ACCESSBIO

For *in vitro* diagnostic use only
CLIA complexity: MODERATE and HIGH
Rx Use only

CareStart™

COVID-19 IgM/IgG

Rapid Diagnostic Test for the Detection of SARS-CoV-2 IgM/IgG Ab

Package Insert

(Instructions for Use)

Table of Contents

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Intended Use

The *CareStart*™ COVID-19 IgM/IgG is an immunochromatographic lateral flow assay intended for the presumptive qualitative detection and differentiation of IgM and/or IgG specific to SARS-CoV-2 in human fingerstick whole blood, venous whole blood, serum or plasma specimens from patients suspected of COVID-19 infection by a healthcare provider. The *CareStart*™ COVID-19 IgM/IgG is an aid in the diagnosis of patients with suspected SARS-CoV-2 infection in conjunction with clinical presentation and the results of other laboratory tests. Results from the *CareStart*™ COVID-19 IgM/IgG should not be used as the sole basis for diagnosis.

This testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate and high complexity tests. SARS-CoV-2 antibodies are generally detectable in human blood specimens during the acute phase of infection or resulting from the previous exposure to the virus. Positive results are indicative of active or recent infection. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities. The *CareStart*™ COVID-19 IgM/IgG is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of the lateral flow *in vitro* diagnostic tests.

Negative results do not preclude SARS-CoV-2 infection and 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.

False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies of non-SARS-CoV-2 or other possible causes.

At this time, it is unknown how long IgM or IgG antibodies may persist in human blood following the infection.

For prescription use only. For *in vitro* diagnostic use only.

Summary and Explanation of the Test

Since the first outbreak reported in December 2019, SARS-CoV-2 has spread rapidly worldwide, and the disease it causes has been named “Coronavirus Disease 2019” (COVID-19). Due to its highly contagious nature and global health crises, SARS-CoV-2 has been designated as a pandemic by the World Health Organization (WHO). SARS-CoV-2 continues to have devastating impacts on healthcare systems and the world economy including the U.S.

To effectively end the SARS-CoV-2 pandemic, systematic screening and detection of both clinical and asymptomatic COVID-19 cases is critical. Particularly, identification of subclinical or asymptomatic cases are important to reduce or stop the infection because these individuals may transmit the virus. As a point-of-care test with a 10 min testing time, *CareStart*™ COVID-19 IgM/IgG allows effective screening of COVID-19 infection in large scale.

Principles of the Test

The *CareStart*™ COVID-19 IgM/IgG test is an immunochromatographic assay for the detection and differentiation of SARS-CoV-2 IgM and/or IgG antibodies in human blood specimens. Control antibody, anti-human IgG, and streptavidin (test line for IgM) are immobilized onto a nitrocellulose membrane to form three distinct lines, the control line, IgG, and IgM test line. The nitrocellulose membrane is attached onto a plastic backing card and combined with other reagents and pads to construct a test strip. The test strip is encased inside a plastic device. Blood samples, including fingerstick whole blood, venous whole blood, serum, or plasma, can be added to the sample well of the test device to initiate a test. The sample specimens are migrating sequentially through filter pad, conjugate pad, nitrocellulose membrane, and absorbent pad. SARS-CoV-2 antibodies in sample specimens interact with the recombinant SARS-CoV-2 antigen that is conjugated to colloidal gold nanobeads and biotin to form an immune complex while it migrates through the conjugate pad. IgM reacts with the gold-conjugated SARS-CoV-2 antigen and biotin-conjugated anti-human IgM. IgG only reacts with the gold-conjugated SARS-CoV-2 antigen. The immune complexes migrate through the nitrocellulose membrane and bind to each respective test line. The IgM immune complexes bind to the streptavidin region (IgM test line) on a membrane to generate a purple-colored line to indicates IgM positive. The IgG immune complexes bind to the anti-human IgG region (IgG test line) on a membrane to generate a purple-colored line to indicates IgG positive. The control line will appear as gold-conjugated chicken IgY binds to the control antibody (anti-chicken IgY) in the control region. The test results should be interpreted 10 minutes after starting the test. The test results should not be interpreted after 15 minutes. The color intensity in the test region will vary depending on the amount of IgM and IgG present in the sample. Any faint colored line(s) in the test region(s) should be considered as positive.

