IMPORTANCE OF ANTIGEN DETECTION FOR THE SCREENING OF COVID-19

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A dissertation submitted to the Department of Mathematic and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Biotechnology

Department of Mathematic and Natural Sciences Brac University
October 2020

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I/We have acknowledged all main sources of help.

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Approval

The thesis/project titled “Importance of antigen detection for the screening of COVID-19”by Suraiya Khan (16136022) of Summer,2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology on [***Date-of-Defense].

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Ethics Statement

No humans or animals were harmed during the making of this study.

Abstract

Since the beginning of the COVID-19 pandemic, laboratories have been using nucleic acid amplification tests, such as real time reverse transcription polymerase chain reaction (rRT-PCR) assays, to detect SARS-CoV-2, the virus that causes the disease. In many countries, access to this form of testing has been challenging. A new technology for COVID-19 detection has become available that is much simpler and faster to perform that currently-recommended nucleic acid amplification tests (NAAT), like PCR. This method relies on direct detection of SARS-CoV-2 viral proteins in nasal swabs and other respiratory secretions using a lateral flow immunoassay (also called an RDT) that gives results in < 30 minutes. Though these antigen detection RDTs (Ag-RDTs) are substantially less sensitive than NAAT, they offer the possibility of rapid, inexpensive and early detection of the most infectious COVID cases in appropriate settings.

Dedication

Dedicated to my mother for always supporting and believing in me

Acknowledgement

First and foremost, I am beholden to the Almighty Allah for blessing me with the good health and knowledge for completing by review paper for my undergraduate study. I have received help from many individual during completing this review paper and I wish to thank them with gratitude and nevertheless my parents, who have always supported me.

It is my radiant sentiment to place on record my best regards and gratitude to **Professor A F M Yusuf Haider, Ph.D.,** Chairperson, Department of Mathematics and Natural Sciences, BRAC University, for his cooperation and encouragement in conducting this study.

This study was completed under the thorough guidance of my Research Supervisor **Dr. Iftekhar Bin Naser,** Assistant Professor, Coordinator of Biotechnology Program, Department of Mathematics and Natural Sciences, BRAC University. He added new directions to my thinking, and acted as a continual stimulus for refinement and depth in the presentation of this work. I am much indebted and would like to express my sincere gratitude to him for his expert guidance and constant support throughout the entire period of my research work.

I convey my deepest acknowledgment and special thanks to the respective **Dr. Firdausi Qadri** (Chairperson) and **Dr. Md. Kaiissar Mannoor** (Senior scientist and Head of ideSHi) for guiding me and allowing me to work at the esteemed organization.

I am obliged to **Mohabbat Hossain** (Research officer of ideShi), who open heartedly helped me to utilize all the required machinery and vital materials to complete the review paper.

I am also indebted to my friends and family who both supported me emotionally with patience to get through the writing procedure. I am also indebted to my faculties and friends as well for helping me intellectually during the planning and writing of my review paper.

Suraiya Khan

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List of Acronyms

SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
Covid-19	Coronavirus Disease of 2019
RT-PCR	Reverse transcription polymerase chain reaction
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
LAMP	Loop Mediated Isothermal Amplification
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
ELISA	Enzyme Linked Immunosorbent Assay
CLIA	Chemiluminescence Immunoassays
LFA	Lateral Flow Assay
IgM	Immunoglobulin M
IgG	Immunoglobulin G
RDT	Rapid Diagnostic Test
HIV	Human Immunodeficiency Virus
NAAT	Nucleic Acid Amplification Technology
NPV	Negative Predictive Value
PPV	Positive Predictive Value
FDA	Food and Drug Administration
EUA	Emergency Use Authorization
EUL	Emergency Use Listing
POCT	Point of Care Testing
NP	Nasopharyngeal
OP	Oropharyngeal
Gavi	Global Alliance for Vaccines and Immunizations
PAHO	Pan American Health Organization
CEPI	Coalition for Epidemic Preparedness Innovations
UNICEF	United Nations Children's Fund

1.1 Introduction

Diagnostic testing plays a crucial role and this pandemic is no exception. Because early clinical presentations of infected patients are non-specific, testing is needed to confirm the diagnosis of COVID-19 in symptomatic patients, as soon as possible, so that these patients can be appropriately isolated and clinically managed. It is also needed for individuals who have come into contact with someone with confirmed COVID-19. Some testing strategies examine only contacts who have symptoms or develop illness of any kind during the 14-day period after contact. Other strategies examine all contacts when identified, regardless of whether they have any symptoms. Studies have shown that a large number of infected individuals might have no symptoms at all, and there is concern that these individuals are still able to shed the virus and transmit infection through saliva droplets as they speak. Diagnostic tests for covid-19 fall into different categories: molecular tests that detect viral RNA, serological tests that detect anti-SARS-CoV-2 immunoglobulins and rapid antigen test. [1]

