truvian™

Easy Check COVID-19 IgM/IgG™

Rapid Diagnostic Test for the Detection of

SARS-CoV-2 IgM/IgG Ab

REF **COV-200601**

For use under Emergency Use Authorization only
For *in vitro* diagnostic use only
For prescription use only
CLIA complexity: MODERATE and HIGH

Package Insert

(Instructions for Use)

Intended Use

Easy Check COVID-19 IgM/IgG™ is an immunochromatographic lateral flow assay intended for the qualitative detection and differen-tiation of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies to SARS-CoV-2 in human serum, plasma (sodium citrate, sodium heparin, or dipotassium EDTA), and venous whole blood (sodium citrate, sodium heparin, or dipotassium EDTA). Easy Check COVID-19 IgM/IgG 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 for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. Easy Check COVID-19 IgM/IgG should not be used to diagnose acute SARS-CoV-2 infection. This testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform moderate or high complexity tests.

Results are for the detection of SARS-CoV-2 antibodies. IgM and IgG antibodies 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 serocon-version.

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

The sensitivity of **Easy Check** COVID-19 IgM/IgG 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 for Easy Check COVID-19 IgM/IgG may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Due to the risk of false positive results, confirmation of positive results should be considered using a second, different SARS-CoV-2 IgG or IgM assay.

Easy Check COVID-19 IgM/IgG is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of the Test

Coronavirus disease 2019 (COVID-19) is a respiratory illness that can spread from person to person. This antibody test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, the virus that causes COVID-19, indicating recent or prior infection, by detecting antibodies to SARS-CoV-2 in human blood specimens. Although not everyone who is infected will develop an antibody response, appropriately validated serology tests, when used broadly, can be useful in understanding how many people have developed an adaptive immune response to the virus and how far the pandemic has progressed. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity.

Principles of the Test

Easy Check is an immunochromato-graphic 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, the IgG test line, and the IgM test line. The nitrocellulose membrane is attached onto a plastic backing card and combined with the other reagents and pads to construct a test strip. The test strip is encased inside a plastic device. Blood samples, including human serum, plasma (sodium citrate, sodium heparin, or dipotassium EDTA), and venous whole blood (sodium citrate, sodium heparin, or dipotassium EDTA), are added to the sample well of the test device to initiate a test. The sample specimens migrate sequen-tially 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 (SARS-CoV-2 nucleocapsid and spike protein S1 RBD) that is conjugated to colloidal gold nanobeads and biotin-conjugated anti-human antibodies to form an immune complex while it migrates through the conjugate pad. IgM antibodies react with the gold-conjugated SARS-CoV-2 antigen and biotin-conjugated anti-human IgM. IgG antibodies only react 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, "M") on the membrane to generate a purple-colored line to indicate a positive IgM result. The IgG immune complexes bind to the anti-human IgG region (IgG test line, "G") on the membrane to generate a purple-colored line to indicate a positive IgG result. The gold-conju-gated chicken IgY migrates through the membrane and binds to the control antibody (anti-chicken IgY) in the control region to generate a red-colored line (control line, "C"). The test results should be interpreted 10 minutes after addition of buffer to the sample well. The test results should not be interpreted after 15 minutes. The color intensity in the test region will vary. 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 a SARS-CoV-2 IgG positive result. The presence of two lines marked by "C" and "M", indicates a SARS-CoV-2 IgM positive result. The presence of three lines "C", "G," and "M", indicates positive results 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, regardless of the presence of "G" or "M" test lines, 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 bottle	1 each	Na_2CO_3, < 0.1% sodium azide as a preservative.
Blood transfer pipette	25 each	For blood transfer.
Package insert	1 each	Instructions for use

*Materials not supplied

- 20 µl micropipette
- Timer
- pair of gloves
- External positive and negative controls (available for purchase separately)

Warnings and Precautions

- For prescription and *in vitro* diagnostic use only. For Use under an Emergency Use Authorization Only.
- This test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform moderate or high complexity tests.
- This test has been authorized only for the presence of IgM and IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmet-ic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- 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 following the addition of buffer to the sample well.
- Do not use if the test device package is damaged.
- Do not use the kit contents beyond the expiration date.
- Do not eat, drink, or smoke in the area where the specimens and kit contents are handled.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
- 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.
- Adding additional blood sample volume to the sample well 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.
- Do not interchange kit contents from different lots.
- Do not re-use any contents in the kit (e.g., blood transfer pipette, test cassette) 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 Easy Check COVID-19 IgM/IgG are stable until the expiration date printed on the outer packaging. Do not use beyond the expiration date.
- The test device must remain in the sealed pouch until use.
- Do not freeze any contents of the kit.

