QuantiVirus™ COVID-19 Tests for Diagnosis of SARS-CoV-2 Infection

The COVID-19 Pandemic

COVID-19, caused by SARS-CoV-2 coronavirus, started as an endemic in Wuhan, China and quickly became a pandemic, reaching more than 180 countries in just a few months. As of September 4, 2020, close to 26.4 million people worldwide have been infected and more than 870,000 people have died. In the United States alone, almost 6.15 million people caught the virus and more than 186,000 lives have been lost.

This disaster has paralyzed the global economy and completely changed peoples' daily lives. While countries are attempting to reopen the economy, large-scale COVID-19 testing is still critical to identify new infections, especially the asymptomatic infections. This allows healthcare workers to provide those infected with proper therapy and quarantine procedures. Before an effective vaccine becomes available, COVID-19 testing has become the routine task for public health to slow down the community spread.

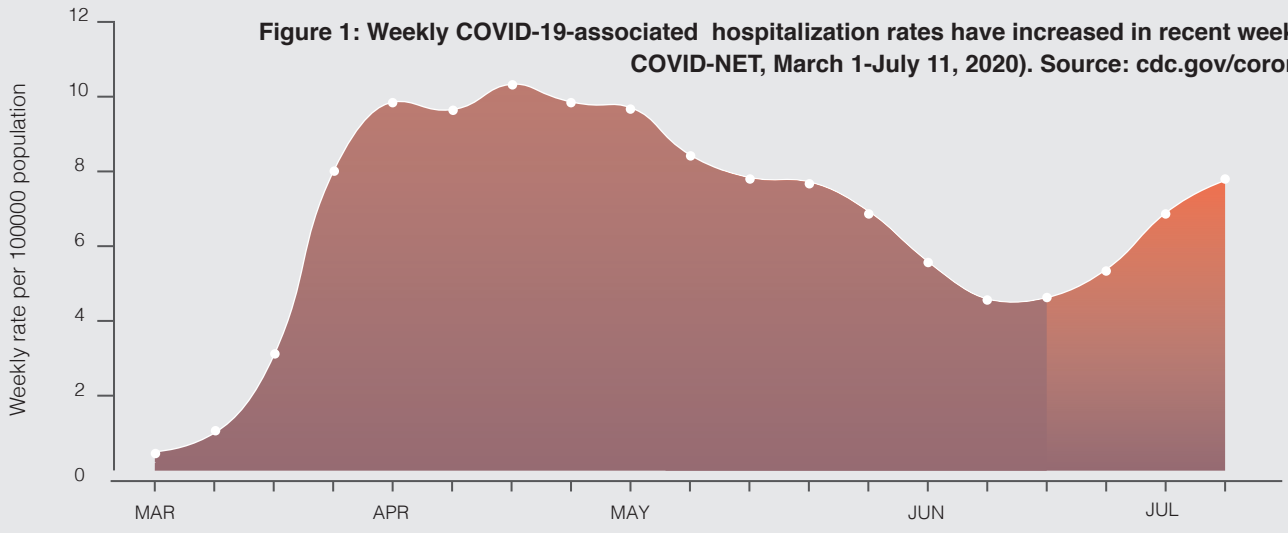


Figure 1: Weekly COVID-19-associated hospitalization rates have increased in recent weeks (from COVID-NET, March 1-July 11, 2020). Source: cdc.gov/coronavirus.

COVID-19 Test Types

Three main types of tests are available: Viral nucleic acid testing, antibody testing, and antigen testing. These tests detect the presence of viral RNA, antibodies, and the viral proteins, respectively.

Although all these tests tell if an individual has been infected with SARS-CoV-2, the RNA virus that causes COVID-19, each test detects the infection from different angles. As of July 30, the FDA has approved 193 COVID-19 tests under EUA: 158 molecular tests (viral RNA), 33 antibody tests, and 2 antigen tests.

