Rapid Antigen Detection Test for Severe Acute Respiratory Syndrome Coronavirus 2: How to Use It Properly?

Tonny Loho[1], Djoko Widodo[2]

[1] Department of Clinical Pathology, Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana, UKRIDA, Jakarta.
[2] Division of Tropical and Infectious Diseases, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta

Corresponding Author:
Tonny Loho, MD., PhD., DMM. Department of Clinical Pathology, Faculty of Medicine, Universitas Kristen Krida Wacana. Jl. Arjuna Utara no. 6, Jakarta 11470, Indonesia. email: tonnyloho@yahoo.com.

ABSTRAK

Angka kejadian kasus Coronavirus 2019 (COVID-19) di Indonesia masih meningkat dan bahkan sangat meningkat pada akhir-akhir ini. Pelacakan kontak, dan surveilens merupakan hal yang penting untuk menemukan kasus di komunitas, termasuk individu tanpa gejala. Diagnosis COVID-19 tergantung pada terdeteksinya RNA virus, antigen virus atau antibodi terhadap virus. Diagnosis molekular menggunakan real time, reverse transcriptase polymerase chain reaction (RT-PCR) merupakan metoda standar/baku emas pada umumnya, tetapi metoda ini belum tersedia secara merata di seluruh Indonesia dan membutuhkan standar laboratorium yang tinggi. Tes cepat antibodi telah banyak digunakan sebagai cara alternatif; tetapi interpretasi hasilnya tidak sederhana sehingga saat ini tidak lagi digunakan untuk skrining penumpang yang akan bepergian keluar kota. Tes cepat antigen (selain PCR) kemudian ditetapkan oleh pemerintah, sebagai salah satu pilihan untuk skrining orang yang akan bepergian melalui darat, laut dan udara. Akibatnya banyak masyarakat yang membeli secara 'online' kit tes cepat antigen dan melakukan tes sendiri di rumah. Tindakan ini menimbulkan pertanyaan seberapa jauh keamanan dan akurasi dalam melakukan tes tersebut. Sebelum suatu tes digunakan sebaiknya ada kajian mengenai sensitivitas dan spesifisitas dibandingkan dengan baku emas dan keterbatasannya. Dalam makalah ini akan dibahas mengenai jenis tes yang tersedia untuk diagnosis laboratorium severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) dengan berbagai keterbatasannya dengan penekanan pada tes cepat antigen dan rekomendasi penggunaan yang tepat dalam praktek sehari-hari.

Kata kunci: *coronavirus disease 2019, tes cepat antigen, severe acute respiratory syndrome coronavirus -2*

ABSTRACT

Cases of coronavirus disease 2019 (COVID-19) in Indonesia are still increasing and even higher in the last few weeks. Contact tracing and surveillance are important to locate cases in the community, including asymptomatic individuals. Diagnosis of COVID-19 depends on the detection of viral RNA, viral antigen, or indirectly, viral antibodies. Molecular diagnosis, using real time, reverse transcriptase polymerase chain reaction (RT-PCR), is the common standard method; however, it is not widely available in Indonesia and requires a high standard laboratory. Rapid, point-of-care antibody testing has been widely used as an alternative; however, interpretation of the results is not simple and now it is no longer used by the Indonesian government as a screening test for people travelling between locations. Thus, the rapid antigen detection test (Ag-RDT) is used by the Indonesian government as a screening test for travellers. As a result, many people buy the kit online and perform self-Ag-RDT at home. This raises the question of how safe and accurate it is to perform self-Ag-

RDT at home. Before a test is applied, it is suggested to research its sensitivity and specificity, as compared to gold standard, and its limitations. In this article, laboratory diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is discussed, with an emphasis on Ag-RDT and the recommendation to use it properly in daily practice.

Keywords: *SARS-CoV-2, COVID-19, antigen testing.*

INTRODUCTION

Cases of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) are still increasing in Indonesia and reached an alarming level, with a positivity rate approached in 30% of the tested people. Contact tracing and active case finding are important strategies to establish and locate asymptomatic carriers in the community. Accurate diagnosis of COVID-19 is required for treatment and/or self-isolation for the patient. The diagnostic approach is mainly using the standard real-time reverse transcriptase polymerase chain reaction (RT-PCR) technique or rapid antibody detection tests (Ab-RDT) as surveillance tools. However, interpretation of the Ab-RDT is not simple and requires sufficient knowledge of the disease's clinical course and viral dynamics. It is therefore that Ab-RDT is no longer used as a screening test for travelers in Indonesia. Instead, the rapid antigen detection test (Ag-RDT) has become the choice for screening, leading people buy the kit online and perform self-testing at home. This raises the question of how safe and accurate it is to perform unsupervised self-Ag-RDT at home. This article will review the available diagnostic tests of SARS-CoV-2, with an emphasis on the appropriate use of Ag-RDT for laboratory diagnosis of SARS-CoV-2 acute infections and asymptomatic persons and its limitations, since it is widely used (or misused) in Indonesia.

