OPTI SARS-CoV-2 Total Antibody Test

IVD CE Rx

For *in vitro* diagnostic use only
For Emergency Use Authorization Only
For Prescription Use only

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OPTIMedical

OPTI SARS-CoV-2 Total Antibody Test

Name and Intended Use

The OPTI SARS-CoV-2 Total Antibody assay is a one-step antigen capture format, Enzyme-Linked Immunosorbent Assay (ELISA), intended for the qualitative detection of total antibodies (including IgM/IgA/IgG) to SARS-CoV-2 in human serum and plasma (K2 EDTA and Sodium Citrate). The OPTI SARS-CoV-2 Total Antibody assay is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The OPTI SARS-CoV-2 Total Antibody assay should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, to perform high complexity tests.

Results are for the detection of SARS CoV-2 total antibodies. Total antibodies (including IgM/IgA/IgG) to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of the OPTI SARS-CoV-2 Total Antibody assay early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results with the OPTI SARS-CoV-2 Total Antibody assay may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The OPTI SARS-CoV-2 Total Antibody assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Clinical Background

A new severe respiratory illness referred to as COVID-19 (Coronavirus Disease 2019) emerged in December 2019 in Wuhan, China. The causative agent was identified as a new *Betacoronavirus* and named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2). The rapid spread of the virus resulted in a global COVID-19 pandemic in 2020.

SARS-CoV-2 is a positive-sense single-stranded RNA virus. This enveloped virus is the seventh described coronavirus known to cause disease in humans. The other coronaviruses infecting humans include four less virulent viruses associated with common colds (HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1) and two additional strains of zoonotic origin causing severe respiratory symptoms which can result in death: SARS-CoV and MERS-CoV (Middle East Respiratory Syndrome Coronavirus).

Most COVID-19 patients show mild to severe respiratory symptoms with fever, cough and myalgia or fatigue. Some patients with confirmed infection are asymptomatic. Most severe forms of the disease are associated with acute pneumonia, respiratory distress, cytokine storms as well as multiple organ dysfunctions. Transmission occurs mainly through inhalation of respiratory droplets and direct or indirect contact with infected individuals. Incubation period reported so far varies generally between 1 and 14 days (mostly 3-7 days). To date there is no specific treatment or vaccine available against COVID-19.

The structural proteins of SARS-CoV-2 include the envelope (E), membrane (M), nucleocapsid (N) and spike (S) proteins. The receptor binding domain (RBD) of the spike protein is the antigen of choice for a serological assay due to its high immunogenicity. Furthermore, RBD is known to be the target of a neutralizing antibody response which may confer protection from future viral infection.

In the absence of vaccine and antiviral treatment the availability of accurate diagnostic tools is key to limit viral transmission. Antibody response to SARS-CoV-2 is detected in most infected individuals at varying times post symptoms onset. Antibody testing can be of great value in conjunction with PCR testing to increase overall diagnostic sensitivity and deploy an efficient pandemic response. Serological testing may aid in identifying infected people at a later stage of the disease when a PCR test is negative. In addition, the use of serological tests can help understand the status of the virus spread and the level of immunity within a given community.

Descriptions and Principles

The OPTI SARS-CoV-2 Total Antibody Test is an enzyme-linked-immunosorbent assay (ELISA) for the detection of antibodies against SARS-CoV-2 in human serum and plasma samples. The test detects total antibodies, including IgG, IgM and IgA.

SARS-CoV-2 Receptor Binding Domain (RBD) recombinant protein is coated in microtiter plate wells and represents the assay solid phase. SARS-CoV-2 Receptor Binding Domain (RBD) protein-Horseradish Peroxidase (HRPO) Conjugate is used as the assay detector. In step 1 of the assay, samples are diluted 1:2 with RBD-HRPO Conjugate. If SARS-CoV-2 antibodies are present, they will bind to the RBD-HRPO Conjugate to form a complex. In step 2 of the assay, the diluted samples are then transferred to microtiter plate wells containing immobilized RBD. If present, SARS-CoV-2 Antibody/RBD-HRPO complexes will bind to/bridge with the immobilized RBD. After a washing step, TMB Substrate is added and reacts with bound complexes to generate a blue color. The color reaction is then stopped with the addition of an acidified Stop solution, shifting color from blue to yellow.

Optical densities (A450 nm) are read and results are calculated by generating a sample to positive control ratio (S/P). The sample to positive ratio is calculated by using the absorbance obtained with the test sample and a positive control (A450 nm), corrected for the absorbance of the negative control.
Color development indicates the presence of SARS-CoV-2 antibodies in the test sample.

