COVID-19 Testing – Abstract

2020 has become the year of COVID-19, with this particular virus being the topic of discussion everywhere we go. Countries around the world are trying to implement practices to prevent the spread of the virus, but undoubtedly people will still become infected. That in mind, we need to be informed about who is currently infected, through the use of diagnostic tests, and who has been infected, through the use of antibody tests. In order to do that, healthcare professionals have the option of 67 different tests, approved for emergency use authorization by the FDA. With that many tests available, it can be difficult to determine what each test does, how these tests work, and which tests work for which patients. In this paper, we will discuss diagnostic tests and antibody tests, along with some of the technology used to process those tests, including RT-PCR, LFIA, ELISA, and CRISPR.

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As the COVID-19 pandemic continues to be a main topic of discussion around the world, information about the virus continues to pour out, more and more every day. A common topic of discussion centers around COVID-19 testing; who has the virus? Who has already had the virus? What are these different tests? It can be very confusing to understand what all of these tests mean and how they relate to further understanding people's status related to COVID-19; but, it's important to distinguish the differences between each test and how they help us learn more during this pandemic.

First, it's important to understand the difference between an antigen and an antibody, two main components that COVID-19 tests are looking for. An antigen is a toxin or other foreign substance (including bacteria, viruses, fungi, etc.) that enters the body and causes an immune response, including production of antibodies. Those antibodies are proteins produced in the blood, by the patient's body, in response to an antigen, to help protect the body. Each antigen that enters the body will cause production of specific antibodies to that antigen; for example, any antibodies that have been produced against this year's seasonal flu will not help in protection against a different virus, such as the common cold. In the simplest of terms, an antigen is a foreign substance, while antibodies are protection made by your body against those antigens.

That information in mind, we can now look at how COVID-19 tests use antigens and antibodies to give us a clearer picture of a person's COVID-19 status. Tests related to the detection of antigens are referred to as diagnostic tests because if a patient tests positive, they determine the patient has an active COVID-19 infection, but if a patient tests negative, they do not have an active COVID-19 infection[13]. The first diagnostic test is a molecular test, also called nucleic acid amplification test (NAAT), which detects presence of viral RNA, the genetic material of COVID-19[6]. The other diagnostic test is an antigen test which detects presence of viral proteins that are building blocks of the COVID-19 virus and lead to production of antibodies. Tests related to the detection of those antibodies are referred to as serological or antibody tests[6]. Antibody tests detect a past exposure to an antigen, in this case COVID-19, not an active infection[14]. It generally takes several days for antibodies to become detectable after initial infection and it is not known at this time how long antibodies remain present in the body after the infection has passed[6]. There are also more specific antibodies that some tests look for, distinguishing between IgM and IgG antibodies. IgM antibodies are the first type of antibody to mobilize and attack against a foreign substance in the body; they are seen earlier on in the course of an infection and taper off fairly quickly[7]. IgG antibodies are mobilized later on during an infection but stay around much longer than IgM antibodies, well after the infection has passed to help provide long term immunity[7].

Currently the tests being used have been provided "Emergency Use Authorization (EUA)" by the FDA[4]. EUA allows the FDA Commissioner to "allow unapproved medical products or unapproved uses of approved medical products to be used in an emergency to diagnose, treat, or prevent serious or life-threatening diseases or conditions…when there are no

adequate, approved, and available alternatives."[3] As of May 8th, 2020, there were 67 COVID-19 tests approved by the FDA for EUA, 55 are NAAT or antigen tests that look for active infection and 12 are antibody tests that look for previous infection[4]. Of the 67 available tests, only 4 can be completed at patient care settings under a CLIA (Clinical Laboratory Improvement Amendment) waiver, given to tests that are deemed simple with a low risk for a false result[4,10]. These tests are considered "point of care" (POC) tests and can be completed at a patient care setting, without being sent to a lab, with results within 15 – 30-minutes[10]. Therefore, the remaining 63 tests related to COVID-19 must be sent off to a lab for testing and interpretation.