THE STRUCTURE OF THE SARS-COV-2 RNA VIRUS

The SARS-CoV-2 genome is a single-stranded RNA, which can act as a messenger RNA (mRNA) for immediate translation of viral proteins. It contains 14 open reading frames (ORFs) following transcriptional regulatory sequences. At the 5' end, there are ORF1a and ORF1b, which are the main transcriptional units.[1]

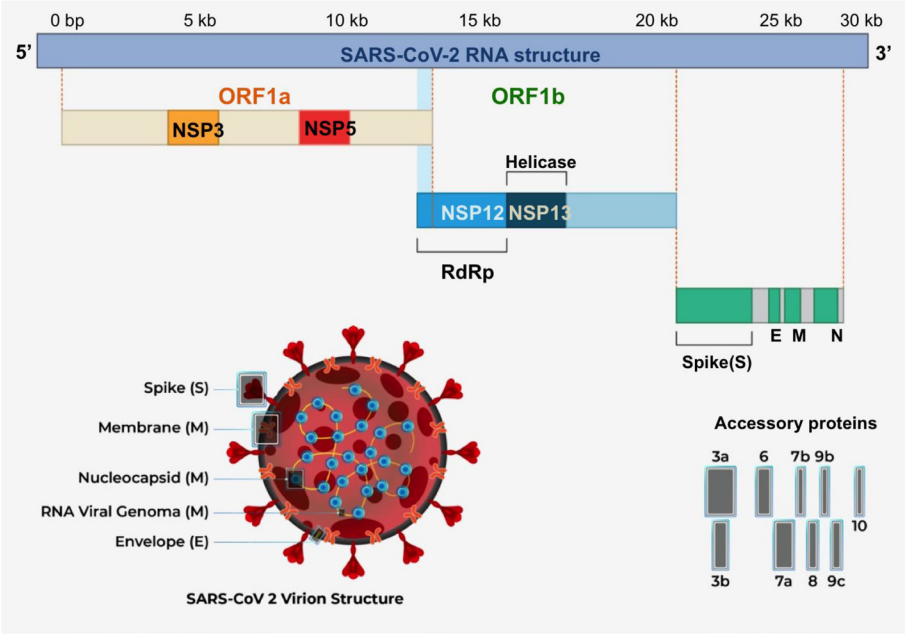

Figure 1. The structure of SARS-CoV-2 genome and virion.

At the 3' end, there are four regions encoding four structural proteins, i.e. spike (S), envelope (E), membrane (M) and nucleocapsid (N), which are the components of the mature virions.[2] The large ORF1a and ORF1b comprise about 67% of the SARS-CoV-2 genome and encode 16 non-structural proteins (NSP1–NSP16) that are involved in transcription and replication.[3] Among these NSPs, NSP12 encodes for RNA-dependent RNA polymerase (RdRp), which is widely used for viral detection in the RT-PCR test. About one-third of the remaining SARS-CoV-2 RNA encode accessory and four structural proteins (S, E, M, and N). The S1 protein is used in rapid antibody testing to elicit the IgM or IgG antibodies response.[3] The schematic representation of SARS-CoV-2 genome is shown in **Figure 1**.

DIAGNOSTIC TESTS

Available COVID-19 diagnostic tests can be grouped into four main categories, i.e. virus isolation, molecular tests that detect viral RNA from a nasopharyngeal swab, serological tests that detect anti-SARS-CoV-2 immunoglobulins (Ig) from whole blood or serum, and serological tests that detect viral antigen from a nasopharyngeal swab.[4]

Virus Isolation

The first category is virus isolation. Virus isolation is laborious, difficult, and not routinely done in clinical laboratories. Usually, only research laboratories have the skill, equipment, and facilities to do it. Isolation of SARS-CoV-2 is essential for diagnostic, research purposes, including assessment of novel therapeutics and detection of new virus strain. Virus culture has been regarded as the reference standard of diagnostics for decades, as it allows for identification and isolation of active, replicating virus. However, more rapid and sensitive molecular techniques, typically nucleic acid amplification tests (NAAT), such as PCR are now the major routine diagnostic tests used in virology diagnostic laboratories. Especially with a novel or emerging virus such as SARS-CoV-2, there are certain circumstances where virus isolation for diagnostic and research purposes remains important, including: 1). To test convalescent sera for neutralizing potential, for instance as therapeutics for SARS-CoV-2 patients in intensive care units. 2). To determine whether infectious virus is present, especially to inform on return to work for previously infectious individuals such as healthcare workers, individuals with persistent PCR positive results on serial follow-up specimens for viral clearance purpose, or when to discontinue transmission-based precautions for patients. 3). As first line testing for SARS-CoV-2, inactivation efficacy of potential preventive or therapeutic compounds. 4). For use as positive controls in the evaluation of molecular assays. 5). For viral genome sequencing to identify new viral strains.[5]

Molecular Test

The second category is the molecular test, also referred to as NAAT or nucleic acid test (NAT) that detect viral RNA. This method can be divided into the RT-PCR and isothermal methods.

RT-PCR METHOD

The RT-PCR is the standard and reliable test for COVID-19 diagnosis and can be performed on various specimens, i.e. nasopharyngeal swab, throat swab, saliva, sputum and bronchoalveolar lavage specimens. There are many commercial kits available using a variety of RNA gene targets. Most tests target one or more region in the viral RNA genome, such as the envelope (E), nucleocapsid (N), spike (S), RNA-dependent RNA polymerase (RdRp), and open reading frame 1 (ORF1).[6] During the RT-PCR process, the viral RNA target is first reversely transcribed to produce complementary DNA (cDNA) sequences. The cDNA is then amplified, beginning with the denaturation of cDNA, annealing of the primer to complementary sequence on the cDNA, and then the elongation of primer. Finally, the amplified cDNA is detected using a fluorescence probe.[7] Among many RT-PCR assays, the RdRp assay shows the highest sensitivity (about 3.8 RNA copies per reaction at 95% detection probability).[8] PCR assay requires specially trained skill to operate, high standard laboratory, controlled temperature cycling, and refrigerated reagent storage.[7]