Reagents		**Volume**
1	SARS-CoV-2 antigen coated plates	5
2	Positive Control	1 x 3.6 mL
3	Negative Control	1 x 3.6 mL
4	RBD Conjugate	1 x 30 mL
A	Substrate Solution	1 x 60 mL
B	Stop Solution	1 x 60 mL
C	Wash Concentrate (10X)	2 x 235 mL
Other Components: Zip lock bag.		1

Note: See table at the end of the insert for a description of symbols used on the insert and labels of this kit.

Storage

Store the reagents at 2–8°C. Reagents are stable until expiration date, provided they have been stored properly.

Materials Required but Not Provided

- Sample dilution plates or racked dilution tubes
- Precision micropipettes or multi-dispensing micropipettes capable of pipetting volumes between 50 µl and 100 µl
- Use only distilled or deionized water for preparation of the reagents used in the test
- Graduated cylinder or equivalent for preparing 1X wash solution
- Laboratory timer
- Plastic plate covers
- Humid Chamber
- 96-well microplate reader (450nm filter, reference filter between 630nm and 650nm)
- Microplate washer (manual, semi-automatic or automatic system)

The OPTI SARS-CoV-2 Antibody Test has been validated using laboratory equipment generally available through major laboratory equipment suppliers (www.vwr.com, www.fisherscientific.com as examples).

Precautions and Warnings

General

- The assay is for *in vitro* diagnostic (IVD) use under the FDA Emergency Use Authorization Only.
- For prescription use only.
- This test has not been reviewed by the FDA.
- Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
- Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Dispose of waste in compliance with local, state, and federal regulations.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- See the end of this insert for reagent hazard and precaution warnings.

Specimen Collection

Blood sample collection tubes are not included in the test kit. Obtain blood samples by venipuncture and centrifuge according to established laboratory guidelines to generate serum or plasma. Refer to CLSI guidelines GP41-A6 and GP44-A4 for the collection, processing and handling of samples.

Transporting Specimens

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens. Store specimens at 2–8°C and ship on ice packs.

Storing Specimens

Specimens can be stored at 2–8°C for up to 72 hours after collection. If a delay in testing is expected, store specimens at –20°C or lower.

Laboratory Practices

- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting, timing, and washing throughout this procedure are necessary to maintain precision and accuracy. Use a separate pipette tip for each sample and control.
- Do not expose TMB solution to strong light or any oxidizing agents. Handle TMB solution with clean glass or plastic ware.
- All wastes should be properly decontaminated prior to disposal. Dispose of contents in accordance with local, regional, and national regulations.
- Care should be taken to prevent contamination of kit components. Do not pour unused reagents back into containers.
- Do not use kit past expiration date and do not intermix components from kits with different lot numbers.

Preparation of Wash Solution

The Wash Concentrate (10X) should be brought to 18–25°C and mixed to assure dissolution of any precipitated salts. The Wash Concentrate (10X) must be diluted 1/10 with distilled/deionized water before use (e.g., 100 mL of concentrate plus 900 mL of water).

Preparation of Samples and Controls

Dilute test samples and controls two-fold (1:2) with Conjugate by diluting 60 µL of sample with 60 µL of Conjugate. Be sure to change tips for each sample and record the position of each sample. Samples should be mixed prior to dispensing into the CoV-2-coated plate. The resulting mix can be kept for a maximum of 60 min at room temperature (18–25°C) before transferring to the antigen-coated microtiter plate.

Test Procedure

All reagents must be allowed to come to 18–25°C before use. Mix reagents by gentle inverting or swirling. Return all assay components to 2–8°C after use. For all incubation steps, to minimize evaporation, cover plates with plastic plate covers and place in a humid chamber or enclosure.

1 Obtain antigen-coated plate(s) and record the sample position. If using partial plates, remove only those wells sufficient for samples to be tested. Place the remaining wells, along with the desiccant, in the extra ziplock bag provided and return to 2–8°C.

2 Dispense 100 µL of DILUTED Negative Control (NC) (DILUTED 1:2) into duplicate wells.

3 Dispense 100 µL of DILUTED Positive Control (PC) (DILUTED 1:2) into duplicate wells.

4 Dispense 100 µL of DILUTED sample (DILUTED 1:2) into appropriate wells.

5 Incubate for 60 minutes at 18–25°C.

6 Remove the solution and wash each well with approximately 300 µL of Wash Solution 5 times. Avoid plate drying between plate washings and prior to the addition of the next reagent. Tap each plate onto absorbent material after the final wash to remove any residual wash fluid.

7 Dispense 100 µL of TMB Substrate into each well.

8 Incubate for 15 minutes at 18–25°C.

9 Dispense 100 µL of Stop Solution into each well.

10 Measure and record the A(450) for samples and controls. Alternatively, plates may be read at 450nm using a reference filter between 630nm and 650nm.