Each of those 67 tests detects RNA, antigens, or antibodies in a specific way with three of the most common methods being RT-PCR (real-time polymerase chain reaction), LFIA (lateral flow immunoassay), and ELISA (enzyme-linked immunosorbent assay). The most common testing technology for COVID-19 is the use of RT-PCR, a type of nucleic acid amplification test (NAAT); therefore, this technology is used diagnostically[6]. RT-PCR is used to make millions of copies of a particular portion of DNA, extracted from the specimen sample (a respiratory sample for COVID-19), to allow it to be analyzed, and in this case, confirm active infection with COVID-19[12]. Because RT-PCR is trying to determine if a patient is actively infected with COVID-19, it is trying to detect COVID-19 itself. Another form of diagnostic technology for COVID-19 is a LFIA which uses the same technology as an over the counter pregnancy test[15]. The LFIA contains a control line to confirm the test is working properly and one or more target lines to see if the target, in this case, COVID-19, is present[9]. Therefore, in the same way as a pregnancy test, once the sample (blood/plasma or a respiratory sample) is placed on the device, if there is only one line present, the patient tests negative for COVID-19[9]. But, if there are two lines present, the patient tests positive for COVID-19 and is actively infected[9]. Lastly, ELISA can be used to detect and measure antibodies or antigens in a specimen sample, usually blood[11]. At this time, only the ELISA test that looks for COVID-19 antibodies has an EUA by the FDA and is known as an Indirect ELISA which tests for antibodies present in the patient's blood, indicating a past infection[4,11]. Additionally, there is a new diagnostic test that was just approved on May 6th, 2020 that uses CRISPR technology[4]. The test kit programs a CRISPR molecule to detect the presence of a specific COVID-19 viral RNA sequence[5].

All of this information in mind, we can see how COVID-19 testing information can be a little jumbled in our brains. However, it's important to remember what is being tested for with each of the 67 EUA tests we have available; are we looking for antigens (indicating a current infection) or antibodies (indicating a previous infection)? As long as we focus on that information, we can better understand what the test results of patients mean and how it relates to their COVID-19 status.

