AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA

Instruction for Use

May. 2022(REV08)

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CE

IVD **For In Vitro Diagnostic Use**

REF **RCE0001K00**

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Table of Contents

For in vitro diagnostic use only.

For prescription use only.

For use under emergency use authorization only.

[INTENDED USE]

The AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma (dipotassium EDTA, sodium heparin, or sodium citrate). The AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA 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. The AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA should not be used to diagnose or exclude 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, that meet requirements to perform high complexity tests.

Results are for the detection of SARS CoV-2 IgG antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies is 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 results to the appropriate public health authorities.

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 AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

Samples should only be tested from individuals who are 15 days or more post symptom onset.

The AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA is only for use under the Food and Drug Administration's Emergency Use Authorization.

[PRINCIPLE]

AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA is a one-step antigen capture format ELISA for qualitative detection of anti-SARS-CoV-2 IgG(RBD) antibodies in human serum and plasma.

First reaction: SARS-CoV-2 IgG(RBD) antibodies in test sample are incubated with sample dilution buffer and recombinant SARS-CoV-2 Receptor Binding Domain (RBD) bound on microwell.

Second reaction: After washing, SARS-CoV-2 IgG(RBD) antibodies from sample bound to antigen on microwell are detected by secondary antibody conjugated to HRP (anti-human IgG conjugated with peroxidase).

Substrate color development reaction: Tetramethylbenzidine (TMB) solution containing hydrogen peroxide is added to the well. If the detection antibody is present, the TMB will oxidize resulting in light blue color development dependent upon the amount of bound detection antibody. The substrate reaction is stopped by adding 1N sulfuric acid. The intensity of color is measured with ELISA reader (spectrophotometer) at 450nm.

[COMPOSITION]

Mark	Reagents	Components	Format
Microwell Plate	12 Antigen coated micro-well plate strip in a frame, ready for use	Recombinant SARS CoV-2 RBD protein	5 plates
Conjugate SOLN	Conjugated antibody solution, ready for use	Poly anti-human IgG conjugated with peroxidase (>0.5 ug/ml), PBS buffer	75 ml, 1 bottle
Sample DIL	Sample dilution buffer, ready for use	PBS buffer, with protein stabilizer (Bovine Serum Albumin)	75ml, 1 bottle
TMB CONC	TMB Solution (101X)	TMB in DMSO (> 1mg/ml)	0.8 ml 1 bottle
Substrate DIL	Substrate dilution buffer	Sodium acetate buffer, H_2O_2 (< 0.1%)	75ml, 1 bottle
Stop SOLN	Stop Solution, ready for use	1N Sulfuric acid	75ml, 1 bottle
Wash SOLN	Washing Solution(20X)	PBS buffer (20X)	110 ml 1 bottle
Control +	Positive Control	Human Serum or plasma (< 50%), PBS buffer	0.8 ml
Control -	Negative Control	Human Serum or plasma (< 50%), PBS buffer	1.3 ml
Calibrator	Calibrator	Human Serum or plasma (< 50%), PBS buffer	1.3 ml
	Plate sealing film	-	10 each
	Protective foil pouch	-	1 each

[MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED]

1. Adjustable micro pipettes and filtered tips
   i. 1~10µl
   ii. 20~200µl
   iii. 200~1000µl
2. ELISA washer
   i. Recommended. Washing of the microwell plates can also be carried out manually
3. ELISA Reader
   i. Wavelength of 450nm
   ii. Reference wavelength range of 650nm
4. Vortex mixer
5. Table top centrifuge
6. Incubator (37°C)
7. Distilled water
8. Corning tube (15ml, 50ml)
9. Micro tube (1.5ml)
10. Digital timer
11. Lab wipes
12. Disposable gloves

[TEST PROCESS]

1. SPECIMEN PREPARATION

- Procure the appropriate sample type
  a. 10µl of either serum or plasma (K2 EDTA, Sodium Heparin, Sodium Citrate) samples can be used in the assay.
     i. If samples are frozen, they must be completely thawed at room temperature prior to testing.
     ii. Solid materials, such as blood corpuscles or blood coagulation components, should be removed by centrifugation prior to use.
- Sample should be properly mixed for 5 to 10 seconds using vortex mixer before centrifugation.

Important:

- Avoid using samples containing inhibitors of horseradish peroxidase (HRP) (e.g. sodium azide), which may result in false-negative.
- Pay close attention to sample quality as hemolysis may lead to inaccurate results.