1.2 Molecular Test (RT- PCR)

Patients may be tested for infection when they present with symptoms, or have had known exposure to COVID-19, or during screening for COVID-19. The standard approach to diagnosis of COVID-19 infection is through laboratory-based testing of swab samples taken from the upper respiratory (e.g. nasopharynx, oropharynx) or lower respiratory tract (e.g. Broncho alveolar lavage or sputum) with RT-PCR. RT-PCR is the primary method for detecting infection during the acute phase of the illness while the virus is still present (whether people are symptomatic or asymptomatic), but can give false negative results. Both the World Health Organization (WHO) and the China CDC (National Health Commission of the People's Republic of China), have produced case definitions for COVID-19 that include the presence of convincing clinical evidence when RT-PCR is negative. The most recent case definition from the China CDC also includes positive serology tests. [2]

RT-PCR tests for SARS-CoV-2 identify viral ribonucleic acid (RNA). Reagents for RT-PCR were rapidly produced once the viral RNA sequence was published. Testing is undertaken in central laboratories and can be very labor-intensive, with several points along the path of performing a single test where errors may occur, although some automation of parts of the process is possible. The amplification process requires thermal cycling equipment to allow multiple temperature changes within a cycle, with cycles repeated up to 40 times until viral DNA is detected. Although the amplification process for RT-PCR can be completed in a relatively short timeframe, the stages of extraction, sample processing and data management (including reporting) mean that test results are typically only available in 24 to 48 hours. Where testing is undertaken in a centralized laboratory, transport times increase this further. The time to result for fully automated RT-PCR assays is shorter than for manual RT-PCR, however most assays still require sample preparation steps that make them unsuitable for use at the point of care. Other nucleic acid amplification methods, including loop-mediated isothermal amplification (LAMP), or CRISPR-based nucleic acid detection methods, that allow amplification at a constant temperature are also being developed. These methods have the potential to reduce the time to produce test results and extraction and sample processing to minutes, but the time for the whole process may still be significant. Laboratory-based molecular tests are most often applied to upper and lower respiratory samples although they are also being used on fecal and urine samples. [2]

1.3 Serological tests (ELISA, LFA and CLIA)

Serology tests to measure antibodies to SARS-CoV-2 have been evaluated in people with active infection and in convalescent cases. Antibodies are formed by the body's immune system in response to infections, and can be detected in whole blood, plasma or serum. Antibody tests are available for laboratory use including enzyme- linked immunosorbent assay (ELISA) methods, or more advanced chemiluminescence immunoassays (CLIA).There are also rapid lateral flow assays (LFA)s for antibody testing that use a minimal amount of whole blood, plasma or serum on a testing strip as opposed to the respiratory specimens that are used for rapid antigen tests.[2] Serological tests have generated substantial interest as an alternative or complement to RT-PCR in the diagnosis of acute infection, as some might be cheaper and easier to implement at the point of care.

A clear advantage of these tests over RT-PCR is that they can identify individuals previously infected by SARS-CoV-2, even if they never underwent testing while acutely ill. As such, serological tests could be deployed as surveillance tools to better understand the epidemiology of SARS-CoV-2 and potentially inform individual risk of future disease. [3]

It has been found that sensitivities were consistently lower with the LFIA method compared with ELISA and CLIA methods. For each test method, the type of immunoglobulin being measured—IgM, IgG, or both—was not associated with diagnostic accuracy. Pooled sensitivities were lower with commercial kits and in the first and second week after symptom onset compared with the third week or later. Pooled specificities of each test method were high. However, stratified results suggested specificity was lower in individuals with suspected covid-19, and that other viral infections could lead to false positive results for the LFIA method. These observations indicate important weaknesses in the evidence on covid-19 serological tests, particularly those being marketed as point-of-care tests. [3]

The use of serology tests for population surveys is not recommended in low prevalence settings as this approach will probably result in more false-positive than true-positive results, even if a test with high specificity is used. For example, if the prevalence of infection is 1% in the general population, a test with 98% specificity will identify two false-positive results for every true positive result. These results could lead to a false sense of security regarding the extent of immunity in the population and premature easing of public health measures on the basis of misleading disease estimates [1].