Figure 1: Types of COVID-19 Tests and What Their Results Mean

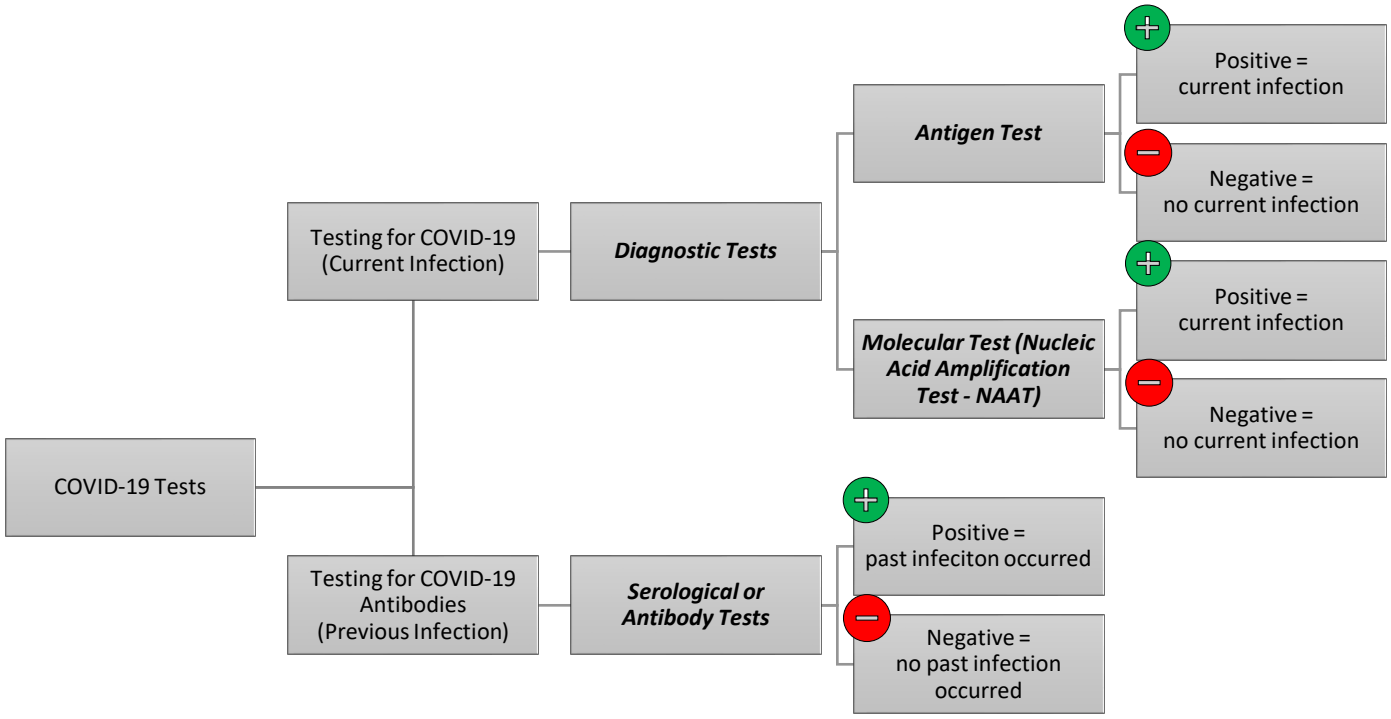

Table 1: Key Terms and Definitions related to COVID-19 Testing	
Antigen	Toxin or other foreign substance (including bacteria, viruses, fungi, etc.) that enter the body and cause an immune response, including production of antibodies
Antibody	Proteins produced in the blood, by the patient's body, in response to an antigen, to help protect the body
Diagnostic Test[13]	Tests that detect a current, active infection by looking for parts of the COVID-19 virus
Molecular Test (Nucleic Acid Amplification Test – NAAT)[6]	Type of diagnostic test that detects presence of COVID-19 RNA, building blocks of the virus
Antigen Test	Type of diagnostic test that detects presence of COVID-19 proteins (these proteins are what cause production of antibodies by the body)
Serological or Antibody Tests[6]	Detect antibodies in the patient's body, produced in response to a COVID-19 infection; used to determine if a patient has had a previous COVID-19 infection, not current
Point of Care (POC) Test[10]	Tests that are deemed simple with a low risk of a false result that can be completed at a patient care setting, without being sent to a lab

Table 2: Popular COVID-19 Testing Technology				
COVID-19 Test		**Looking For?**	**What is it?**	**Prevalence**
RT-PCR[4,6,8]	Real Time-Polymerase Chain Reaction	Looking for viral RNA (current infection)	Technology to determine the result of some diagnostic tests; RT-PCR is used to make millions of copies of a particular portion of DNA, extracted from the specimen sample, allowing it to be analyzed and confirm or refute active COVID-19 infection	52 of the 67 available COVID-19 tests use this technology
LFIA[4,9]	Lateral Flow Immunoassay	Looking for antigens or antibodies (current or past infection respectively)	Uses the same technology as an over the counter pregnancy test, a control line confirms the test is working properly and a target line determines if the target (COVID-19 proteins or antibodies) is present	3 of the 67 available COVID-19 tests use this technology
ELISA[4,11]	Enzyme-Linked Immunosorbent Assay	Looking for antibodies (past infection)	Two-step process involving binding of COVID-19 antibodies (if present in the patient's sample) to COVID-19 antigens in the lab, in a dish; followed by a second reaction that causes a color change if COVID-19 antibodies are present, indicating the patient had a previous COVID-19 infection	2 of the 67 available COVID-19 tests use this technology
CRISPR[4,5]	Clustered Regularly Interspaced Short Palindromic Repeats	Looking for viral RNA (current infection)	This technology programs a CRISPR molecule to detect the presence of a specific COVID-19 viral DNA sequence (made from its viral RNA) to determine the patient is positive for current COVID-19 infection	1 of the 67 available COVID-19 tests use this technology

Table 3: Advantages and Disadvantages of COVID-19 Testing Technology				
COVID-19 Test Type	**Looking For**	**Detect Active or Past Infection?**	**Advantages**	**Disadvantages**
RT-PCR[4,8,12]	Viral RNA	Active	• 2 PCR tests available for POC testing • Good for pathogens that are difficult to culture + quicker results compared to culturing	• Results in ~6 – 8hrs • Majority of PCR tests require being sent off to a lab • Detects dead and live organisms and does not distinguish between the two • Need specialized equipment to run • RNA extraction step can be time-consuming and lack of availability of reagents is a concern right now
Lateral Flow[4,9,15]	Antigen or Antibodies	Active or Past, respectively	• Results in 15 – 30min • Antigen test may be completed as a POC test • Simple, one-step test with no additional equipment necessary	• Inaccurate sample volume decreases precision • Non-fluid samples must be pre-treated (use viral transport media here)
ELISA[2,11]	Antibodies*	Past	• High sensitivity and specificity • Can complete many tests at once • Easy to perform • Quantitative (can determine the concentration of an antigen/antibody in a sample)	• Must be completed in the lab • The earliest results take 24hrs to return, if done locally and days to weeks if sent off to a distant lab
CRISPR[1,5]	Viral RNA	Active	• Results in ~1hr • Simple and efficient • Only need basic equipment, found in most labs	• Must be completed in the lab • Slightly less sensitive compared to PCR-based tests • RNA extraction step can be time-consuming and lack of availability of reagents is a concern right now
*Can detect antigens, but no ELISA tests to detect COVID-19 antigens have an EUA at this time[4,11]				

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