2. TEST PREPARATION

Preparation of washing solution

① Before beginning analysis, prepare necessary amount of washing solution with 20X washing concentrate and dilute it to 20-fold with distilled or deionized water at room temperature.

② For each well, approximately 3mL of washing solution is used but additional volume may be needed depending on the washing method or instruments used.

$$\text{1X Washing solution volume} = 3\ \text{ml} \times (\text{sample number} + 6)$$

⚠ **Caution**

- Use distilled or deionized water only, with exception to any deionized water prepared by polystyrene ion-exchange resin because such preparation may result in inactivation of HRP.
- Avoid using metal containers for storage.

Preparation of substrate solution (TMB)

① Dilute proper amount of Tetramethylbenzidine (TMB) concentrate with substrate dilution buffer in accordance with the number of wells which will be used before using substrate solution.

② For each well, 100µl of substrate solution is used but additional volume may be needed for the solution loss by repeated pipetting.

1X Substrate solution volume = 100µl x (Sample number + 6)

⚠ **Caution**

- Avoid using metal containing containers or pipette which may result in color development of prepared substrate solution.
- Use distilled water only in order to avoid contamination with metal ions often found in tap water.
- Avoid any body contact with TMB concentrate which contains DMSO and mix well before dilution with buffer solution.
- Take care not to contaminate substrate solution with sodium azide, a commonly used preservative, which is also an inhibitor of HRP.
- TMB concentrate may occasionally freeze during storage in refrigerator, but it can still be used after complete dissolution.
- Diluted TMB solution should be prepared again if the color of the solution is not colorless anymore (e.g. turns from colorless to blue).

3. ASSAY PROCESS

①Bring the microwell plate to room temperature about 30 minutes prior to use and mix well.

②Determine the number of wells needed for the test as below.

One (1) well is used to test one (1) sample.

For each test, 6 additional wells (2 negative controls, 2 positive controls and 2 calibrators) are needed apart from the number of test samples.

Number of wells = Sample number + 6

③After recording the position of the wells for control reagents and test samples, dispense 100µl of sample diluent into each well except for substrate blank.

④Dispense 10µl of negative, positive control, calibrator and test samples into each well, gently shake for about 5 ~ 10 seconds making certain of no spillage. If necessary, cover the plate with sealer.

⑤Incubate the sealed plate at 37±1°C for 60±5 minutes.

⑥After reaction, remove the plate sealer and wash 5 times as follows. First, remove any remaining contents of the wells with an aspirator then fill completely with washing solution and leave the plate for 5□ 15 seconds. Repeat the process of aspiration 5 times and invert the plate and tap on absorbent tissue to remove excess washing solution.

⑦Except for substrate blank, dispense 100µl of conjugate solution into all wells and seal with plate sealer.

⑧Incubate the sealed plate at 37±1°C for 30±1 minute. After reaction, take off the plate sealer, remove any remaining contents of the wells with an aspirator and wash 5 times with washing

solution. Invert the plate and tap on absorbent tissue to remove excess washing solution.

⑨ Dispense 100µl of substrate solution into all wells including the substrate blank.

⑩ Incubate at 18 ~ 30°C in the dark place, for 30±1 minute.

⑪ Dispense 100µl of stop solution into all wells including substrate blank.

⑫ If there is moisture under the plate wipe the bottom of plate with a lab wipe since moisture can interfere with measurement results. Read the microwell plate at a wavelength of 450nm. For dual wavelength readers, set the reference wavelength of 650nm.

⚠ Caution

- DO NOT open the microwell plate until it has come to room temperature for 30 minutes. The coated antigen is sensitive to moisture.
- Any unused wells should be stored at 2 ~ 8°C in the supplied foil zipper bag with desiccant
- DO NOT touch the bottom of any unused wells

[QUALITY CONROL]

Positive and negative controls are provided with the kit. These controls are required as internal controls in order to identify reagent failure. Two (2) replicates of the positive control and two (2) replicates of the negative control must be used with each test.

The negative control is produced by pooling several previously-confirmed negative serum or plasma samples. The positive control is produced by pooling several previously-confirmed positive serum or plasma samples and diluted with pooled negative specimen.

The optical density (OD) value for each negative control must be greater than or equal to -0.005 and less than or equal to 0.2. If one of two values is outside this range, the test must be repeated once.