11 Calculations:

Controls

$$NC\bar{x} = \frac{A1\ A(450) + A2\ A(450)}{2} \qquad PC\bar{x} = \frac{A3\ A(450) + A4\ A(450)}{2}$$

Validity criteria

$NC\bar{x} < 0.150$	$0.250 \leq PC\bar{x} < 1.00$

For invalid assays, technique may be suspect and the assay should be repeated following a thorough review of the package insert.

Samples

$$S/P = \frac{\text{Sample A(450)} - NC\bar{x}\ A(450)}{PC\bar{x} - NC\bar{x}}$$

The presence or absence of antibody to the antigen is determined by first calculating the S/P value of each sample.

12 Interpretation:

Negative	Positive
$S/P < 0.25$	$S/P \geq 0.25$

Limitations

- The assay performance characteristics have not been established for visual result determination.
- The assay performance characteristics have not been established for matrices other than those specified.
- The assay should not be used to diagnose or exclude acute infection. Results are not intended to be used as the sole basis for patient management decisions.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
- A negative result for an individual subject indicates absence of detectable anti-SARS-CoV-2 antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity.
- False positive results due to cross-reactivity with antibodies to other coronaviruses can occur.
- Assay performance characteristics have not been established for testing cord blood, for testing neonates, for prenatal screening, or for general population screening.
- Samples that are hemolyzed should be avoided for analysis with this assay.
- Results from immunosuppressed patients must be interpreted with caution.
- The assay has not been evaluated with samples collected <14 days after symptoms onset.
- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.

Performance Characteristics

Analytical Sensitivity and Specificity

Reactivity/Inclusivity

Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is exceptionally limited at present).

Cross-Reactivity

The following disease-state samples were tested on the OPTI SARS-CoV-2 Total Antibody Test to assess cross-reactivity. One (1) sample out of 108 yielded a positive result on the OPTI SARS-CoV-2 Total Antibody Test. The results are summarized in the following table.

Clinical Condition	Number tested	Number Positive
Human Coronavirus OC43	12	0
Human Coronavirus 229E	12	0
Human Coronavirus HKU1	12	0
Human Coronavirus NL63	12	0
Haemophilus influenzae	7	0
Mycoplasma pneumoniae	4	0
anti-Influenza A IgG	5	**1**
anti-Influenza B IgG	5	0
anti-Respiratory Syncytial Virus IgG	5	0
anti-Hepatitis A Virus	5	0
anti-Hepatitis B Virus	5	0
anti-Hepatitis C Virus	5	0
HIV Seropositive	10	0
RF	5	0
Antinuclear Antibodies (ANA)	4	0
Total	**108**	**1**

Interfering substances

Antibody-negative and positive SARS-CoV-2 samples spanning the test dynamic range were spiked with the following materials, at noted concentrations and tested on the OPTI SARS-CoV-2 Total Antibody Test. No false positives or false negatives were observed.

		Positive Sample		Positive Sample		Positive Sample		Negative Sample		Negative Sample	
Substance Tested	**Conc.**	**S/P**	**Result**	**S/P**	**Result**	**S/P**	**Result**	**S/P**	**Result**	**S/P**	**Result**
Control	NA	1.66	Positive	0.92	Positive	0.58	Positive	0.01	Negative	0.01	Negative
Cholesterol	30 mg/mL	1.66	Positive	1.10	Positive	0.62	Positive	0.01	Negative	0.00	Negative
Hemoglobin	10 mg/mL	1.50	Positive	0.96	Positive	0.54	Positive	0.01	Negative	0.09	Negative
Bilirubin	0.4 mg/mL	1.78	Positive	0.90	Positive	0.54	Positive	0.01	Negative	0.01	Negative

Class Specificity

Not applicable - the OPTI SARS-CoV-2 Total Antibody Test is intended for the detection of total antibody with no differentiation between different immunoglobulins.

Clinical Sensitivity/Positive Percent Agreement (PPA)

The clinical sensitivity was determined by evaluating the OPTI SARS-CoV-2 test with samples collected from a total of 155 patients where the time between onset of symptoms and blood collection was noted and from 201 patients where time post PCR result was recorded.