The presence of two lines marked by “C” and “G” indicates SARS-CoV-2 IgG positive. The presence of two lines marked by “C” and “M”, indicates SARS-CoV-2 IgM positive. The presence of three lines “C”, “G,” and “M”, indicates positive for both SARS-CoV-2 IgG and IgM. The appearance of only the control line “C” indicates negative. If the control line does not appear, the test result is not valid. With an invalid result, it is recommended to repeat the test using a new, unopened device following the instructions.

Reagents and Materials Provided

Contents Name	Quantity (in a kit)	Description
Test device	25 each	Foil pouched test device containing one test strip which is encased on plastic device cassette.
Assay buffer vial	1 each	< 0.1% sodium azide as a preservative.
Blood transfer pipette	25 each	For blood transfer.
Package insert (instructions for use)	1 each	

* Materials not supplied

- Alcohol swab
- Pair of gloves
- Biohazard or sharps container
- Blood lancet
- Timer / Pen
- Sterile gauze or cotton

Precautions

IVD use

- For prescription use and *in vitro* diagnostic use only.
- As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
- Immediately use after opening the test device in the pouch.
- Immediately add the assay buffer to the test device after the specimen is applied.
- In order to obtain accurate results, the test must follow this package insert.
- Do not interpret the test result before 10 minutes and after 15 minutes starting the test.
- Do not use if the test device package is damaged.

Safety and handling

- Do not use the kit contents beyond the expiration date.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
- Do not eat, drink, or smoke in the area where the specimens and kit contents are handled.
- Dispose of used contents as biohazardous wastes in accordance with federal, state, and local requirements.
- Nitrile or latex gloves should be worn when performing this test.
- If the assay buffer contacts the skin or eye, flush with copious amounts of water.
- Handle all specimens as though they contain infectious agents.
- The excessive blood sample may cause false positive or invalid results.
- Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- Reagents contain sodium azide, which is harmful if inhaled, swallowed, or exposed to skin. Contact with acids produces a very toxic gas. If there is contact with skin, wash immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Contamination and inhibition

- Do not interchange kit contents from different lots.
- Do not re-use any contents in the kit as they are single-use only.

Storage and Stability

- Store the test kit as packaged between 1 ~ 30°C.
- The reagents and materials in the *CareStart*™ COVID-19 IgM/IgG are stable until the expiration date printed on the outer packaging.
- The test device must remain in the sealed pouch until use.
- Do not freeze any contents of the kit.

Quality Control

Internal Quality Control: The *CareStart*™ COVID-19 IgM/IgG contains a built-in internal procedural control that is included in the test device. A red-colored line appearing in the control region "C" is designed as an internal procedural control. The appearance of the procedural control line indicates that sufficient flow has occurred, and the functional integrity of the test device has been maintained. If the procedural control line does not develop in 10 minutes, the test result is considered invalid and retesting with a new device is recommended. If the internal procedural control line is still absent in the retest, please contact the manufacturer or distributor.

External Control: The commercially available external controls may be used to demonstrate that the test device and test procedure perform properly. It is recommended to follow the laboratory regulations or quality control procedures to perform external controls in *CareStart*™ COVID-19 IgM/IgG.

NOTE: The external controls are not included in *CareStart*™ COVID-19 IgM/IgG.

Specimen Type

Acceptable specimen types for testing with the *CareStart*™ COVID-19 IgM/IgG are human fingerstick whole blood, venous whole blood, serum, or plasma. Proper specimen collection methods must be followed. Inadequate specimen collection and/or improper specimen handling may yield a false results; therefore, specimen collection requires specific training and guidance due to the importance of specimen quality to obtain accurate test results.