Patients at an early stage in the disease course, or asymptomatic or paucisymptomatic patients, might have low antibody concentrations that could give false-negative results. Patients' disease stage and severity are important points to consider, along with the population being tested. The estimated level of risk can be considered before using a serology test, because of the changing false-positive rate or low positive predictive value across different populations. We suggest countries consider risk levels before using serology tests and creating public health guidance. Scaling up testing, particularly at the community level, allows for better estimates of risks, which in turn allows more effective public health measures to be put into place than would be otherwise.[1]

1.4 Rapid antigen detection test (RDT)

Antigen-detection diagnostic tests are designed to directly detect SARS-CoV-2 proteins produced by replicating virus in respiratory secretions and have been developed as both laboratory-based tests, and for near-patient use, so-called rapid diagnostic tests, or RDTs. The diagnostic development landscape is dynamic, with nearly a hundred companies developing or manufacturing rapid tests for SARS-CoV-2 antigen detection .

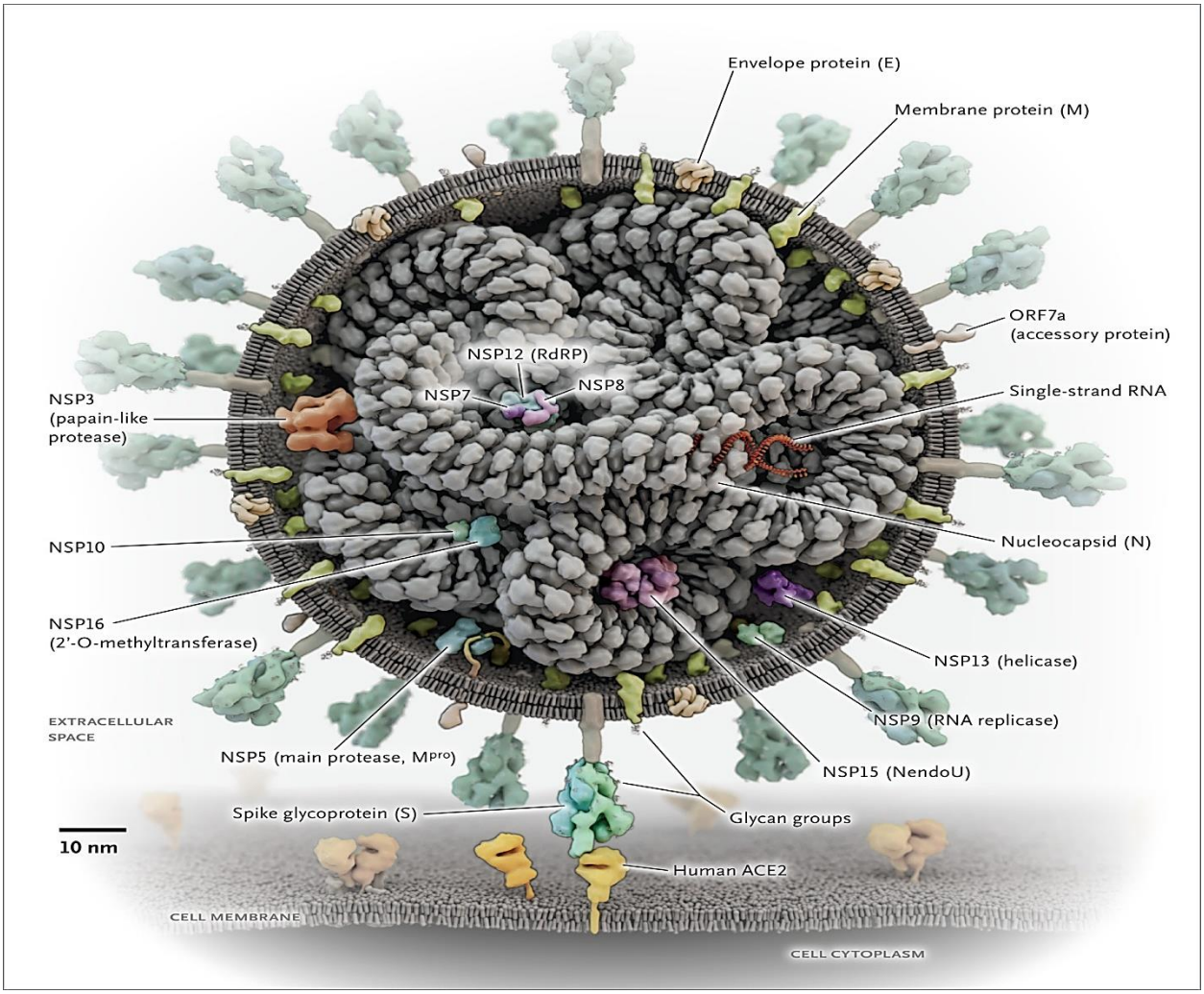


Figure 1.1 : The SARS-Cov-2 Virion and its proteins (Source: https://www.nejm.org/doi/full/10.1056/NEJMcibr2007042)