The mean OD of positive control is calculated by averaging the two OD values obtained for positive control. The OD value for positive control must be greater than or equal to 0.800 and the difference between two values must be less than or equal to 0.650. If any of two values is (are) outside the criteria, the test must be repeated once.

[INTERPRETATIOIN OF THE RESULT]

The mean optical density of calibrator is calculated by averaging the two OD values obtained for calibrator. The OD value for each calibrator must be greater than or equal to -0.005 and less than or equal to 0.2. If the OD value is measured between -0.005 to 0.000, it should be rounded up to 0.000 for calculation of mean OD. If one of two values is outside this range, the test must be repeated.

The mean optical density of positive control is calculated by averaging the two OD values obtained for positive control. The OD value for positive control must be greater than or equal to 0.800 and the difference between two values must be less than or equal to 0.650. If any of conditions are outside the criteria, the test must be repeated.

Calculate cut-off value according to the following formula:

Cut-off = Mean OD of calibrator + 0.230

Calculate the S/Co (Signal to cutoff ratio) according to the following formula

S/Co = Sample OD /Cut-off

Controls	Interpretation	Validation Criteria
Negative Control (NC)	Valid	The OD for each negative control must be greater than or equal to -0.005 and less than or equal to 0.2. ($-0.005 \leq NC < 0.2$)
Positive Control (PC)	Valid	The OD value for positive control must be greater than or equal to 0.800 and the difference between two values must be less than or equal to 0.650. ($PC \geq 0.8$ and $\lvert PC_1 - PC_2 \rvert < 0.65$)

S/Co Value	Interpretation	Remark
S/Co < 1.0	Negative	Samples with S/Co less than 1.0 are considered nonreactive and no further testing is required. IgG antibodies for SARS-Cov-2 are not detected.
S/Co ≥ 1.0	Positive	Samples with S/Co greater than or equal to 1.0 are considered as reactive. IgG antibodies for SARS-CoV-2 are detected.

[STORAGE CONDITION]

This kit should be stored at 2 ~ 8 °C. Please refer to the printed expired date on the product package.

The reagents are stable for 15 days from opening date when stored at 2 ~ 8 °C.

[ANALYTICAL PERFORMANCE]

1. Analytical Specificity

Cross-reactivity

The cross-reactivity of the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA was evaluated by testing SARS-CoV-2 seronegative specimens from patients with antibodies to other coronaviruses or medical conditions. A total of 76 specimens from 10 different categories were tested.

There was no cross-reaction (false positive results) observed with the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA in any of the specimens that were tested. The results are summarized in the table below.

Panel	N	Negatives	NPA %
anti-HCV	27	27	100
anti-HBV	7	7	100
anti-HIV	19	19	100
anti-229E (alpha coronavirus)	4	4	100
anti-NL63 (alpha coronavirus)	5	5	100
anti-229E (alpha coronavirus), anti-NL63 (alpha coronavirus)	4	4	100
anti-NL63 (alpha coronavirus), MERS-CoV	1	1	100
MERS-CoV	5	5	100
ANA	3	3	100
RF	1	1	100
Total	**76**	**76**	**100**

Class Specificity

AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA assay demonstrates class-specific reactivity only to human IgG isotypes. No binding interactions were observed with anti-SARS-CoV-2 IgM antibodies.

Interference

Potential interfering substances, IgA, IgD, IgM, and IgE were tested in human serum to determine if they were above physiologically relevant levels, and whether they could cause false positives or false negatives on AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA.

Two (2) positive specimens and one (1) negative specimen were spiked with potential interfering substances and elevated IgA, IgD, IgM, IgE. The samples were subsequently tested in duplicates and interference was observed with hemoglobin, unconjugated bilirubin, total cholesterol, total protein, IgA, IgD, and IgM. The used substances are summarized in the table below.

Interfering substance	Test Concentration	Interfering substance	Test Concentration
Cholesterol	250 (mg/dL)	IgA	350 (mg/dL)
Hemoglobin	500 (mg/dL)	IgM	250 (mg/dL)
Bilirubin	20 (mg/dL)	IgE	43000 (ug/dL)
Protein (Albumin)	12 g/dL	IgD	3 (mg/dL)

2. Matrix Equivalency

To investigate the utility of plasma samples, five (5) negative matrix sets of serum and corresponding plasma (K2 EDTA, Sodium Heparin, and Sodium Citrate plasma from same donors) were evaluated at four (4) different concentrations (negative, high negative, low positive, high positive). The results are summarized in the table below.