Specimen Collection and Test Procedures

Specimen Collection Procedure

Procedural Notes

- U Use commercially available blood lancet and alcohol swab for specimen collection.
- Use only provided blood transfer pipette for sample loading to the test device.

- Collect the specimen wearing safety gloves to avoid contact and contamination.
- Process the test of the sample immediately after collection.
- Only those samples that are clean, clear, and with good fluidity can be used for the assay (do not use thickened or high-fat blood samples).

Fingerstick Whole Blood:

Disinfect the finger with an alcohol swab. Allow the finger to dry thoroughly prior to proceeding. Using a sterile lancet, puncture the skin of the finger and collect 10 µL blood sample using the provided blood transfer pipette to add the blood to the test device.

Venous Whole Blood:

Draw venous whole blood following the general laboratory procedures by a trained operator. Collect the blood sample in a commercially available blood collection tube containing anticoagulants including citrate, heparin, or EDTA. Swirl the tube gently as needed.

Serum:

Collect venous whole blood into a container NOT containing anticoagulants. Wait for the blood clot and separate the serum by centrifugation.

Plasma:

Collect venous whole blood into a container containing anticoagulants. Separate the plasma by centrifugation.

Specimen Storage:

It is recommended to process the test of the sample immediately after collection. If specimens cannot be tested immediately, store them at 2 to 8°C after collection up to 72 hours. The venous whole blood should be tested within 72 hours and avoid freezing the sample. The serum and plasma can be frozen at -20°C or colder for longer storage.

Test Procedures

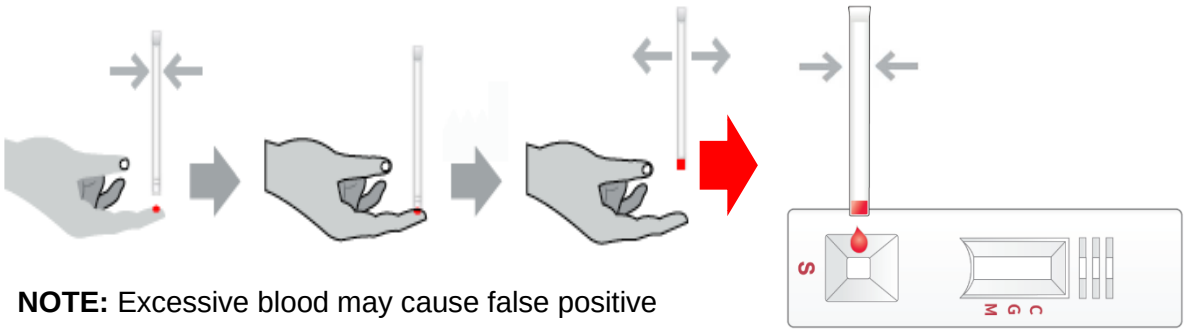
Procedural Notes

- Allow test devices, reagents, and specimens to warm up to room temperature (15~30°C) before testing.
- Remove the *CareStart*™ COVID-19 IgM/IgG test device from its foil pouch immediately before testing.
- The *CareStart*™ COVID-19 IgM/IgG kit IS INTENDED to be used only with human fingerstick whole blood, venous whole blood, serum or plasma specimens.

1. Place a device on a clean, flat surface after removing it from the pouch. Write the patient's ID on the device if required.

2. ***a) Transfer Fingerstick Whole Blood:***

Press the top part of the provided blood transfer pipette and touch the blood by the pipette tip while pressing the pipette. Fill the pipette with blood sample up to **the blue marked line (approximately 10 µl)** by releasing the press slowly. Add the blood sample to the **sample well "S"** of the test device by pressing the top part of the blood transfer pipette.