The manual method is laborious and takes about eight hours for the result to show. The automated method can be divided into ordinary and rapid results, which is also referred to as the point of care test (POCT) method. In the ordinary automated methods, RNA extraction takes place in one instrument, whereas amplification and detection take place in separate instruments. Results are ready in about two to three hours. However, the instrument can test many specimens simultaneously, 16 or 32 specimens in one run of extraction, taking 10 to 30 minutes and 96 specimens of amplification, which usually takes 90 minutes. In the POCT method, RNA extraction, amplification, and detection are done in one instrument and the result is ready in 45 to 60 minutes. But the instrument can only test one specimen in one run, for example, GeneXpert® (single-plex), Biofire®, and Qiastat®, which are multiplex. Single-plex means that one cartridge can only detect one pathogen such as SARS-CoV-2 only. Multiplex means that one cartridge can detect many pathogens (around 19 virus and bacteria, including SARS-CoV-2) simultaneously. A cartridge is a tool that contains all the reagents for extraction, amplification, and detection of the pathogen in the specimen. After the specimen is added into the cartridge, the cartridge is inserted into the instrument and the result will show after 45 – 60 minutes. GeneXpert detects two nucleic acid targets, i.e. N2 (nucleocapsid) and E (envelope); N2 is more specific for SARS-CoV-2. The lowest limit of detection (LOD) for this assay, as claimed by the manufacturer, is 250 copies/mL.[9] The GeneXpert instrument is widely available in Indonesia but the cartridge is currently not available here due to the high demand in Europe and America.

CYCLE THRESHOLD (CT) VALUES

SARS-CoV-2 genomic load is assessed by the number of amplification cycles needed for a positive PCR test (i.e. the cycle threshold or Ct value). Ct values have an inverse relationship to viral loads and thereby provide a surrogate measurement of the viral genomic load. Consequently, the higher the viral load, the lower the Ct value of a specimen and vice versa.[9]

Ct values are not comparable between tests and may not be comparable between different lots of the same test, as they are dependent on various factors such as specimen collection, storage, transport, time from collection, nucleic acid target, primers and probes, extraction method, amplification method, instruments used, etc. Therefore, if the same sample from an individual is tested with two different tests, or even the same test from different lots, they are likely to return different Ct values, even if both tests return a "positive" test result.[10]

ISOTHERMAL METHOD

The isothermal method is another PCR method that utilizes fixed temperature in amplification; for instance, Abbott ID NOW®, which is available in Indonesia. This technique does not require nucleic acid denaturation by thermocycling but instead, targeting a unique region of the RdRp genome of SARS-CoV-2 RNA and amplifying it, and is detected by fluorescent-labeled molecular beacons. The result can be read in 5–13 minutes but the sensitivity is lower, as compared to the RT-PCR method.[9]

WHAT ARE THE CLINICAL INDICATIONS FOR MOLECULAR TESTING?

The International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) Task Force on COVID-19 has issued interim guidelines on molecular testing of SARS-CoV-2. The clinical indications for molecular testing are: 1). To diagnose viral infection in the acute phase of symptomatic illness (0-<14 days). 2). To assist in clinical assessment of asymptomatic, pre-symptomatic or mildly symptomatic patients with known exposure to positive COVID-19 cases. 3). To assist in screening of asymptomatic, pre-symptomatic or mildly symptomatic individuals in various contexts, including but not limited to: prior to scheduled surgery or delivery, travel, hospital discharge, return to work/school and to manage small outbreaks (retesting should be considered).[11]

WHAT ARE THE PRIORITIZED POPULATIONS FOR MOLECULAR TESTING?

According to the IFCC, the prioritized populations are: 1) Patients with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g. cough, shortness of breath) and all individuals having been in contact with a confirmed or probable COVID-19 case in the last 14 days (in resource limited settings). 2) Higher risk groups and settings, including the elderly and patients with pre-existing conditions (e.g. cardiovascular disease, diabetes, cancer, hypertension etc).[11]

MOLECULAR TEST INTERPRETATION AND LIMITATIONS

IFCC guidelines mention that positive molecular test results could mean: 1). SARS-CoV-2 RNA has been detected in the specimen and the patient should be considered presumptively infected. 2). Active viral replication and potential for viral transmission cannot be concluded. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.[11]

Negative molecular test results could mean: 1). SARS-CoV-2 RNA was not present in the specimen above the limit of detection of the assay. 2). SARS-CoV-2 infection cannot be ruled out and this one test result should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. 3). Re-testing should be considered if: (i) infection is still suspected after considering other differential diagnoses, (ii) molecular testing is being used for hospital discharge or (iii) analytical inhibition is suspected.[11]

SEROLOGICAL TESTS THAT DETECT ANTIBODIES

The third category is serological tests that detect anti-SARS-CoV-2 immunoglobulin. Antibodies are immunoglobulin molecules that are produced by a specific immune response after an infection. They can be found in the patients' whole blood, plasma, or serum and are specific to the pathogen. Antibodies created in response to SARS-CoV-2 infection are IgM, IgG, and IgA; IgM usually occurs first, followed by IgG and IgA (**Figure 2**). However, recent reports observed three patterns of seroconversion, i.e. synchronous (both IgM and IgG in the same time); IgM prior to IgG; and IgM later than that of IgG. Patients achieved seroconversion of IgG or IgM within 20 days after symptom onset.[12]