Quality Control

Internal Quality Control:

Easy Check COVID-19 IgM/IgG contains a built-in internal procedural control 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:

It is recommended to follow the laboratory regulations or quality control procedures to perform external controls in Easy Check COVID-19 IgM/IgG. Controls are available through Access Bio under catalog number: # SCLM-02571 or SCLM-10071.

NOTE: The external controls are available for separate purchase.

- Positive External Control: Mixture of human chimeric SARS-CoV-2 IgM and IgG spike S1 antibodies in heat inactivated SARS-CoV-2 antibody negative confirmed serum.
- Negative External control: Heat inactivated SARS-CoV-2 antibody negative confirmed serum.

Specimen Type

Acceptable specimen types for testing with the Easy Check COVID-19 IgM/IgG test are human serum, plasma (sodium citrate, sodium heparin, or dipotassium EDTA), and venous whole blood (sodium citrate, sodium heparin, or dipotassium EDTA). Proper specimen collection methods must be followed. Inadequate specimen collection and/or improper specimen handling may yield 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 Handling Procedures

Procedural Notes

- Collect the specimen wearing safety gloves to avoid contact and contamination.
- **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 anticoag-ulants including sodium citrate, sodium heparin, or dipotassium 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 anticoagu-lants (sodium citrate, sodium heparin, or dipotassium EDTA). Separate the plasma by centrifugation.

1. Easy Check COVID-19 IgM/IgG can be performed using human serum, plasma (sodium citrate, sodium heparin, or dipotassium EDTA), and venous whole blood (sodium citrate, sodium heparin, or dipotassium EDTA).

2. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.

3. Testing should be performed immediately after specimen collection. Do not leave the serum and plasma specimens at room temperature beyond 8 hours. Serum and plasma specimens may be stored at 2-8°C for up to 48 hours. For long term storage, serum and plasma specimens should be kept below -20°C for up to one month. It is recommended to test whole blood specimens immediately after blood collection.

4. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens cannot be frozen and thawed more than once.

5. If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

Test Procedures

Procedural Notes

- Allow test devices, reagents, and specimens to equilibrate up to room temperature (15~30°C) prior to testing.
- Remove the Easy Check COVID-19 IgM/IgG test device from its foil pouch immediately before testing.
- The Easy Check COVID-19 IgM/IgG kit IS AUTHORIZED to be used only with human serum, plasma (sodium citrate, sodium heparin, or dipotassium EDTA), and venous whole blood (sodium citrate, sodium heparin, or dipotassium EDTA) specimens.
- Use only provided blood transfer pipette or micropipette for sample loading to the test device.

Description of Symbols

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 **Batchcode**
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.

Prescription-only

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.

Contains sufficient for <n> tests
Indicates the total number of IVD tests that can be performed with the IVD.

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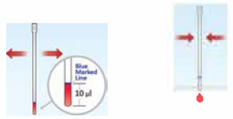
Document number: IFU-0003 Revision number: A Effective date: 2020-07-28

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. Transfer venous whole blood, serum or plasma sample:

a) using a provided blood transfer pipette:

Press the top part of the provided blood transfer pipette and touch the sample by the pipette tip while pressing the pipette. Releasing the press slowly to fill the pipette with sample up to **the blue marked line (approximately 10 µl).** 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) using a micropipette:

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

3. Open the cap and invert the assay buffer bottle and hold vertically above the sample well. Squeeze the bottle 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 the result **at 10 minutes.** The test results should not be read earlier than 10 minutes. Test results should not be read after 15 minutes.

Interpretation of Results

NOTE: The test results should be read and interpreted not earlier than 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):

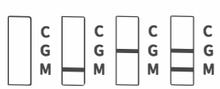
The color intensity in the test region will vary. Any faint colored line(s) in the test region(s) should be considered as positive.

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. Re-run the test using a new test device. If the same invalid result persists, contact the manufacturer or distributor before continuing to test samples.