Viral RNA Tests

The most sensitive test is the nucleic acid test which tells if an individual is currently infected, since the viral RNA is only present at the infection stage. A sensitive test can detect 50 copies or less of viral RNA per ml of sample.

However, the test can still generate false-negative results due to incorrect sampling, improper processing and storage of samples or a too early infection. Healthcare professionals recommend that the viral RNA test be conducted at 7 to 8 days after infection or 3 to 4 days after the symptoms appear to prevent false-negative results.

The high sensitivity of the test is attributed to the amplification and detection of the viral RNA through reverse transcriptase (RT)-qPCR with properly designed primers and TaqMan probes that exhibit good PCR amplification efficiency and broad and linear dynamic range as well as high inclusibilty and specificity. Although the viral RNA level is low, RNA reverse transcription and DNA amplification allows chemical signal of fluorescence to be detected during this qRT-PCR test. A sensitive test detects the presence of one to three viral genes as targets (Orf1ab, E, and N genes, for instance). Although qRT-PCR, isothermal amplification, and NGS techniques have all been approved for genetic testing of COVID-19 by the FDA EUA, qRT-PCR is still the gold standard.

Antibody Tests

The antibody test, as opposed to the viral RNA test, does not tell if a patient is currently infected because antibody production takes time and may stay in the blood for a period of time even after the virus is gone. Upon infection, the patient's innate immune system is activated first, then the adaptive immune response stimulates the body to produce antibodies. Thus, the antibody test is not a sensitive test for early infections. Instead of being used as an infection screening test, the antibody test can only tell if a person has been exposed to the SARS-CoV-2 virus. Starting production after infection for 5 days or more, the antibody concentration peaks at two weeks for IgM and three weeks for IgG types. The positive antibody test can only tell if an individual has been infected in the past if the test has no cross-reaction with other coronaviruses.

However, if the test is negative, it does not guarantee that the person is not currently infected or has not been infected. The person may have been infected but has not produced antibodies yet, or the antibodies have disappeared in the body after the person has recovered for some time.

The importance of the antibody test includes:

- Help estimate the virus spread in the community
- May be chosen as donors for plasma therapy if the antibody is tested positive
- May be immune from the second infection, although no clear evidence supports the immunity
- May help to decide the priority of vaccinations, so people who do not have the antibodies yet can get the vaccination first

Antigen Tests

The third type of testing, antigen testing, is not as common as the first two types. Antigen tests detect virus replicates in the infected cells. Therefore, the antigen test can tell the current infection status.

Since specific antibodies against these chosen antigens must be identified and produced before development of such antigen tests, antigen tests take a longer time to develop. Antigen detection may not be as accurate compared to the first two tests, however they are fast and easy to operate, and thus are often used at home as a point-of-care test, or in communities or remote areas where healthcare is not easily available.

Table 1: Comparison of three types of COVID-19 tests

	RT-PCR (Nucleic Acid Test)	Antibody Test	Antigen Test
Detection	Viral RNA	Antibody produced against virus antigens	Antigens such as proteins and glycans from virus
Infection Detection	Current infection	Exposure to virus	Current infection
False-Negative Cause	Improper assay design, incorrect sampling, improper processing and storage of samples or a too early infection	Infected but antibody not produced yet	Early infection that does not have enough antigen present
False-Positive Cause	Non-specific assay design with cross-reactivity with other coronaviruses or PCR contamination	Antibody used is reactive to other coronaviruses	Antibodies used against other coronaviruses' antigens
Sensitivity	Highly-sensitive	Less sensitive	Less sensitive
Technical Requirement	High	Medium or low	Low

DiaCarta's COVID-19 Tests

DiaCarta has developed three COVID-19 tests: two qRT-PCR tests detecting one gene (SARS-CoV-2 Multiplex Test for Orf1ab gene) or three viral genes (SARS-CoV-2 Test for Orf1ab, E and N genes), and one anti-SARS-CoV-2 IgG test based on xMAP technology developed by Luminex. The molecular tests using qRT-PCR received the FDA EUA approval on April 8, 2020 and July 21, 2020.