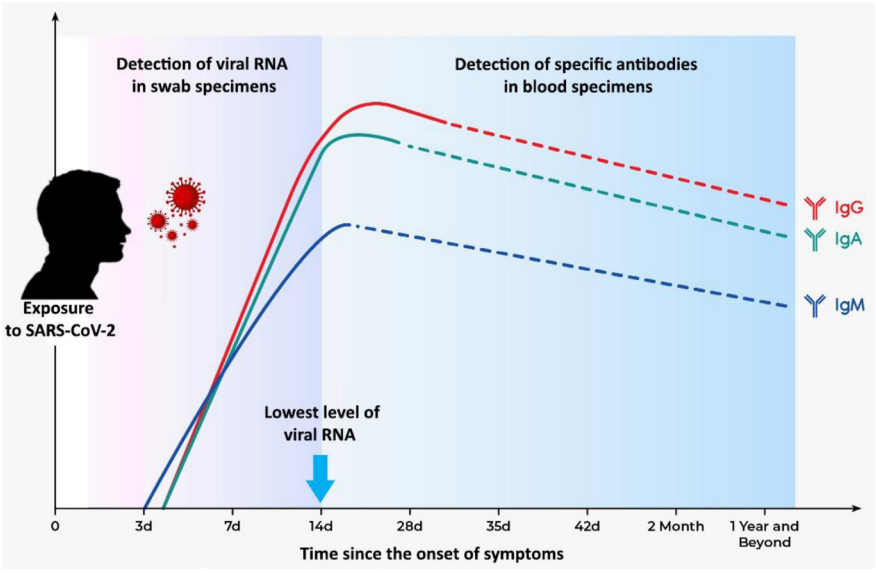

Figure 2. Schematic illustration of antibodies against SARS-CoV-2, viral RNA and their window period of detection.

METHOD OF ANTIBODY TESTING

Serological tests for COVID-19 antibody testing method can be classified into two main categories, the first is laboratory-based (lab-based) and the second is POCT-based. The lab-based method consists of two methods i.e. the enzyme-linked immunosorbent assay (ELISA), and the chemiluminescence immunoassay (CLIA).

The ELISA is a semi-quantitative in vitro determination of human antibodies of the IgG, IgM and IgA against SARS-CoV-2. The CLIA is a fully automated quantitative method using magnetic microbeads coated with SARS-CoV-2 recombinant antigen to detect IgG (quantitative) and IgM (semi-quantitative) antibodies against the SARS-CoV-2.[13] Antibody testing with the ELISA and CLIA methods is convenient, with a high-throughput and high efficiency.[14] Antibody detection by the CLIA method has been proven to be a complimentary method for rapid and sensitive laboratory diagnosis when RT-PCR test of nasopharyngeal swabs showed negative results in highly suspected positive COVID-19 patients.[15]

LATERAL FLOW IMMUNOASSAY (LFIA) METHOD/RAPID TEST/ ANTIBODY-BASED RAPID DIAGNOSTIC TEST (ABRDT)

The POCT-based method/AbRDT consists of only one method i.e. lateral flow immunoassay (LFIA) which can be done quickly, anywhere; hence the term "rapid" antibody test. The LFIA method is also called a lateral flow test (LFT), lateral flow assay (LFA), dipstick, pen-side test, quick test, rapid test, or test strip. The LFIA is a qualitative assessment of the presence of an analyte from a patient's specimen. The basic mechanism of LFIA is hydrating and transporting the reagents as they react with the specimen across the band, through the chromatographic lateral flow (**Figure 3**). When the patient's specimen flows through the device, anti-SARS-CoV-2 antibodies (IgM or IgG, or both), will bind to the SARS-CoV-2 antigens that are fixed on the test pad. The antigens are labeled with gold colorimetric reagent; the IgM antibodies will bind to the IgM (M) band and the IgG antibodies will bind to the IgG (G) band. However, if there are no antibodies in the patient's specimen; there will be no labeled complexes bound to the antigen and no bands are shown. The colloidal gold will continue to the control band zone and will be captured to indicate that the fluid has fully passed the device. A red-colored band will appear in the control zone of all valid tests, regardless of their positivity for COVID-19. The results show within 15 minutes and the test can be used as a rapid screening of SARS-CoV-2 at different stages of acute infection.16 Currently, there are hundreds of brands of rapid tests; the Foundation for Innovative New Diagnostics (FIND) lists almost 200 rapid COVID-19 antibody tests that have passed the European standard and bear the "Conformité Européenne" (CE) marks.[17]

INTERPRETATION OF ABRDT

The typical rapid test cassette has a control band "C" and two antibody bands, i.e. the "M" band that indicates the presence of IgM and the "G" band for IgG antibody. A valid test should show a "positive" control band. If both IgM and

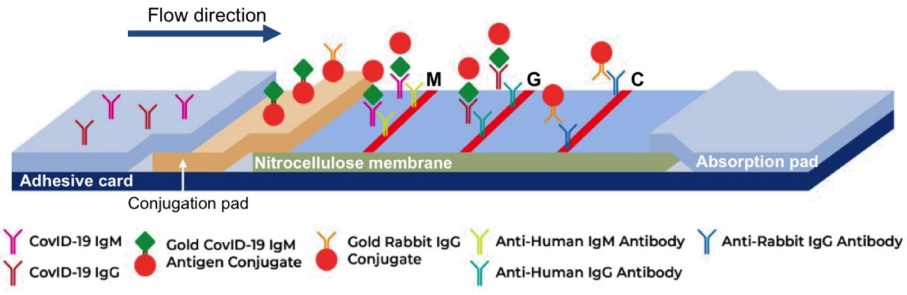

Figure 3. Schematic diagram of a lateral flow immunoassay test.

