis
Abbott Launches Molecular Point-of-Care Test to Detect Novel Coronavirus in as Little as Five Minutes, https://abbott.mediaroom.com/2020-03-27-Abbott-Launches-Molecular-Point-of-Care-Test-to-Detect-Novel-Coronavirus-in-as-Little-as-Five-Minutes	Not a scientific article
FDA Emergency Use Authorisation: Abbott Real-Time SARS-CoV-2 Assay, https://www.fda.gov/media/136255/download	The full text could not be obtained
COVID 2019 PHEIC: Global research and innovation forum: towards a research roadmap	Not a scientific article
COVID-19 IgG/IgM Rapid Test, http://mybodysphere.com/sars2covid19.html	Not a scientific article
COVID-19 "Coronavirus" IgG/IgM Rapid Test Cassette, https://www.confirmbiosciences.com/coronavirus-test	Nuclear diagnosis,

FDA Emergency Use Authorizations, https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations	Not a scientific article
Abbott realtime SARS-CoV-2 assay, https://www.molecular.abbott/us/en/products/infectious-disease/RealTime-SARS-CoV-2-Assay	Not a scientific article

Table 3: Sensitivity of the Present Diagnostic Tests

SARS-CoV-2 test	Days after symptom onset		
	1-7	8-14	15-39
RNA by RT-PCR	67%	54%	45%
Total Antibody	38%	90%	100%
IgM	29%	73%	94%
IgG	19%	54%	80%

The reason for the negative antibody findings in patients might due to the lack of blood samples at the later stage of illness. The median sero-conversion time for Ab, IgM and then IgG were day-11, day-12 and day-14, separately. The presence of antibodies was among patients within 1-week since onset, and rapidly increased to IgM and IgG since day-15 after onset. In contrast, RNA detectability decreased in samples collected before day-7 during day 15-39. Combining RNA and antibody detections significantly improved the sensitivity of pathogenic diagnosis for COVID-19 ($p<0.001$), even in early phase of 1-week since onset ($p=0.007$). Moreover, a higher titer of Ab was independently associated with a worse clinical classification ($p=0.006$)[12].

Discussion

Virus isolation is not recommended as routine diagnostic procedure due to lack of permissive cell lines, time to results, labour and expertise requirements, and the lack of commercial antisera for culture confirmation. When rapid antigen testing and/or molecular assays are neither availed nor stable, serology can be used as an additional diagnostic tool and can aid investigation of an ongoing outbreak and retrospective assessment of the attack rate or extent of outbreak**.**

Though they may sound complex, PCR tests are a pretty reliable way of testing for infectious diseases, which is why they are used widely to test for COVID-19. However, some countries have run into issues when trying to scale up their testing capabilities.

One reason for this is simply the fact that the test takes time. It can take a few hours to get results; couple this with lab testing capacities and it places a limit on how many tests a single lab can carry out in a day. A small research lab might be able to run around 80 tests a day; much larger labs with multiple machines may be able to run between 1000 to 2000. Another limitation is the availability of reagents needed to run the tests. We mentioned previously that some companies sell RNA extraction kits for this step of the process. Global demand for these tests in the wake of the pandemic has led to a shortage, limiting the number of tests carried out.

In some cases, the tests haven't functioned as intended. The tests originally provided for diagnosis in the USA contained different sets of primers for different purposes. This included one which targeted a genetic sequence found in all coronaviruses as a form of control test. This part of the test didn't function correctly; while it didn't stop them from being usable, it caused confusion as to whether results were positive or not, slowing diagnosis.

Contamination or degradation can also cause issues. These can lead to false positives (when someone doesn't have the virus but the test says they do) or false negatives (when someone does have the virus but the test says they don't). A final big limitation of this type of testing is that it can only tell you if someone has the virus at the time of testing. It can't tell us whether they've had the virus but have subsequently recovered before testing. This is pretty useful to know, as if someone's had the virus and recovered, they'll be immune to catching it again (at least for a while).

A type of test that can tell us whether someone has previously had the virus is an antibody-based test. Your body produces antibodies to fight off infections. These antibodies remain in your blood for some time after infection, and tests can detect them. Currently, some companies are working on antibody tests for the SARS-CoV-2 virus, and it's expected that they'll be rolled out quickly once they're available.

There are other tests which could prove useful too. Another type looks for specific proteins on the surface of viruses. They are faster than the PCR testing, but also less sensitive, so there's more chance of an inaccurate result. The World Health

Organization has stressed the importance of testing as part of the worldwide response to the COVID-19 pandemic. There's significant variability in the number of tests carried out in different countries. China and South Korea have both run around 300,000 tests, whereas the United Kingdom and the United States have only run 50,000 and 40,000 respectively. Less testing means it's harder to track the spread of the infection and isolate and track contacts of the infected.

Until testing capacity increases and more types of test become available, countries are advising their citizens to self-isolate if they think they may have symptoms of the virus. Some may only know for sure if they've had the virus once antibody tests are available but following this advice and that on social distancing is the best way to prevent its spread.

Conclusion

Detection and monitoring of confirmed cases of COVID-19 is crying need. Adequate testing capacity for SARS-CoV-2 is lacking worldwide, preventing people from accessing care. When rapid antigen testing and/or molecular assays are neither availed nor stable, serology can be used as an additional diagnostic tool and can aid investigation of an ongoing outbreak and retrospective assessment of the attack rate or extent of outbreak.

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