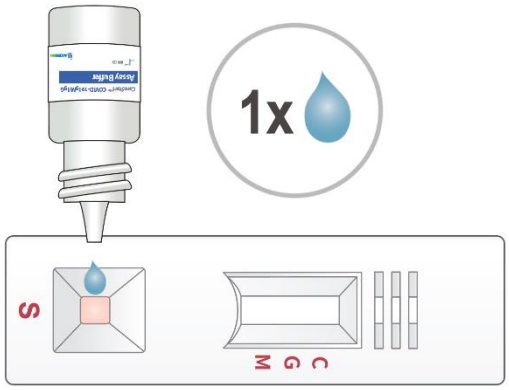


NOTE: Excessive blood may cause false positive or invalid test results.

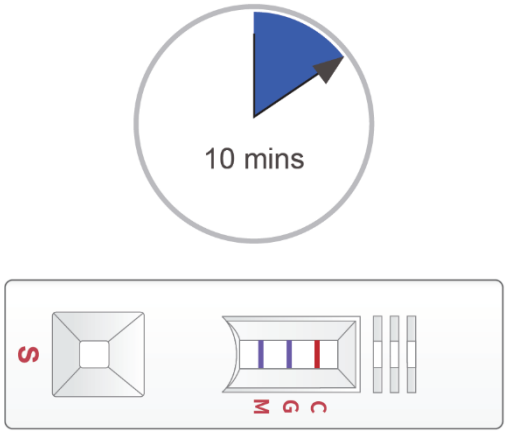
b) Transfer Venous Whole Blood, Serum, and Plasma:

Collect **10 µl** of the **venous whole blood, serum, or plasma** sample using a micropipette. Add the 10 µl collected sample to the **sample well "S"** of the test device.

3. Open the cap and invert the assay buffer vial and hold vertically above the sample well. Squeeze the vial gently to add **one (1) drop** of the assay buffer solution to the **sample well "S"** immediately after sample loading.



4. Start a timer. Read and interpret the test results **after 10 minutes**. Do not interpret the test result after 15 minutes.



Interpretation of Results

NOTE: The test results should be read and interpreted 10 minutes after the sample application and the reading and interpretation of the results should not exceed 15 minutes. The test results should not be interpreted using any instruments.

IgM Positive: two distinct lines appear.

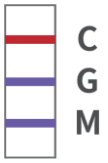
One red-colored line next to "C" and one purple-colored line next to "M" indicates SARS-CoV-2 IgM positive result.

IgG Positive: two distinct lines appear.

One red-colored line next to "C" and one purple-colored line next to "G" indicates SARS-CoV-2 IgG positive result.

IgM/IgG Positive: three distinct lines appear.

One red-colored line next to "C", one purple-colored line next to "M", and one purple-colored line next to "G" indicates SARS-CoV-2 IgM and IgG positive result.



Result with faint colored line(s):

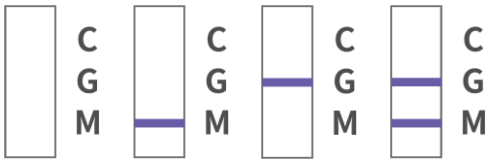
The color intensity in the test region will vary depending on the amount of IgM and IgG present in the sample. Any faint colored line(s) in the test region(s) should be considered as positive.

Negative:

Only one line next to "C" indicates a negative result.

Invalid: no control line appears.

If the control line "C" is not visible, the result is invalid. It is recommended that the specimen be re-tested. If the same invalid result persists, contact the manufacturer or distributor before continuing the test.