Most Ag-RDTs for COVID-19 use a sandwich immunodetection method employing a simple-to-use lateral flow test format commonly employed for HIV, malaria and influenza testing. Ag-RDTs are usually comprised of a plastic cassette with sample and buffer wells, a nitrocellulose matrix strip, with a test line with bound antibody specific for conjugated target antigen-antibody complexes and a control line with bound antibody specific for conjugated-antibody. In the case of

SARS-CoV-2 RDTs the target analyte is often the virus' nucleocapsid protein, preferred because of its relative abundance. Typically, all materials that are required to perform the test, including sample collection materials, are provided in the commercial kit, with the exception of a timer. [4]

After collecting the respiratory specimen and applying it to the test strip, results are read by the operator within 10 to 30 minutes with or without the aid of a reader instrument. Most of the currently manufactured tests require nasal or nasopharyngeal swab samples, but companies are carrying out studies to assess the performance of their tests using alternative sample types such as saliva, oral fluid and sample collection systems to potentially expand options for use and to facilitate safe and efficient testing. [4]

1.4.1 Test performance of RDT

The performance of an Ag-RDT is determined by the sensitivity and specificity of the test to detect a SARS-CoV-2 infection compared with a reference standard, NAAT (generally rRT-PCR). Specificity and sensitivity are two fundamental requirements for an effective diagnosis. It is also important that the testing method is user friendly. [5]

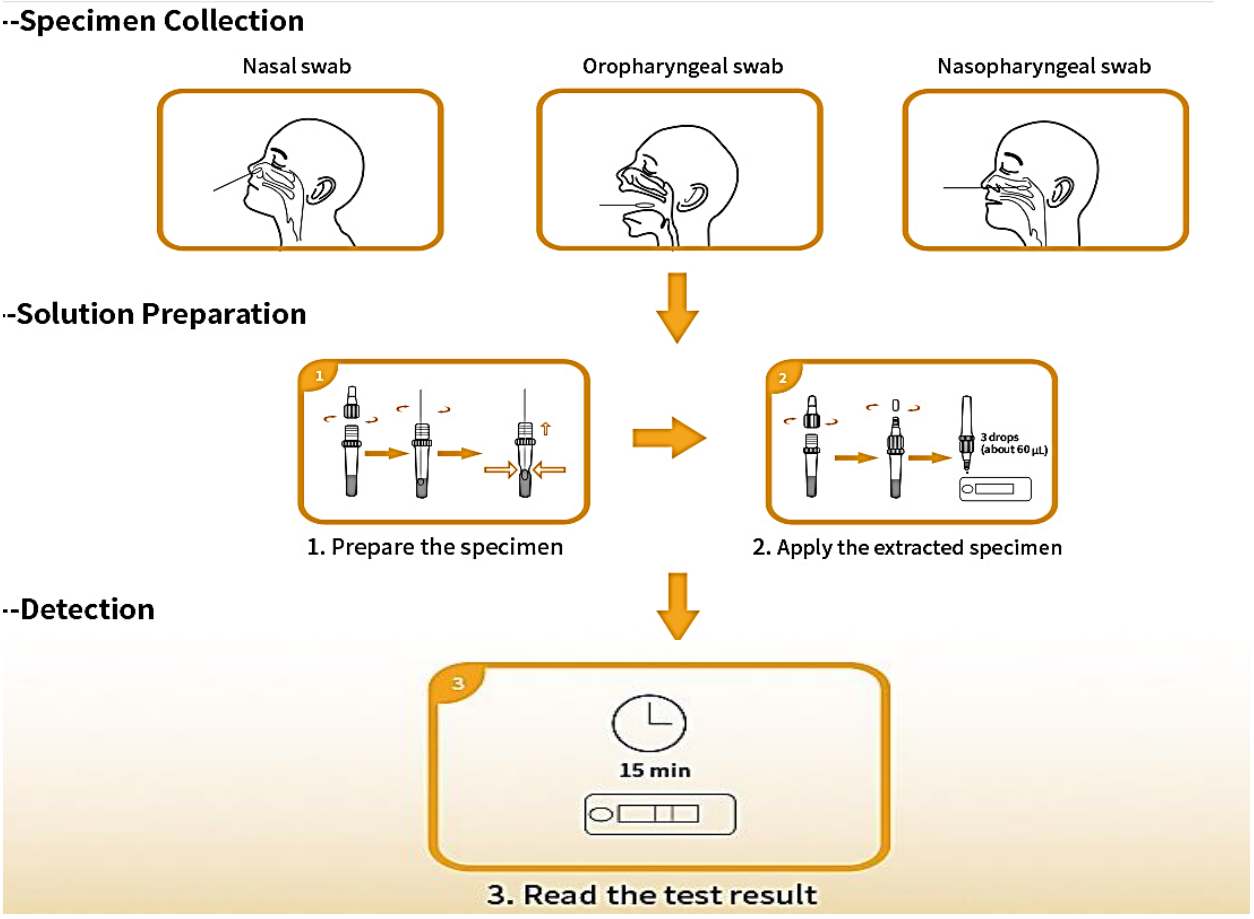
Sensitivity - is the percentage of cases positive by a NAAT reference standard that are detected as positive by the Ag-RDT under evaluation.