IgG bands are positive but there is no control band, the test is considered invalid. There are four possibilities of rapid test results with separate IgG and IgM bands (**Figure 4**). Color intensity does not indicate anything semi-quantitatively and all color changes should be regarded as positive result. However, the interpretation of the results should consider whether the person is symptomatic or asymptomatic, especially when both antibodies are negative.[18]

WHAT IS THE BEST SEROLOGICAL METHOD?

Comprehensive meta-analyses have reported some differences in test technology with the CLIA method appearing more sensitive compared to ELISA or LFA for assays targeting IgG and IgM.[19] Low sensitivity has been reported for currently available Ab-RDT.[20] IFCC interim guidelines suggest that currently available Ab-RDT compare poorly in sensitivity to lab-based assays and should not be used without extensive clinical and analytical validation. When used, negative results with a high suspicion of infection should be followed up with a lab-based assay.[11]

WHAT ARE THE CLINICAL INDICATIONS FOR SEROLOGICAL TESTING OF ANTIBODIES AGAINST SARS-COV-2?

The IFCC recommend the following indications should be regarded as supported by current evidence and clinical value: 1). To serve as adjunct to molecular testing in patients presenting with suggestive clinical features (> 14 days post symptom onset), but molecular testing for SARS-CoV-2 is negative, undetermined, or unavailable. 2). To serve as adjunct to molecular testing where persistently positive molecular tests occur in the absence of infectious virus, such as late, after resolved infection. 3). To assist in the diagnostic work-up of multi-system inflammatory syndrome in children (MIS-C).[11]

Beside the above mentioned indications, there are several other indications that should be regarded as potentially valuable in the future: 1). To identify previous infection in non-hospitalized individuals (asymptomatic and symptomatic) and ascertain community exposure via seroprevalence surveys. 2). To quantitatively evaluate the degree of antibody response in COVID-19 patients. 3). To assist in identification of potential convalescence plasma donors. 4). To assist in identification of immunity and evaluation of antibody response to future vaccines. 5). To assist in monitoring the progression of herd immunity.[11]

WHAT KIND OF POPULATIONS THAT SHOULD BE PRIORITIZED FOR SEROLOGICAL TESTING OF ANTIBODIES AGAINST SARS-COV-2?

The IFCC suggests that patients presenting with possible Covid-19 symptoms but who were negative by molecular testing (e.g. delayed clinical onset) should be tested.[11]

ASSAY SELECTION

The available SARS-CoV-2 serological assays usually detect IgM, IgG, IgA, or total antibodies. The specific dynamics of IgM, IgG, and IgA response and their relationship to each other are not well elucidated, but could

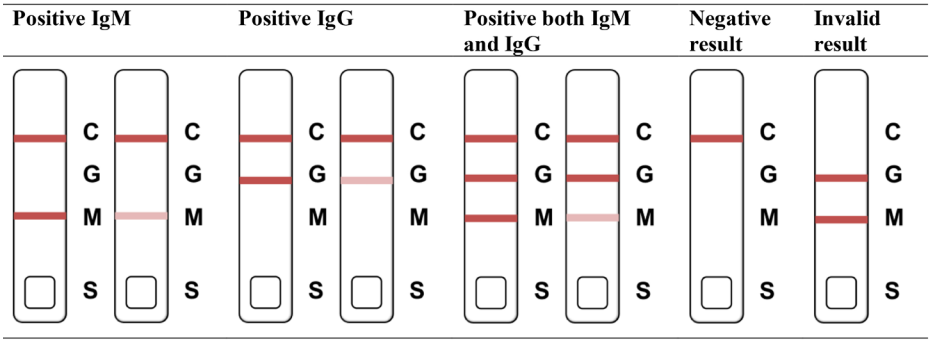

Figure 4. Illustration of different rapid test results.

potentially impact assay performance.[11,12] Miller et al[21] found that serological sensitivity increased with days post symptom onset with > 50% of patients seropositive by at least one antibody isotype after Day-7, > 80% after Day-12, and 100% by Day-21. In comparison to IgM, IgG last longer after infection and could be used for serological monitoring or surveillance.[22]

Another important factor to consider in assay selection is antigenic target. There are two antigenic targets i.e. the spike glycoprotein (S) and the nucleocapsid protein (N) of SARS-CoV-2. The S protein consist of two subunits, the N-terminal S1 unit, which contains the receptor binding domain (RBD) and the C-terminal S2 subunit.[23] Some commercially available assays only target the RBD region of S1 because it is claimed to have a greater correlation to antibody neutralization activity compared to other antigenic targets. But according to the IFCC, proper neutralization assays should be considered the only technique that can determine the neutralization capacity of the sera.[11] After SARS-CoV-2 vaccination, it is recommended to measure the neutralizing antibody titer to convince that the vaccination result is already protective.