Limitations

1. For use under an Emergency Use Authorization only.

2. Use of the Easy Check COVID-19 IgM/IgG is limited to laboratory personnel who have been trained. Not for home use or point of care (POC) use.

3. The test is limited to the qualitative detection of anti-COVID-19 antibody levels in human serum and ACD plasma samples and does not indicate the quantity of the antibodies. The intensity of the test line does not necessarily correlate to SARS-CoV-2 antibody titer in the specimen.

4. The test results should be interpreted 10 minutes after starting the test. The test results should not be interpreted after 15 minutes.

5. This test can only be used for the analysis of human serum, plasma (sodium citrate, sodium heparin, or dipotassium EDTA), and venous whole blood (sodium citrate, sodium heparin, or dipotassium EDTA) samples. Do not use Easy Check COVID-19 IgM/IgG with fingerstick (capillary) whole blood samples.

6. Negative results do not preclude SARS-CoV2 infection and should not be used as the sole basis for patient management decisions. IgM antibodies may not be detected in the first several days of infection; the sensitivity of the Rapid COVID-19 IgM/IgG Combo Test Kit early after infection is unknown. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

7. A negative result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody used in the test.

8. The test may have lower sensitivity for IgG and IgM detection in symptomatic individuals prior to 15 days since symptom onset.

9. Direct testing with a molecular diagnostic test should be performed to evaluate for acute SARS-CoV-2 infection in symptomatic individuals.

10. Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection or to determine infection status.

11. It is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity.

12. Positive results may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for an alternative serology test to confirm an adaptive immune response. 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.

13. This test will only indicate the presence of SARS-CoV-2 IgM and/or IgG antibodies in the specimen.

14. 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.

15. This device has been evaluated for use with human specimen material only.

16. This test cannot rule out diseases caused by other bacterial or viral pathogens.

17. This device should not be used for the screening of donated blood.

Conditions of Authorization for Laboratories

The Easy Check COVID-19 IgM/IgG Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and other authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

Authorized Laboratories using Easy Check COVID-19 IgM/IgG ("your product" in the conditions below), must adhere to the Conditions of Authorization indicated in the Letter of Authorization are listed below:

1. Authorized laboratories using Easy Check COVID-19 IgM/IgG will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

2. Authorized laboratories using Easy Check COVID-19 IgM/IgG will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Easy Check COVID-19 IgM/IgG are not permitted.

3. Authorized laboratories that receive Easy Check COVID-19 IgM/IgG will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

4. Authorized laboratories using Easy Check COVID-19 IgM/IgG will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

5. Authorized laboratories will collect information on the performance of Easy Check COVID-19 IgM/IgG and report to Division of Microbiology Devices (DMD)/Office of Health Technology7 (OHT7)-(Office of In Vitro Diagnostics and Radiological Health (OIR)/Office of Product Evaluation and Quality (OPEQ)/Center for Devices and Radiological Health (CDRH) (via email: CDRH EUA Reporting@fda.hhs.gov) and Access Bio, Inc. Technical Support (via email: TShelp@accessbio.net) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the assay of which they become aware.

6. All laboratory personnel using Easy Check COVID-19 IgM/IgG must be appropriately trained in immunochromatographic techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use tEasy Check COVID-19 IgM/IgG in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the Easy Check COVID-19 IgM/IgG .

7. Access Bio, Inc., authorized distributors, and authorized laboratories using Easy Check COVID-19 IgM/IgG will ensurethat any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[a] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform moderate or high complexity tests" as "authorized laboratories".

Performance Characteristics

Clinical Agreement

The clinical performance of Easy Check COVID-19 IgM/IgG was evaluated using retrospectively collected SARS-CoV-2 serum and plasma samples at 3 sites by 10 operators in the U.S. A total of 246 samples, 211 plasma (47 positive and 164 pre-COVID) and 35 serum (17 positive and 18 negative), collected from the U.S were tested in this study. A total of 164 pre-COVID samples were collected before December 2019 in the U.S. All the collected serum and plasma samples were confirmed by the FDA EUA authorized SARS-CoV-2 RT-PCR methods as comparators.

All the negative and positive samples were tested in a blinded fashion. Each sample was assigned with a unique subject identifica-tion code during collection 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 Easy Check COVID-19 IgM/IgG testing procedures.