Limitations

1. This test has not been reviewed by the FDA.
2. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic test should be considered to rule out infection in these individuals.
3. Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to determine infection status.
4. This test may complement the diagnostic accuracy of quantitative polymerase chain reaction (qPCR) tests, but it is not meant to compare this test's clinical sensitivity and specificity with those of a molecular test since the performance of these tests is affected by virus titers and patient's immunity against SARS-CoV-2.
5. 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.
6. This test will only indicate the presence of SARS-CoV-2 IgM and/or IgG antibodies in the specimen from both viable and non-viable SARS-CoV-2 virus.
7. The detection of SARS-CoV-2 IgM/IgG antibodies is dependent upon proper specimen collection, handling, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
8. Results from the device should be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
9. This device has been evaluated for use with human specimen material only.
10. False-negative results may occur if the concentration of the target antibodies in the clinical specimen is below the detection limits of the device.
11. This device is a qualitative test and does not provide information on the viral load or antibody concentration present in the specimen.
12. This test cannot rule out diseases caused by other bacterial or viral pathogens.
13. The prevalence of infection will affect the test's predictive values.
14. Positive and negative predictive values are highly dependent on prevalence. False-negative test results are more likely during peak activity when the prevalence of the disease is high. False-positive test results are more likely during the periods of low SARS-CoV-2 activity when prevalence is moderate to low.
15. This device should not be used for the screening of blood donated by individuals.

Performance Characteristics

Clinical Evaluation

Due to the difficulty of conducting a prospective study, the clinical performance of *CareStart*™ COVID-19 IgM/IgG was evaluated using retrospectively collected SARS-CoV-2 serum samples. A total of 47 positive and 50 negative specimens were collected from subjects in Korea University Guro Hospital, Republic of Korea, with an IRB approval. A total of 21 positive and 20 negative specimens collected from the U.S patients were purchased from Cantor BioConnect. All the collected samples were confirmed by commercially available SARS-CoV-2 RT-PCR as a comparator method.

All the negative and positive samples were tested in a blinded fashion. Each sample was assigned with a unique subject identification code during preparation and randomized prior to the testing. The expected results of the samples were completely blinded to the operators in this study. All the samples were tested according to the *CareStart*™ COVID-19 IgM/IgG testing procedures.

A total of 138 samples were considered evaluable for the clinical evaluation study. The performance of the *CareStart*™ COVID-19 IgM/IgG was compared to the comparator method and presented in the table below:

***CareStart*™ COVID-19 IgM/IgG Performance against the Comparator Method**

CareStart™ COVID-19 IgM/IgG		Comparator (RT-PCR)		
		Positive	Negative	Total
Positive	IgG+/IgM+	43	0	43
	IgG+/IgM-	4	1	5
	IgG-/IgM+	15	1	16
Negative	IgG-/IgM-	6	68	74
Subtotal		68	70	138
Positive Percent Agreement (PPA)		91.2% (95% CI: 82.1% – 95.9%)		
Negative Percent Agreement (NPA)		97.1% (95% CI: 90.2% – 99.2%)		

A total of 68 positive samples were collected from 33 symptomatic individual subjects to observe any potential seroconversion status. Serum samples were collected and tested from 1 to 32 days after the provided symptom onset date. The evaluation study results are presented in the table below:

CareStart™ COVID-19 IgM/IgG Performance against Days from Symptom Onset to Blood Collection

Days from symptom onset to blood collection	Number of RT-PCR positive samples	*CareStart*™ COVID-19 IgM/IgG performance against days from symptom onset to blood collection							
		IgM – / IgG –		IgM + / IgG –		IgM + / IgG +		IgM – / IgG +	
		%	Count	%	Count	%	Count	%	Count
≤7 days	28	11%	3/28	11%	3/28	71%	20/28	7%	2/28
8-14 days	13	8%	1/13	8%	1/13	69%	9/13	15%	2/13
15-21 days	10	10%	1/10	0%	0/10	70%	7/10	20%	2/10
22-28 days	11	0%	0/11	0%	0/11	27%	3/11	73%	8/11
29-32 days	6	17%	1/6	0%	0/6	67%	4/6	17%	1/6
Total	**68**		**6**		**4**		**43**		**15**

While the sample size was relatively small (N = 68), the profile of IgM and IgG positivity seemed to show an expected host immune response. For example, in the acute stage of the infection (≤ 7 days post-symptom onset), 11% (3/28) was negative, 11% (3/28) IgM positive, 71% (20/28) IgM/IgG positive, and 7% (2/28) IgG positive. As time lapsed since the symptom onsets, most of the samples became either IgM/IgG or IgG positive.