QuantiVirus™ SARS-CoV-2 Test and QuantiVirus™ SARS-CoV-2 Multiplex Test

Both FDA EUA-approved qRT-PCR tests are used for high-throughput testing using common qPCR instruments with different sample types. Table 2 compares the two tests.

Table 2: Comparison of QuantiVirus™ SARS-CoV-2 and QuantiVirus™ SARS-CoV-2 Multiplex Test

	QuantiVirus™ SARS-CoV-2 Test	QuantiVirus™ SARS-CoV-2 Multiplex Test
FDA EUA Approval	Approved on April 7, 2020	Approved on July 21, 2020
CE/IVD Marked	CE/IVD Marked	CE/IVD Marked
Viral Genes Detected	Orf1ab, E and N	Orf1ab
Negative Control	Human RNase P	Human RNase P
Sample Configuration	One sample, one reaction	One sample, one reaction
Throughput	381 (384 well-plate) or 93 (96-well plate)	381 (384 well-plate) or 93 (96-well plate)
Sample Types	Nasopharyngeal Swabs, Oropharyngeal Swabs and Sputum	Nasopharyngeal Swabs, Oropharyngeal Swabs and Sputum
Analytical Sensitivity	100 copies per mL sample	50 copies per mL sample
Benefit over other Tests	More confidence	Cost-effective

The Test Mechanism

The mechanism for detecting the viral RNA has two steps. The first step is to extract RNA (not included in the testing kit) from the samples (NP, OP, or saliva samples); the second step is to use a one-step qRT-PCR to make cDNA using viral RNA and amplify the DNA sequence of the viral genes. To improve the test throughput, the viral target genes and the negative control are all detected in one reaction using probes labeled with different colors of fluorophores with well separated emission wavelength on a validated qPCR instrument. Both tests are validated on the most common qPCR instruments including Thermo Fisher Scientific QuantStudio 5, 7500 Fast Dx, Bio-Rad CFX384 and Roche LightCycler 480 II. These qRT-PCR tests in general have high analytical sensitivity, suggesting that even a low viral load at early infection can be efficiently identified by the test.

Our tests can detect 50 to 100 copies of virus per mL of sample. Another advantage of the qPCR assay is that the assay has a wide linear range (10^6 order of magnitude). This dynamic range allows large variations of target gene copies atdifferent stages of infection from the same patient, or from different patients to be detected efficiently without sample dilutions.

One important parameter to evaluate a testing kit is to test the specificity, in this case, the cross-reaction with other SARS-CoV-2-related coronaviruses and other common respiratory flora. Our tests do not show any cross-reactivity with eight other known coronaviruses we collected. Clinical sensitivity and specificity are also critical for a successful diagnostic kit. Our tests provide 97% sensitivity and 100% specificity, offering high testing accuracy and confidence in patient test results. These tests rank top 10 among all the tests granted with FDA EUA based on low limit of detection according to the evaluation from Nature Biotechnology published on August 20, 2020.

Clinical Sample Testing

Both tests are compared with the CDC kit and Abbott kit for clinical samples testing. All these kits have shown the same testing results for both positive and negative samples (Table 3). Additionally, more clinical sample testing indicated that the QuantiVirus™ SARS-CoV-2 Test Kit has high diagnostic accuracy with Positive Percentage Agreement (PPA) of 97.7 (95% CI: 0.88-1.0), Negative Percentage Agreement (NPA) of 100% (95% CI: 0.94-1.0) and Overall Percentage Agreement (OPA) of 98.8% (Table 4).