A total of 246 samples were considered evaluable in this study.

Easy Check COVID-19 IgM/IgG Performance against the Comparator Methods

For IgG antibody detection, the positive percent agreement (PPA) of Easy Check COVID-19 IgM/IgG was 96.88% (62/64) (95% CI of 89.30 – 99.13%) and negative percent agreement (NPA) was 99.45% (181/182) (95% CI of 96.95 – 99.90%). For IgM antibody detection, the PPA was 89.06% (57/64) (95% CI of 79.10 - 94.60%) and NPA was 99.45% (181/182) (95% CI 96.95 - 99.90%).

The overall PPA (either IgG positive or IgM positive counted as positive) was 98.44% (63/64) (95% CI of 91.67 – 99.72%) and overall NPA was 98.90% (180/182) (95% CI 96.08 – 99.70%).

IgG results stratified by days post-onset of symptoms

Days from Symptom Onset	Total number of samples	Non-reactive	Reactive	PPA	95% CI
0 - 7	-	-	-	-	-
8 - 14	1	0	1	100% (1/1)	20.65 – 100%
≥ 15	62	2	60	96.77% (60/62)	88.98 – 99.11%
Unknown	1	0	1	100% (1/1)	20.65 – 100%

IgM results stratified by days post-onset of symptoms

Days from Symptom Onset	Total number of samples	Non-reactive	Reactive	PPA	95% CI
0 - 7	-	-	-	-	-
8 - 14	1	0	1	100% (1/1)	20.65 – 100%
≥ 15	62	7	55	88.71% (55/62)	78.48 - 94.42%
Unknown	1	0	1	100% (1/1)	20.65 – 100%

Independent Clinical Agreement Validation

The Easy Check COVID-19 IgM/IgG from Truvian was tested on Jun 2, 2020 at the Frederick National Laboratory for Cancer Research (FNLCR) sponsored by the National Cancer Institute (NCI). The test was validated against a panel of previously frozen samples consisting of 30 SARS-CoV-2 antibody-positive serum samples and 80 antibody-negative serum and plasma samples. Each of the 30 antibody-positive samples was confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 30 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the Easy Check COVID-19 IgM/IgG. The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers.

All antibody-negative samples were collected prior to 2020 and include: i) Seventy (70) samples selected without regard to clinical status, "Negatives" and ii) Ten (10) samples selected from banked serum from HIV+ patients, "HIV+". Testing was performed by one operator using one lot of the Easy Check COVID-19 IgM/IgG. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).

For the evaluation of cross-reactivity with HIV+, it was determined whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman). The results and data analysis are shown in the tables below:

	Comparator Method Antibody Positive			Collected pre-2020 Antibody Negative		
CareStart™ COVID-19 IgM/IgG	IgM+, IgG+	IgM+, IgG-	IgM-, IgG+	Negative	HIV+	Total
IgM+, IgG+	27					27
IgM+, IgG-				1	0	1
IgM-, IgG+	3			1		4
IgM-, IgG-				68	10	78
Total	30			70	10	110

Measure	Estimate	Confidence Interval
IgM Sensitivity	90.0% (27/30)	74.4%; 96.5%
IgM Specificity	98.8% (79/80)	93.3%; 99.8%
IgG Sensitivity	100% (30/30)	88.7%; 100%
IgG Specificity	98.8% (79/80)	93.3%; 99.8%
Combined Sensitivity	100% (30/30)	88.7%; 100%
Combined Specificity	97.5% (78/80)	91.3%; 99.3%
Combined PPV for prevalence = 5.0%	67.8%	35%; 88.4%
Combined NPV for prevalence = 5.0%	100%	99.4%; 100%
Cross-reactivity with HIV+	0.0% (0/10), not detected	-

Important limitations of the study:

1. Samples were not randomly selected, and sensitivity and specificity estimates may not be indicative of the real-world performance of the device
2. These results are based on serum and plasma samples only and may not be indicative of performance with other sample types, such as whole blood, including finger stick blood.
3. Information about anticoagulants used is not known.
4. The number of samples in the panel is a minimally viable sample size that still provides reasonable estimates and confidence intervals for test performance, and the samples used may not be representative of the antibody profile observed in patient populations.