CROSS REACTIVITY

There are six other human coronaviruses that can infect humans, i.e. NL63, OC43, 229E, and HKU1, SARS-CoV (2003), and MERS-CoV, which may compromise the test specificity.[24] Several other respiratory viruses (e.g. influenza A virus, respiratory syncytial virus, rhinovirus, para influenzae virus, etc.) are also potentially cross react with SARS-CoV-2 antibodies.[25] Recent studies showed that cross-reaction occurs between SARS-CoV and SARS-CoV-2.[26,27] This is because the SARS-CoV-2 shares 74.5% genome identity with SARS-CoV, both exhibiting a similar well-conserved structure.[26] However, there is no circulating SARS-CoV in the human population today since the outbreak has ended in 2003. Therefore, false positive results from SARS-CoV antibodies reaction are unlikely to happen.[28] Most studies of cross-reactivity were done using the ELISA or CLIA methods.[25]

SEROLOGICAL TEST THAT DETECT ANTIGEN

The fourth category is the serological test that detects viral antigen in a nasopharyngeal swab. This antigen rapid detection test (Ag-RDT) is based on antigen antibody reaction which is conducted on membrane technology with colloidal gold labeling immunochromatographic assay. The test uses monoclonal antibodies highly specific to the SARS-CoV-2 protein antigen.[29] The result may show within 15 minutes. The principle of this test is similar to Ab-RDT (**Figure 3**). The collected specimen is that of a nasopharyngeal swab.

HOW TO COLLECT THE NASOPHARYNGEAL SWAB CORRECTLY?

The healthcare worker should adhere to the WHO infection prevention and control guidelines and use personal protective equipment such as, N-95 mask, gloves, eye protection, and gown while collecting the specimen.[30]

Procedures

Label the vial with the patient's name, date of birth, and time collected. Insert the flexible swab into the nostril, parallel to the palate. If you detect resistance to the passage of the swab, retract, and try reinserting it at a different angle, closer to the floor of the nasal canal. The swab should reach a depth equal to the distance from the nostrils to the outer opening of the ear. Rotate the swab gently and then leave it in place for a few seconds. Carefully remove the swab without touching the sides of the nostril. Open the transportation vial and place the swab into the transport medium. Break the swab at the scored line and recap. Place the specimen in the large inner pocket of the plastic biohazard bag provided Remove gloves and perform hand hygiene, remove mask and perform hand hygiene.[31]

IS IT POSSIBLE TO CONDUCT THE AG-RDT AT HOME?

Some people purchase an Ag-RDT kit online and perform the test at home. This scenario could lead to unsafe practice of infection prevention and control measures such as not wearing the prescribed N-95 mask or gloves, no hand

hygiene, and no eye protection, which may cause infection from the positive SARS-CoV-2 person/s tested. Also, inadequate nasopharyngeal swab could lead to false negative results. A study by McCulloch et al32 found that unsupervised home, self-collected mid nasal swabs led to 80.0% sensitivity compared to clinician-collected nasopharyngeal swabs for detection of SARS-CoV-2 infection.

SENSITIVITY AND SPECIFICITY OF AG-RDT AND ITS IMPLICATIONS

According to WHO's latest interim guidance (11 September 2020), Ag-RDT's sensitivity compared to RT-PCR is lower, ranging from 0 – 94%, but specificity is high (> 97%). Ag-RDT is 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). 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 presented 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-RDT is higher.[33]

ARE THERE ANY CERTAIN QUALIFICATIONS FOR AGRDT?

The WHO requires that SARS-CoV-2 Ag-RDT meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity, compared to a NAAT reference assay that can be used to diagnose SARS-CoV-2 infection in the range of settings where NAAT is not available or where prolonged turn-around times preclude clinical utility.[33]

WHAT ARE THE BEST SCENARIOS IN WHICH TO APPLY AG-RDT?

The WHO also suggest the appropriate scenarios for use of SARS-CoV-2 Ag-RDTs include the following: 1). To respond to suspected outbreaks of SARS-CoV-2 in remote settings, institutions, and semi-closed communities where NAAT is not immediately available. Positive Ag-RDT results from multiple suspected subjects is highly suggestive of a SARS-CoV-2 outbreak and would allow for early implementation of infection control measures. Where possible, all specimens yielding positive Ag-RDT results (or at least a subset) should be transported to laboratories with NAAT capability for confirmatory testing. 2). To support outbreak investigations (e.g. in closed or semi-closed groups including schools, prisons, care-homes, dormitories, cruise ships, and work-places, etc.) In NAAT-confirmed SARS-CoV-2 outbreaks, Ag-RDT could be used to screen at-risk individuals and rapidly isolate positive cases (and initiate other contact tracing efforts) and prioritized sample collection from Ag-RDT-negative individuals for NAAT. 3). To monitor trends in disease incidence in communities, and particularly among essential and healthcare workers during outbreaks or in regions of widespread community transmission where the positive predictive value and negative predictive value of an Ag-RDT result is sufficient to enable effective infection control. 4). Where there is widespread community transmission, Ag-RDT may be used for early detection and isolation of positive cases in healthcare workers, health facilities, prisons, care homes, schools, and for contact tracing. 5). Testing of asymptomatic contacts of cases may be considered, even if the Ag-RDT is not specifically authorized for this use, since asymptomatic cases have been demonstrated to have viral loads similar to symptomatic cases, though in such a situation, a negative Ag-RDT should not remove a contact from quarantine requirements.[33]

IN WHAT TYPE OF CONDITIONS IS IT NOT RECOMMENDED TO USE AG-RDT?