Specificity- is the percentage of cases negative by a NAAT reference standard that are detected as negative by the Ag-RDT under evaluation. [4]

Data on the sensitivity and specificity of currently available Ag-RDTs for SARS-CoV-2 have been derived from studies that vary in design and in the test brands being evaluated. They have shown that sensitivity compared to NAAT in samples from upper respiratory tract (nasal or nasopharyngeal swabs) appears to be highly variable, ranging from 0-94% but specificity is consistently reported to be high (>97%).

Although more evidence is needed on real-world performance and operational aspects, Ag-RDTs are most likely to perform well in patients with high viral loads (Ct values ≤25 or >106 genomic virus copies/mL) which usually appear in the pre-symptomatic (1-3 days before symptom onset) and early symptomatic phases of the illness (within the first 5-7 days of illness) (12). This offers the opportunity for early diagnosis and interruption of transmission through targeted isolation and cohorting of the most infectious cases and their close contacts.

Patients who present more than 5-7 days after the onset of symptoms are more likely to have lower viral loads, and the likelihood of false negative results with Ag-RDTs is higher. Despite these expected limitations in performance, if correctly performed and interpreted, Ag-RDTs could play a significant role in guiding patient management, public health decision making and in surveillance of COVID-19. At minimum, Ag-RDTs would need to correctly identify significantly more cases than they would miss (sensitivity ≥80%) and have very high specificity (≥97-100%). Based on these performance parameters, this interim guidance proposes several potential roles for Ag-RDT and offers general recommendations for selection of tests and key considerations for their implementation. [4]