Figure 2: Product Configuration for QuantiVirus™ SARS-CoV-2 Test Kit

**This product configuration image only shows the 24-reaction and 48-reaction pack size. 480-reaction pack size has 7 vials.*

Figure 3: Product Configuration for QuantiVirus™ SARS-CoV-2 Multiplex Test Kit

**This product configuration image only shows the 24-reaction and 48-reaction pack size. 480-reaction pack size has 7 vials.*

Table 3: Clinical Sensitivity: 100% agreement with real patient samples previously tested with CDC assay and Abbott m2000 assay

Sample ID	Abbott m2000	CDC Assay	DiaCarta´s QuantiVirus™ Assay
A		Detected	Detected at 1:100 dilutions
B		Detected	Detected at 1:1000 dilutions
C	Not Detected	Not Detected	Not Detected
D	Not Detected	Not Detected	Not Detected
E	Not Detected	Not Detected	Not Detected
F	Not Detected	Not Detected	Not Detected
G	Not Detected	Not Detected	Not Detected
H	Not Detected	Not Detected	Not Detected
I	Not Detected	Not Detected	Not Detected
J	Detected	Detected	Detected
K	Detected		Detected
L	Detected		Detected
M	Detected	Detected	Detected
N	Not Detected	Not Detected	Not Detected

Table 4. Clinical sample testing with QuantiVirus™ SARS-CoV-2 Test Kit

Patient Samples	N	QuantiVirus™ SARS-CoV-2 Test			PPA（95% CI）	NPA (95% CI)
		Detected	Inconclusive	Not Detected		
Positive	44	43	0	1	97.7%(0.88-1.0)	100%(0.94-1.0)
Negative	56	0	0	56		

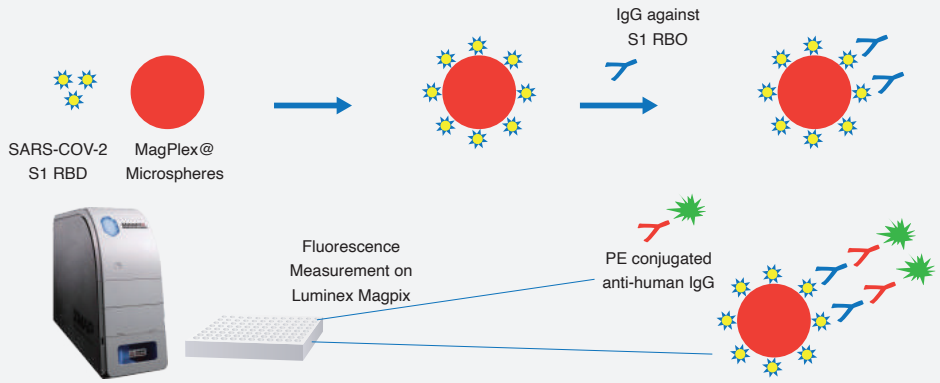
QuantiVirus™ Anti-SARS-CoV-2 IgG Test

How does the test work?

The QuantiVirus™ Anti-SARS-CoV-2 IgG test detects the antibody IgG, produced in response to SARS-CoV-2 in the blood. The test, developed using Luminex xMAP technology, is a two-step immunoassay. It detects anti-SARS-CoV-2 spike protein 1 (S1) receptor-binding domain (RBD) IgG antibody in human serum or plasma specimens using either Luminex 200 or MAGPIX instruments. The test takes approximately 3 hours per run with a 96-well plate capable of testing 92 patient samples.

Briefly, recombinant spike protein 1 (S1) RBD was covalently coupled to the surface of MagPlex® Microspheres (magnetic beads) via a carbodiimide linkage using xMAP® Antibody Coupling (AbC) kit. The S1 RBD protein coated magnetic beads and human specimens were mixed and incubated at room temperature. The IgG antibodies present in human specimens against S1 RBD protein (the antigen) will bind to the coated magnetic beads. After washing, PE conjugated anti-human IgG antibody was added to the reaction mixture and incubated at room temperature. After washing, PE fluorescence of each well in a 96-well microplate was measured on Luminex 200 or MAGPIX® instrument for Median Fluorescence Intensity (MFI). Interpretation of the testing results was performed by calculating the MFI ratio of each sample to the average MFI of two blank wells.