The WHO recommend that Ag-RDT should not be used: 1). In individuals without symptoms, unless the person is a contact of a confirmed case. 2). For airport or border screening at points of entry. 3). In screening prior to blood donation or elective surgery. 4). Where there are zero or only sporadic cases. 5). Appropriate biosafety and infection prevention and control measures (IPC) are lacking. 6). Management of the patient does not change based on the result of the test.33

The CDC recommendations for performing Ag-RDT is divided into three categories: 1). Testing a symptomatic person – high pretest probability. 2). Testing an asymptomatic person who has had close contact with a person with COVID-19 – moderate pretest probability. 3). Testing an asymptomatic person with no known exposure to a person with COVID-19 – low pretest probability.[34]

ALGORITHM OF TESTING A SYMPTOMATIC PERSON – HIGH PRETEST PROBABILITY

When testing a person who has symptoms associated with COVID-19, indicating that pretest probability is high, the healthcare provider generally can interpret a positive antigen test to indicate that the person is infected with SARS-CoV-2. A negative antigen test result for a symptomatic person should be confirmed with an FDA-authorized NAAT (**Figure 5** and **6**).[10]

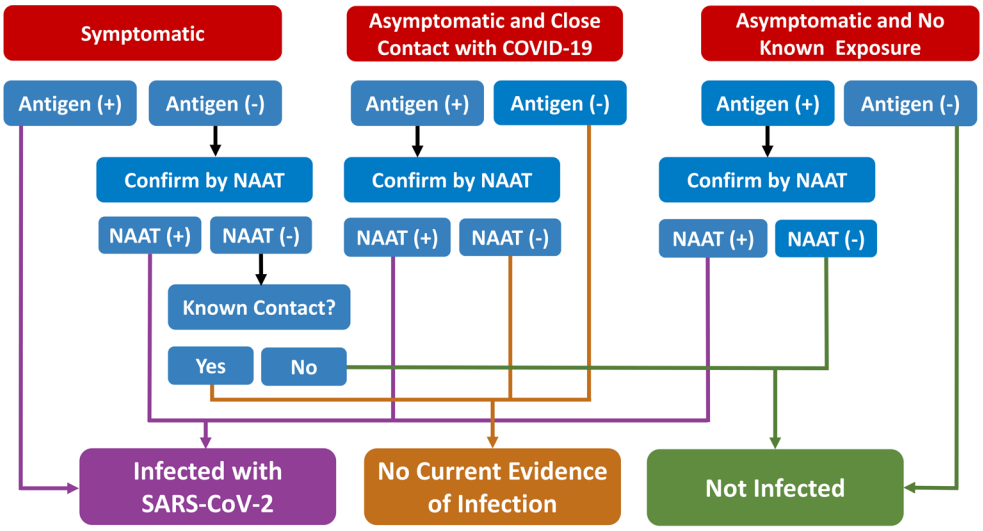

Figure 5. Testing algorithm of symptomatic, asymptomatic and close contact with COVID-19 and asymptomatic and no known exposure individuals.

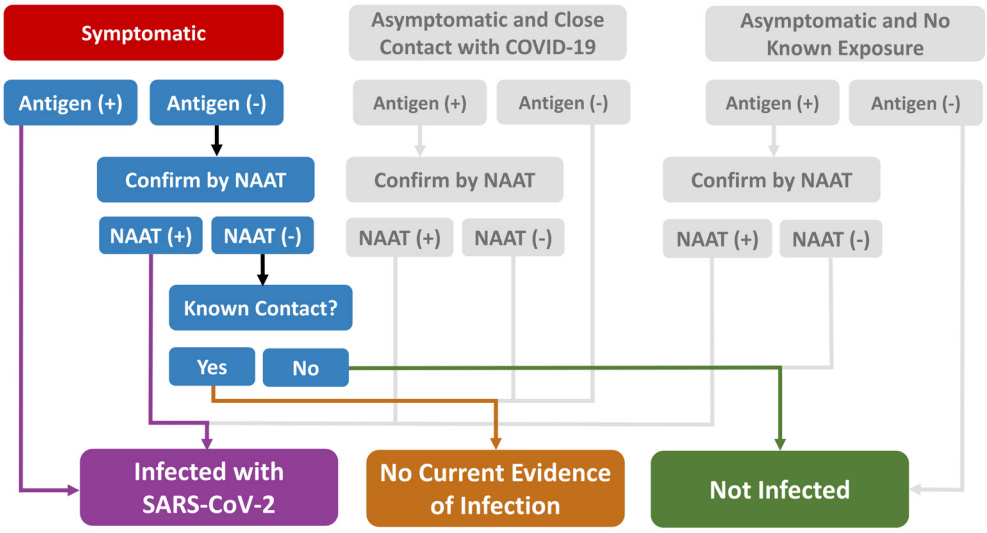

Figure 6. Testing algorithm of symptomatic individuals.

ALGORITHM OF TESTING AN ASYMPTOMATIC PERSON WHO HAS HAD CLOSE CONTACT WITH A PERSON WITH COVID-19 – MODERATE PRETEST PROBABILITY

When testing a person who is asymptomatic and has had exposure to a person with COVID-19 within the last 14 days, indicating that the pretest probability is moderate, the healthcare provider should confirm a positive antigen test result with an FDA-authorized NAAT (**Figure 7**). Persons who receive a positive antigen test result should undergo confirmatory testing and should isolate while awaiting results of the confirmatory testing.

TESTING AN ASYMPTOMATIC PERSON WITH NO KNOWN EXPOSURE TO A PERSON WITH COVID-19 – LOW PRETEST PROBABILITY

Healthcare providers should consider pretest probability when using antigen tests as screening tests, and confirmatory testing may be required for clinical management and public health decision-making.

When testing a person who is asymptomatic and has not had any known exposure to a person with COVID-19 within the last 14 days, indicating that the pretest probability is low, the healthcare provider generally can interpret a negative antigen test to indicate that the person is not infected with SARS-CoV-2. If the prevalence of SARS-CoV-2 infection is not low in the community, clinical judgement should consider whether this negative antigen test result should be followed by a confirmatory NAAT. See the antigen testing algorithm when pretest probability is low (**Figure 8**).