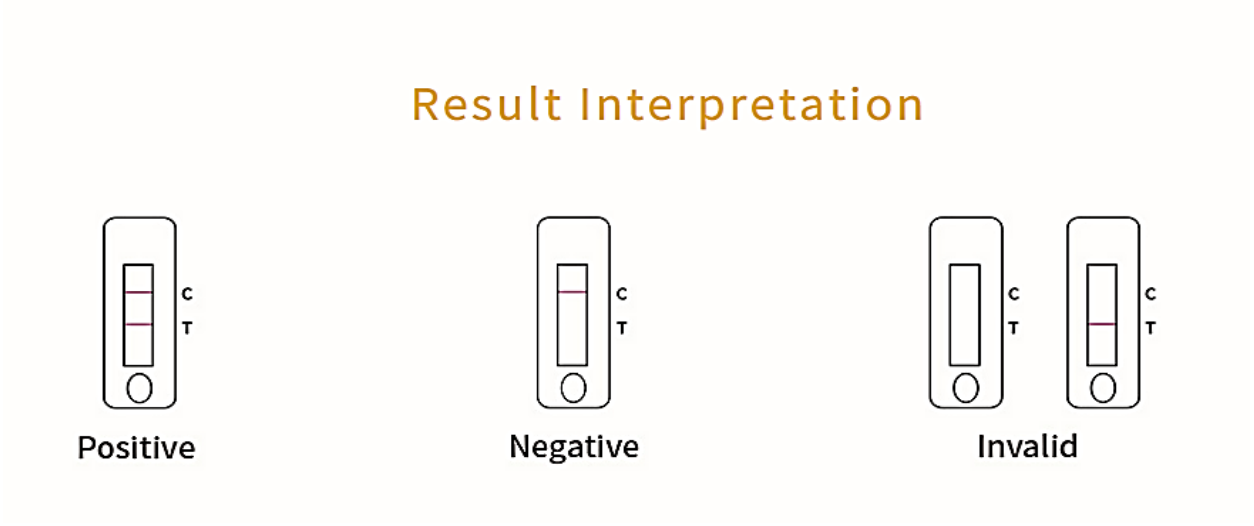
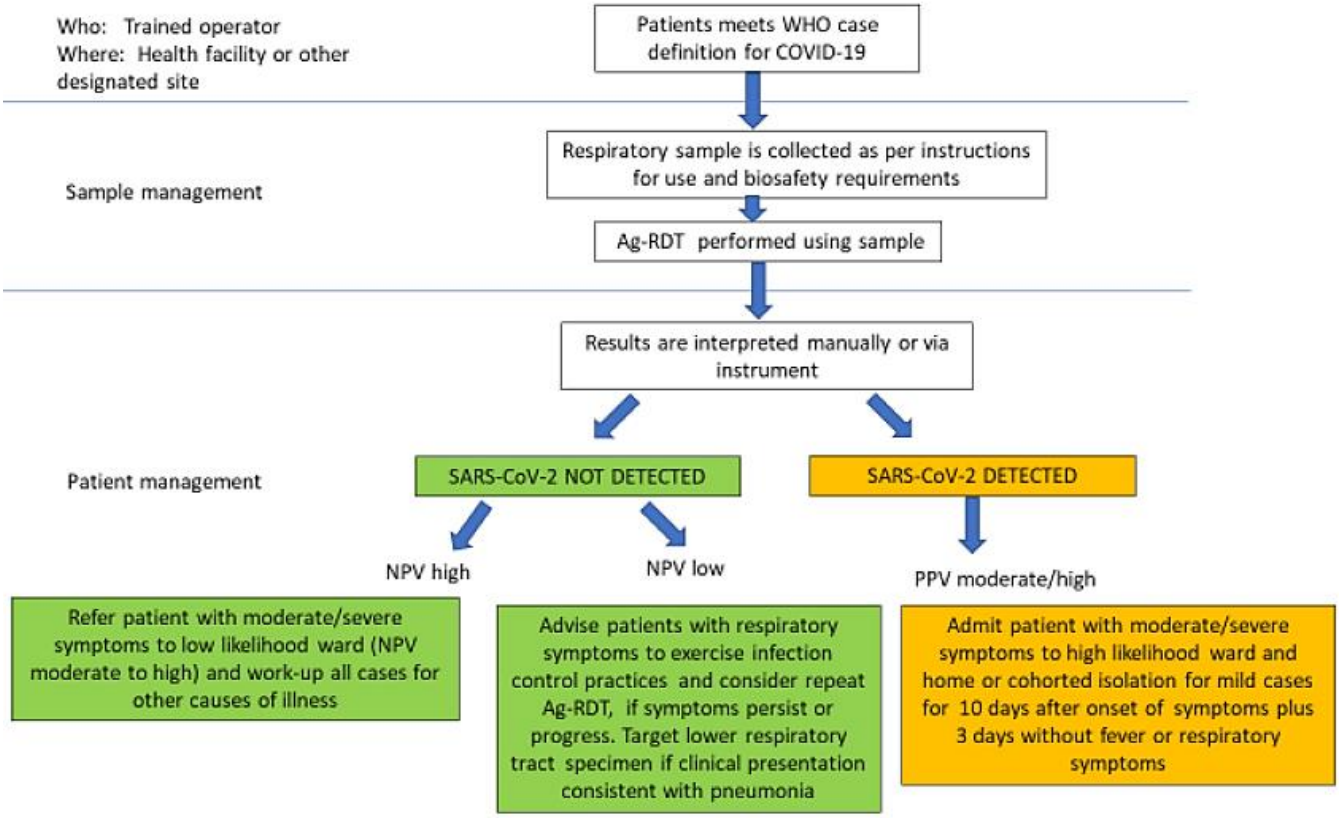


Figure 1.2 Steps for Ag detection test ***(Source: https://www.vivachek.com/en/prods/sarscov2-agrapidtest.html?gclid=EAIaIQobChMIgoij9fTy6wIVRCQrCh33WwUwEAAYASAAEgI9ZvD_BwE)***