Figure 4. The high-throughput immunoassay for anti-SARS-CoV-2 IgG detection



Clinical Performance

Seventy-seven (77) different positive serum samples were used in the evaluation of positive percent agreement (PPA). Two hundred and twenty-six (226) serum or EDTA plasma samples collected from healthy donors prior to the outbreak of COVID-19 were used in the evaluation of Negative Percent Agreement (NPA).

Not surprisingly, the samples taken longer days (more than 15 days) after the symptom onset provide much higher PPA value (96.72%) compared to the samples taken a few days after the symptom onset. The NPA was 98.23%.Thirty (30) serum samples were also further evaluated by comparing to Abbott SARS-CoV-2 IgG antibody test which has been approved by FDA EUA. The results showed 100% concordance between the two assays.

Table 5. Determination of positive percent agreement (PPA) and negative percent agreement (NPA)

Category	Days from Symptom Onset	Number of Samples	IgG Positive	IgG Negative	PPA and NPA (95% CI)
COVID-19 Positive	0 - 7 days	7	3	4	PPA:42.86%(9.90%~81.59%)
	8 - 14 days	9	6	3	PPA:66.67%(29.93% to 92.51%)
	≥15 days	61	59	2	PPA:96.72%(88.65%~99.60%)
COVID-19 Negative	n/a	226	4	222	NPA:98.23%(95.53%~99.52%)

Cross-Reactivity

Cross-reactivity of the QuantiVirus™ Anti-SARS-CoV-2 IgG Test was evaluated by using serum or plasma samples which are positive for IgG antibodies to the pathogens such as Influenza A or B. The result indicates that no cross-reactivity was found in any of the samples tested.

Figure 5: Product Configuration for QuantiVirus™ Anti-SARS-CoV-2 IgG test

The test has been filed to FDA for EUA approval and is CE-marked.

**This product configuration image only shows the 96-reaction pack size. Please refer to product IFU for 384-reaction and 960-reaction pack size kit configuration.*

DiaCarta CLIA-Certified COVID-19 Clinical Laboratory Testing Service

In addition to providing high-quality COVID-19 diagnostic tests described above, DiaCarta also provides a high-throughput COVID-19 testing service for the reopening of businesses and schools. Managed by the LIMS (Laboratory Information Management System), the whole clinical laboratory workflow is seamless from sample collection to reporting of results. The DiaCarta Clinical Laboratory is Clinical Laboratory Improvement Amendments (CLIA) certified and qualified for performing high complexity testing. Our test has been approved by the US FDA under an Emergency Use Authorization (EUA) and was validated in accordance with the FDA Guidance Document Policy.

DiaCarta COVID-19 Testing Service Highlights

CLIA-CERTIFIED

» Fast Turnaround Time
- Turnaround time within 24-48 hours on receipt of samples.
- Lab capability is 5,000 tests per day and increasing.
- High throughput PCR cyclers.

» Automated System for Sample Processing
- Electronic or manual accessioning of patient data
- Automated RNA extraction

» COVID-19 Testing by qRT-PCR (Nucleic Acid Test)
- qRT-PCR is the gold standard for COVID-19 testing
- Utilizing DiaCarta's FDA EUA approved RT-PCR assay
- Highly sensitive RT-PCR assay

» Secured Web Portal for Test Reporting
- Automated test results generation
- Digital access to test results for customers

COVID-19 Testing Service Workflow

For Client: From Sample Collection to Test Results

Turnaround Time within 24-48 Hours on Receipt of Samples

01 Order the test by contacting order@diacarta.com

02 Receive sample collection kits from DiaCarta

03 Complete sample collection & sample labeling

04 Ship the samples to DiaCarta's clinical laboratory

05 Access/Download the test results online

Test Process within DiaCarta - Automated System & Quality Results

Clinical Laboratory Improvement Amendments (CLIA) Certified Lab and FDA EUA Approved RT-PCR Test