CONFIRMATORY TESTING WHEN USING ANTIGEN TESTS

As the antigen testing algorithm indicates, confirmatory testing may be needed regardless of the symptom or exposure status of the person being tested, or the pre-test probability. Confirmatory testing should take place as soon as possible after the antigen test, and not longer than 48 hours after the initial antigen testing. If more than 48 hours separate the two specimen collections or if there have been opportunities for new exposures, a NAAT should be considered as a separate test – not a confirmation of the earlier test. If the results are discordant between the antigen test and the confirmatory NAAT, generally, the confirmatory test result should be interpreted as definitive for the purpose of clinical diagnosis.

CONCLUSION

Ag-RDT is easy to perform and can be done in remote areas where NAAT is not available. The sensitivity of Ag-RDT is lower than NAAT

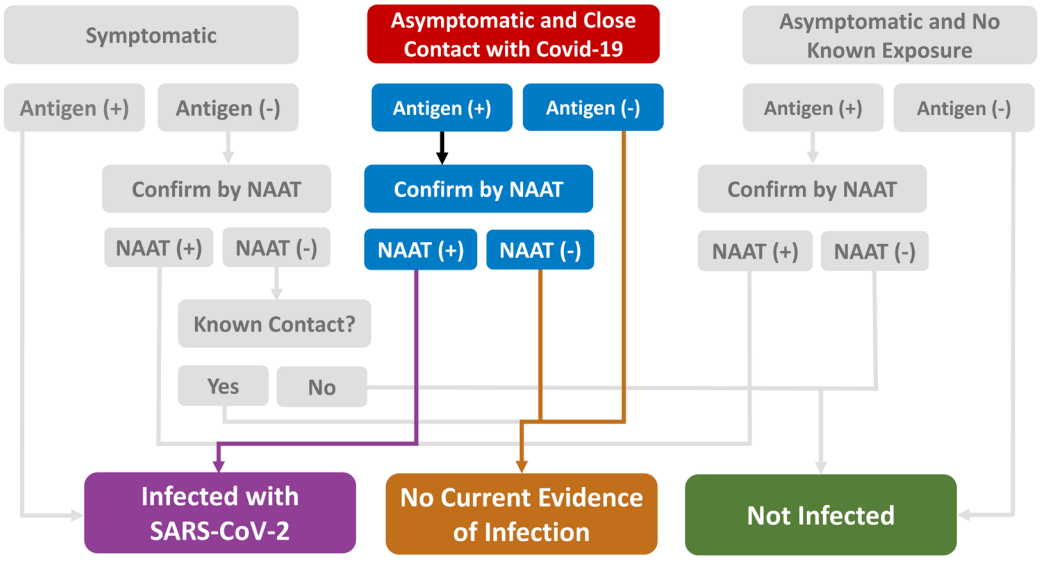

Figure 7. Testing algorithm of asymptomatic and close contact with Covid-19 individuals.

and usually can only detect SARS-CoV-2 in high viral loads (Ct values ≤25), which makes its applications best 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). 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. Ag-RDT with self-collected mid nasal swabs lead to lower sensitivity of the self-Ag-RDT and more false negative results. Inadequate personal protective equipment leads to higher risk of viral transmission to the examiners. A positive Ag-RDT result is recommended to be confirmed by NAAT in less than two days within testing. The use of Ag-RDT is not recommended in settings or populations with low expected prevalence of disease, for example, screening at points of entry, blood donation, and elective surgery.

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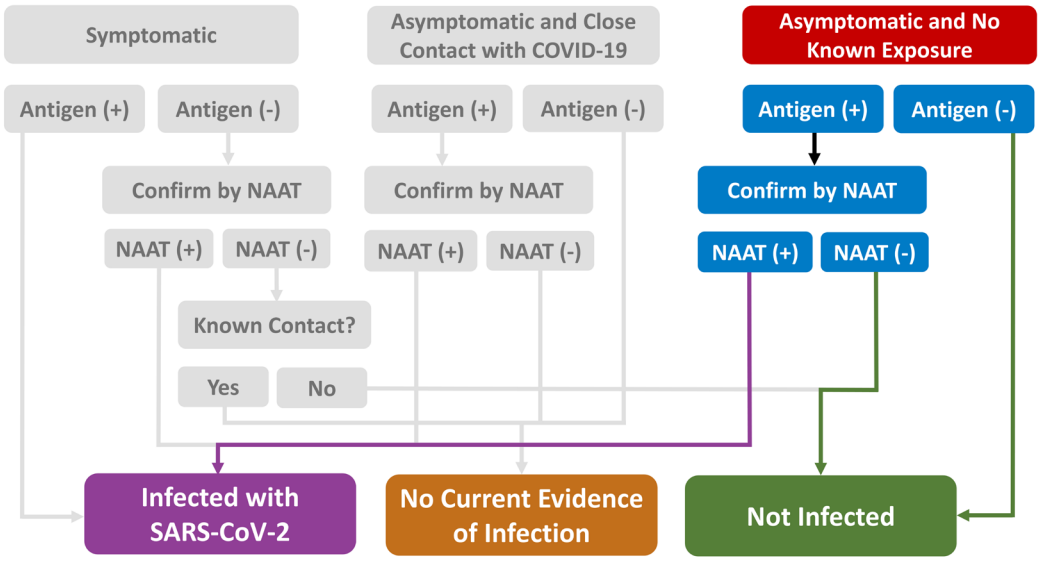

Figure 8. Testing algorithm of asymptomatic and no known exposure individuals.

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