NPV- negative predictive value; PPV – positive predictive value

Figure 1.3: Flowchart demonstrating the potential use of antigen-based RDTs (that meet minimum performance criteria) in settings of widespread community transmission and where there is no NAAT capacity. ***(Source: https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays)***

1.4.2 Factors influencing test performance

Many factors may affect the performance of antigen-detecting RDTs. Consequently, findings in clinical settings may be variable. The following should be taken into account:

• **Patient factors:** such as the time from illness onset and immune status sample type (upper or lower respiratory tract), quality and processing, including storage conditions and dilution in viral transport medium
• **Viral factors:** including the concentration and duration of viral antigen shedding and structural variation in the target antigen, cross reactivity with other viruses
• **Specific protein target**: as some antigens are produced in higher concentrations than others, e.g. nucleocapsid versus spike proteins
• **Product design or quality issues including**:
- Insufficient antibody quantity or affinity for the target antigen(s)

- Poor packaging and exposure to heat and humidity during improper transport and/or storage, which can degrade antibodies in the test
- Unclear or incorrect instructions that can affect test performance

• **Inadequate training or competency of the test operator**: which may lead to error in preparing the antigen-detecting RDT, performing the test or interpreting the result, with erroneous conclusions. [4]

1.4 Discussion

The rapidly emerging SARS-CoV-2 pandemic is causing tremendous public health challenges worldwide .Timely detection and isolation of cases and their contacts are considered crucial to help curtail this unprecedented pandemic. This strategy relies on robust, rapid, and easy-to-perform diagnostic tools that can be used to test large numbers of samples in a short period of time.

Generally, the ease-of-use and rapid turnaround time of Ag-RDTs offers the potential to expand access to testing and decrease delays in diagnosis by shifting to decentralize testing of patients with early symptoms. The trade-off for simplicity of operation of Ag-RDTs is a decrease in sensitivity compared to NAAT. Very few of the SARS-CoV-2 Ag-RDTs have undergone stringent regulatory review. Only four tests have received United States Food and Drug Administration (FDA) Emergency Use Authorization (EUA), and another two tests have been approved by Japan's Pharmaceutical and Medical Devices Agency. Only three companies have submitted documents toward WHO's Emergency Use Listing (EUL) procedure [4]

To date, the recommended diagnostic method for SARS-CoV-2 infection (known as Covid-19) is real-time reverse-transcription polymerase chain reaction (RT-PCR), which was introduced in January 2020, and is now applied using WHO or CDC protocols as well as various commercial assays. The enormous gap between the large number of patients/contacts and the laboratory capacities to perform RT-PCR in a timely manner is a mayor limitation of current public health containment strategies. RNA detection is the most frequently used method for the identification of COVID-19 patients because this method is extremely sensitive due to the power of nucleic acid amplification and also highly specific by using complementary nucleic acid probe/primer for the identification of a particular RNA. The major drawback in such an emergency situation is probably the requirement of well-trained personnel and lengthy testing time (usually 3–4 h) to run the test. [6]

Other options include serological tests, but due to their diagnostic limitations in early infections, these tests are currently not recommended for case detection. Serology testing targeting on viral-induced antibodies are given different information as those for viral RNA and proteins from SARS-CoV-2. Although the protein testing method is similar, the targets are not part of the virus and the testing specimens used can be quite different as compared to those for viral protein detection.Therefore, there is a critical demand for alternative assays such as antigen detection tests, which, in contrast to antibody tests, can detect the presence of the virus itself in respiratory samples. Tests detecting SARS-CoV-2-specific antigen have recently been developed and many of them are now commercially available. However, the real-world performance of these assays is uncertain and their validation is therefore of high priority.[5]

Among possible test formats, rapid diagnostic tests (RDTs) should be prioritized, since they are timely, easy to perform, and can serve as point-of-care testing (POCT)

Any rapid antigen diagnosis of SARS-CoV-2 meeting the following criteria:
• Portable or mains-powered device
• Minimal sample preparation requirements
• Minimal biosafety requirements
• No requirement for a temperature-controlled environment
• Test results available within 2 hours of sample collection [2]