Cross-Reactivity (Exclusivity)

The cross-reactivity study was conducted by testing a total of 87 plasma or serum samples, including 14 non-SARS-CoV-2 pathogens and one (1) autoantibody. All the plasma and serum samples tested as negative showed no cross-reactivity and resulted in 100% agreement between Easy Check COVID-19 IgM/IgG test result and the expected result as presented in the table below:

Samples	Sample number and type	Test results (# ofpositive / # of replicate)	
		IgM Results	IgG Results
Anti-Influenza A	5 plasma	0/5	0/5
Anti-Influenza B	5 plasma	0/5	0/5
Anti-HCV	8 plasma, 2serum	0/10	0/10
Anti-HBV	5 serum	0/5	0/5
Anti-229E (alpha coronavirus)	3 plasma	0/3	0/3
Anti-NL63 (al pha coronavirus)	7 plasma	0/7	0/7
Anti-OC43 (beta coronavirus)	9 plasma	0/9	0/9
Anti-HKU1 (beta coronavirus)	5 plasma	0/5	0/5
Antinuclear antibodies (ANA)	5 serum	0/5	0/5
Anti-res piratory syncytialvirus	1 plasma, 5serum	0/6	0/6
Anti-HIV	10 plasma	0/10	0/10
Anti-Dengue virus	5 plasma	0/5	0/5
Anti-T. pallidum (Sy philis)	5 serum	0/5	0/5
Anti-Rhinovirus	2 plasma	0/2	0/2
Anti-Hepatitis B	5 plasma	0/5	0/5

Interfering Substances

To assess substances with the potential to interfere with the performance of Easy Check COVID-19 IgM/IgG, SARS-CoV-2 IgM, IgG positive, and negative samples were tested with the addition of potentially interfering substances. The Easy Check COVID-19 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 Easy Check COVID-19 IgM/IgG. None of the IgG positive samples tested produced false negative in all biotin concentrations tested.

Class Specificity

Easy Check COVID-19 IgM/IgG was evaluated to determine that the assay accurately detects each SARS-CoV-2 IgM and IgG antibody class on its corresponding test lines. A total of five (5) IgM and IgG positive serum samples were treated with DTT to determine class specificity of the test. All samples treated with DTT showed no visible IgM line with the Easy Check COVID-19 IgM/IgG, whereas the IgG results were not affected by DTT treatment. IgM and IgG results after DTT treatment showed 100% agreement to the expected results.

Matrix Equivalency

The matrix equivalency study was performed by spiking SARS-CoV-2 IgM/IgG positive sample into negative sample matrices for serum, venous whole blood, and plasma using different anticoagulants. All testing matrices were collected from the same donor and a total of five donors were evaluated in Easy Check COVID-19 IgM/IgG. The venous whole blood samples were collected from each individual in four different containers to prepare serum (no anticoagulant), and three matrices each of venous whole blood (sodium citrate, sodium heparin, and dipotassium EDTA) and plasma (sodium citrate, sodium heparin, and dipotassium EDTA). To prepare positive sample panels, each sample matrix was spiked with SARS-CoV-2 IgM/IgG positive serum sample at low positive and moderate positive levels and randomized for testing. The samples were tested in duplicate with Easy Check COVID-19 IgM/IgG. All the test results of seven different matrices from the individual showed 100% agreement to the expected results.

Sample type	Test results (# of positive / # of replicate (% agreement))					
	LP results		MP results		Negative results	
	IgM	IgG	IgM	IgG	IgM	IgG
Venous whole blood (EDTA)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	0/10 (100%)	0/10 (100%)
Venous whole blood (Heparin)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	0/10 (100%)	0/10 (100%)
Venous whole blood (Citrate)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	0/10 (100%)	0/10 (100%)
Plasma (EDTA)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	0/10 (100%)	0/10 (100%)
Plasma (Heparin)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	0/10 (100%)	0/10 (100%)
Plasma (Citrate)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	0/10 (100%)	0/10 (100%)
Serum	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	0/10 (100%)	0/10 (100%)

Technical Support

For questions, or to report a problem, please call Technical Support at **+1-888-744-8948** (Available Hours: Mon. to Fri.: 8 a.m. – 5 p.m.) or **techsupport@truvianhealth.com**

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).