The following table describes the clinical sensitivity by time of sampling post Onset of Symptoms:

OPTI SARS-CoV-2 Total Antibody Results

Days from Onset of Symptoms	Total PCR Positive samples	Number Reactive	Number Non-Reactive	PPA	95% CI
</=7	0	0	0	NA	NA
8 to 14	1	0	1	0%	-2.9% ; 82.9%
>/=15	154	148	6	96.1%	91.5% ; 98.4%
Total samples	155				

The following table describes the clinical sensitivity by time of sampling post PCR positive result:

OPTI SARS-CoV-2 Total Antibody Results

Days from PCR Positive result	Total PCR Positive samples	Number Reactive	Number Non-Reactive	PPA	95% CI
</=7	2	2	0	100%	28.9% ; 100%
8 to 14	9	8	1	88.9%	54% ; 99.8%
>/=15	190	184	6	96.8%	93.1% ; 98.7%
Total samples	201				

Clinical Specificity/Negative Percent Agreement (NPA)

The clinical specificity was determined by evaluating the OPTI SARS-CoV-2 test with samples collected in 2019, prior to the appearance of SARS-CoV-2.

OPTI SARS-CoV-2 Total Antibody Results

Healthy Donors	Matrix	Total Samples	Number Reactive	Number Non-Reactive	Negative Percent Agreement (NPA)	95% CI
UK 2019	Serum	98	1	97	99.0%	93.8% ; 100%
UK 2019	Plasma	99	3	96	97.0%	91% ; 99.3%
USA 2019 #1	Serum	50	2	48	96.0%	85.6% ; 99.6%
USA 2019 #2	Plasma	50	1	49	98.0%	88.3% ; 100%
	Healthy Total	297	7	290	97.6%	95.1% ; 98.9%

Clinical condition						
2019 Collections	**Various**	**108**	**1**	**107**	**99.1%**	**94.3% ; 100%**

Grand Total 2019	**405**	**8**	**397**	**98.0%**	**96.1% ; 99.1%**

Matrix Equivalency

Serum derived from both Red top and SST collections, as well as Plasma derived from Sodium Citrate and Dipotassium EDTA were accounted for during assay development. For one clinical study, paired SST serum and Dipotassium EDTA tubes were collected from 57 unique patients. Regression analysis was performed for the comparison of K2 EDTA plasma to SST serum; results are shown in the figure below.

	K2 EDTA plasma
n	57
OD range (serum)	0.039-4.43
OD range (plasma)	0.0103-5.04
Regression equation (y = plasma, x = serum)	-0.010 + 1.0614 x
95% C.I. of intercept	-0.06342 - 0.04284
95% C.I. of slope	1.038 - 1.085
Coefficient of determination R2	0.9934

Precision Analysis

A single site precision study was undertaken and included kit controls (Negative and Positive), along with Negative, Weak Positive and Moderately Positive samples. Samples were tested in triplicate, by 2 operators, 2 times per day over 5 days, using one lot of the OPTI SARS-CoV-2 Total Antibody Test. Results are presented in the table below.

			Between Operator		Between Day		Bewtween run		Within run		Overall	
Sample	**N**	**Mean OD**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Kit Positive Control	59	0.64	0.01	1.3	0.02	2.6	0.01	1.5	0.02	3.4	0.03	4.7
Kit Negative Control	60	0.01	0.00	9.3	0.00	5.7	0.00	4.9	0.00	15.5	0.00	19.6
Negative 1	60	0.02	0.00	3.3	0.00	4.6	0.00	3.4	0.00	10.5	0.00	12.4
Positive 1	60	0.35	0.02	5.9	0.02	6.9	0.01	3.0	0.02	5.9	0.04	11.2
Positive 2	60	0.52	0.05	8.7	0.03	6.5	0.02	3.2	0.02	4.2	0.06	12.1
Positive 3	60	0.70	0.01	1.4	0.01	1.4	0.00	0.1	0.01	1.6	0.02	2.6
Positive 4	60	0.88	0.02	2.4	0.05	6.1	0.02	1.9	0.03	3.1	0.07	7.5
Positive 5	60	1.07	0.02	1.7	0.01	1.2	0.00	0.3	0.02	1.4	0.03	2.5

For technical assistance on the OPTI SARS-CoV-2 Total Antibody Test:

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Contact your IDEXX area manager or distributor or visit our website: www.optimedical.com

WARNING

H317, H412, P261, P280, P302 + P352, P333 + P313

Positive Control / Negative Control / Conjugate / Stop Solution / Wash Solution (10X) – May cause an allergic skin reaction. Harmful to aquatic life with long lasting effects. Avoid breathing mist/vapours. Wear protective gloves. IF ON SKIN: Wash with plenty of water. If skin irritation or rash occurs: Get medical advice/attention.

Symbol Descriptions

LOT	Batch Code (Lot)
SN	Serial Number
REF	Catalog Number
IVD	In vitro diagnostic
EC REP	Authorized Representative in the European Community
CONTROL +	Positive Control
CONTROL −	Negative Control
	Use by date
	Date of manufacture
	Manufacturer
	Temperature limitation
	Consult instructions for use
	Major change in the user instructions
CE	CE marking - European conformity

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