01 Specimens delivered to DiaCarta CLIA Lab

02 Specimens accessioned at DiaCarta lab via LIMS (ensuring positive sample identification)

03 Viral RNA extracted from collected specimens

04 RT-qPCR test completed with extracted viral RNA

05 Results exported to LIMS System (data reviewed by technologist and approved by medical director)

06 Results available to customers via LIMS system

Contact DiaCarta TODAY for the COVID-19 Testing Service

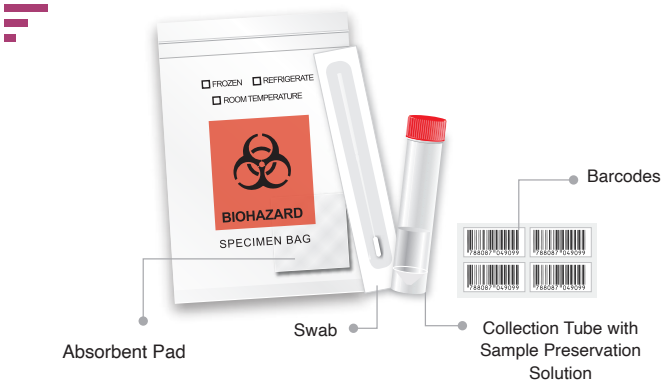
- Public health departments that are looking for additional resources to help test for COVID-19 in their community
- Healthcare facilities that are looking for outsourcing their testing services
- Businesses that are looking to reopen and want to test their employees periodically
- Schools that are planning to reopen and want to test their teachers and students periodically

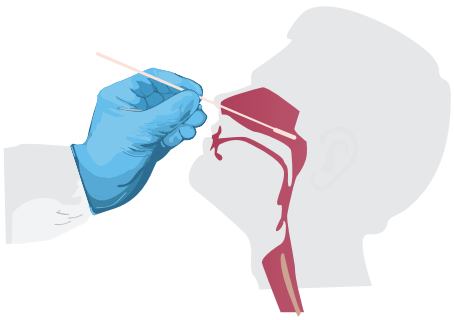
Contact DiaCarta at order@diacarta.com, or call us at 1-800-246-8878.

Sample Collection kits

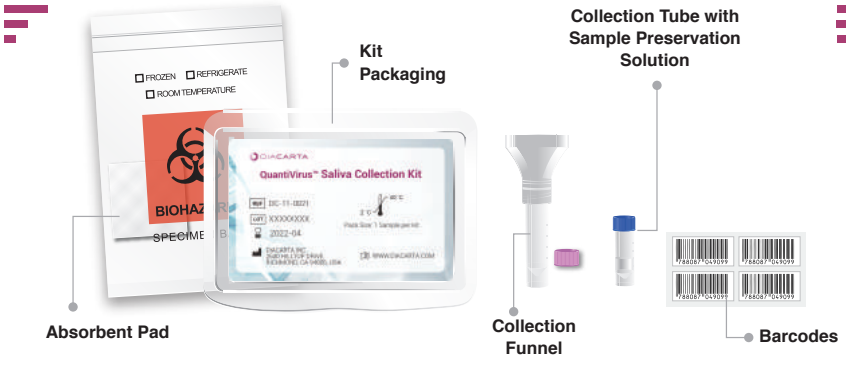
DiaCarta provides sample collection kits for nasopharyngeal (NP) sampling, oropharyngeal (OP) sampling as well as saliva sampling.

Nasopharyngeal and Oropharyngeal Sample Collection Kit





Saliva Sample Collection Kit



Summary

The COVID-19 pandemic has continued to threaten our lives, although we are fighting the battle and leading the effort in discovering effective drugs and vaccines. We must try to control the virus spread as much as possible until a promising vaccine emerges. One of the most effective and critical tools to achieve this is to increase the scale of COVID-19 testing.

DiaCarta is ready to support the community for COVID-19 testing with products for every step of the workflow:

- FDA EUA approved qRT-PCR kits for your testing facilities
- NP, OP, and saliva sample collection kits
- Clinical laboratory services supporting your COVID-19 testing needs