One study evaluated novel antigen-based RDT for the detection of SARS-CoV-2 in respiratory specimens from suspected Covid-19 cases. The fluorescence immunochromatographic SARS-CoV-2 antigen test was evaluated using universal transport medium with nasopharyngeal (NP) and oropharyngeal (OP) swabs from suspected Covid-19 cases. Diagnostic accuracy was determined in comparison to SARS-CoV-2 real time (RT)-PCR. In this study total 127 samples were included; 82 were RT-PCR positive. Median patients' age was 38 years, 53.5% were male, and 93.7% were from the first week after symptom onset. Overall sensitivity and specificity were 93.9% and 100%, respectively, with a diagnostic accuracy of 96.1%. Sensitivity was significantly higher in samples with high viral loads [6]

Table 1.1- Sensitivity and specificity of antigen detection test in total and in different 254 subgroups of samples (*Source : Lorena Porte, Paulette Legarraga, Valeska Vollrath, Ximena Aguilera, José M Munita, Rafael Araos, Gabriel Pizarro, Pablo Vial, Mirentxu Iruretagoyena, Sabine Dittrich, and Thomas Weitzel - 2020)*

Samples		RT-PCR		Antigen Detection Test			
				Positive n	Negative n	Sensitivity N%	Specificity %
All		Positive	82	77	5	93.9	100%
		Negative	45	0	45		
Gender	Male	Positive	44	43	1	97.7	100%
		Negative	24	0	24		
	Female	Positive	38	34	4	89.5	100%
		Negative	21	0	21		
Days post symptom onset	0-7	Positive	76	72	4	94.7	100%
		Negative	42	0	42		
	8-12	Positive	5	4	1	80	100%
		Negative	3	0	3		

The evaluated RDT showed a high sensitivity and specificity in samples mainly obtained during the first week of symptoms and with high viral loads, despite the use of a non-validated sample material. The assay has the potential to become an important tool for early diagnosis of SARS CoV-2, particularly in situations with limited access to molecular methods. The assay was easy to use and provided results in a timely manner. [6]

Table 1.2: Situations where SARS-CoV-2 Ag-RDTs should not be used (Source: World Health Organization, 2020)

Do not use SARS-CoV-2 Ag-RDTS:	Explanation
In individuals without symptoms unless the person is a contact of a confirmed case	Pre-test probability (the likelihood, before testing, that the patient has the disease based on epidemiology, case contact, clinical findings) is low.
Where there are zero or only sporadic cases	Ag-RDTs are not recommended for routine surveillance purposes or case management in this setting. Positive test results would likely be false positives. Molecular testing is preferred
Appropriate biosafety and infection prevention and control measures (IPC) are lacking	To safeguard health workers, respiratory sample collection for any test from patients with suspected COVID-19 requires that operators wear gloves, gown, mask and face shield or goggles.
Management of the patient does not change based on the result of the test	If test-positive and test-negative patients will be treated the same way because of unknown or low PPV and/or NPV, then there is no benefit to testing
For airport or border screening at points of entry	Prevalence of COVID-19 will be highly variable among travelers, and it is therefore not possible to determine PPV and NPV of test results. Positive and negative tests would require confirmatory testing to increase PPV and NPV for decision making.
In screening prior to blood donation	A positive RDT result would not necessarily correlate with presence of viremia. Asymptomatic blood donors do not meet the definition of a suspect case [4].

However, the availability of established diagnostic technologies have enabled researchers to plug-and-play in the design of COVID-19 diagnostics. Such technologies took decades to optimize, but they are now playing an important role in identifying and managing the spread of COVID-19. Lessons learned from the 2002 SARS outbreak have guided the development of COVID-19 identification and detection. Transmission electron microscopy was used to identify the morphology of the virus, genome sequencing was used to confirm the identity of the virus, and sequence data were used to help design PCR primers and probes. SARS-CoV took 5 months to be identified. The same techniques were used to identify SARS-CoV-2 in only 3 weeks [7].

1.5 Conclusion

WHO is working closely with different groups and evaluating the performance and operational characteristics of commercialized SARS-CoV-2 antigen detecting RDTs to systematically compile the evidence as it emerges and coordinate updates. Besides performing these diagnostic tests ; as a developing country, Bangladesh should also make stronger efforts to have a vaccine with the help of Global Alliance for Vaccines and Immunizations (Gavi), UNICEF, WHO, Pan American Health Organization (PAHO), the Coalition for Epidemic Preparedness Innovations (CEPI), PAHO, World Bank, the Bill and Melinda Gates Foundation, and others. China, India, the UK, the USA and Russia are now the most potential vaccine-producing countries. It is significant to keep in touch with them all. Besides, the government needs to have constant contact with international bodies to have promised vaccine doses from them on the principle of equitable distribution.

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