Cross-Reactivity (Exclusivity)

Cross-reactivity of the *CareStart*™ COVID-19 IgM/IgG was evaluated by testing the serum samples containing antibodies to non-SARS-CoV-2 pathogens listed in the table below. A total of five individual samples were tested for each non-SARS-CoV-2 pathogen. All the serum samples tested as negative showed no cross-reactivity on the *CareStart*™ COVID-19 IgM/IgG as presented in the table below:

- Influenza A and B
- HCV
- HBV
- HIV
- Dengue virus
- Antinuclear antibodies (ANA)
- Respiratory syncytial virus
- Syphilis

Interference Substances

To assess substances with the potential to interfere with the performance of the *CareStart*™ IgM/IgG, SARS-CoV-2 IgM, IgG positive, and negative samples were tested with the addition

of potentially interfering substances. The *CareStart*™ IgM/IgG test performance was not affected by any of eight potentially interfering substances tested.

- Acetaminophen
- Acetylsalicylic acid
- Albendazole
- Chloroquine diphosphate
- HAMA
- Hemoglobin
- Ibuprofen
- Rifampicin

The interfering effects of biotin concentrations ranging between 10 ng/ml and 100 µg/ml were tested in a separate study. Biotin concentrations up to 2.5 µg/ml did not lead to false results. Biotin concentrations >5 µg/ml can cause false-negative IgM results with the *CareStart*™ COVID-19 IgM/IgG. None of the IgG positive samples tested produced false negative in all biotin concentrations tested.

Matrix Equivalency

The matrix equivalency study was conducted to determine the specimen types that can be used with *CareStart*™ IgM/IgG. This equivalence study was designed to compare the performance of the *CareStart*™ IgM/IgG in human whole blood, serum or plasma. The SARS-CoV-2 positive and negative serum samples were spiked into the human negative whole blood, serum or plasma to prepare the contrived samples of IgG and IgM. All demonstrated positive and negative contrived sample types, including whole blood, serum, and plasma, were equivalence in *CareStart*™ COVID-19 IgM/IgG.

Test line	Test results (# of positive / # of replicate)							
	Fingerstick whole blood		Venous whole blood		Serum		Plasma	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
IgM/IgG positive sample	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
IgM/IgG negative sample	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Technical Support

For questions, or to report a problem, please call Technical Support at +1-888-898-1270 (Available Hours: Mon. to Fri.: 8 a.m. – 5 p.m.) or TShelp@accessbio.net (24/7 available).

Test system problems may also be reported to the FDA using the MedWatch reporting system (phone: 1-800 FDA-1088; fax: 1-800 FDA-1078: or http://www.fda.gov/medwatch).

Description of Symbols

Symbol	Descriptions
IVD	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
	Consult instructions for use Indicates the need for the user to consult the instructions for use.
	Manufacturer Indicates the medical device manufacturer.
LOT	Batch code Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Do not re-use Indicates a medical device that is intended for one use, or uses on a single patient during a single procedure.
	Use by date Indicates the date after which the medical device is not to be used.
Rx	Prescription-only

Symbol	Descriptions
REF	Catalog number Indicates the manufacturer's catalog number so that the medical device can be identified.
	Caution Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
	Date of manufacture Indicates the date when the medical device was manufactured.
	Temperature limit Indicates the temperature limits to which the medical device can be safely exposed.
	Do not use if the package is damaged Indicates a medical device that should not be used if the package has been damaged or opened.
Σ	Contains sufficient for <n> tests Indicates the total number of IVD tests that can be performed with the IVD.

Manufacturer:
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Tel: 732-873-4040
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