Result	Matrix Type		
	EDTA plasma	Heparin plasma	Citrate plasma
N (Total)	40	40	40
PPA % (vs Serum)	100	100	100
NPA % (vs Serum)	100	100	100
Total Agreement %	100	100	100
Regression Equation (S/Co) Y =Plasma, X=Serum	0.9219x+0.0045	0.9090x+0.0007	0.9479x-0.0071
Slope (95% C.I)	0.8601 - 0.9949	0.8738 - 0.9700	0.8984 – 1.0129
R^2	0.9643	0.9862	0.9803

[CLINICAL PERFORMANCE]

1. Positive Percent Agreement Studies

Two Positive Percent Agreement (PPA) studies were conducted with retrospective serum specimens from subjects where SARS-CoV-2 infection status was confirmed with an FDA emergency use authorized RT-PCR test.

For PPA study #1, the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA displayed a 16.7% PPA in study subjects confirmed positive for infection status by RT-PCR within 0 to 7 days. From 8 to 14 days post-RT-PCR confirmation, the PPA was 83.3%, and for ≥15 days post-RT-PCR confirmation the PPA was 100%.

The following table provides PPA by time of sampling from RT-PCR confirmation:

		AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA		
Days from PCR confirmed	Number of Samples Tested	Positive results	PPA	95% CI
0-7 days	12	2	16.7 %	4.70 - 44.8%

8-14 days	12	10	83.3 %	55.2 - 95.3%
≥15 days	41	41	100 %	91.4 – 100.0%

For PPA study #2, the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA displayed a 35.3% PPA for ≤ 7 days from symptom onset. From 8 to 14 days from symptom onset, the PPA was 87%, and for ≥15 days post-symptom onset the PPA was 98.9%.

The following table provides PPA by time post-symptom onset:

		AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA		
Days from Symptom Onset	**Number of Subjects Tested**	**IgG Positive results**	**IgG PPA**	**95% CI**
0-7 days	**17**	**6**	**35.3%**	17.3-58.7%
8-14 days	**46**	**40**	**87.0%**	74.3-93.9%
≥15 days	**87**	**86**	**98.9%**	93.8-99.8%

2. Negative Percent Agreement

Two Negative Percent Agreement (NPA) studies were conducted with serum specimens from 536 study subjects: 336 serum samples collected before the COVID-19 pandemic started and were presumed negative for the purposes of the study and 200 serum samples confirmed negative for SARS-CoV-2 infection status by an FDA emergency use authorized RT-PCR test.

The following tables provide NPA results with samples collected before and after the start of the COVID-19 pandemic:

	Pre-Pandemic Specimens AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA	
Number of Subjects Tested	**IgG Negative Results**	**IgG NPA (95% CI)**
336	**336**	**100% (CI: 97.3-100%)**

	Confirmed Negative Specimens AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA	
Number of Subjects Tested	**IgG Negative Results**	**IgG NPA (95% CI)**
200	**200**	**100%** **(CI: 98.1-100%)**

[INDEPENDENT CLINICAL AGREEMENT VALIDATION STUDY]

AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA was tested on April 01, 2022 at the Frederick National Laboratory for Cancer Research (FNLCR), a Federally Funded Research and Development Center (FFRDC) 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 were 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 COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma). The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods.

All 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+".

For evaluation of cross-reactivity with HIV+, it was evaluated 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.

Summary Results

AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA	Comparator Method			Total
	Antibody Positive	Antibody Negative		
		Negative	HIV+	
IgG +	29	0	0	29
IgG -	1	70	10	81
Total	30	70	10	110

Summary Statistics

Measure	Estimate	Confidence Interval
IgG Sensitivity	96.7% (29/30)	(83.3%; 99.4%)
IgG Specificity	100% (80/80)	(95.4%; 100%)
PPV for prevalence = 5.0%	100%	(48.9%; 100%)
NPV for prevalence = 5.0%	99.8%	(99.41; 100%)
Cross-reactivity with HIV+	0.0% (0/10), not detected	

Limitation of this study

• Samples were not randomly selected, and sensitivity and specificity estimates may not be indicative of the real-world performance of the device.

• 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.

• 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.

[WARNINGS AND PRECAUTIONS]

This product can only be used by those who received professional training in in vitro diagnostic tests.

For in vitro diagnostic use

This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories

This product has been authorized only for detecting the presence of IgG antibodies to SARS-CoV-2, not for any other viruses or pathogens

The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner

[SAFETY PRECAUTIONS]

CAUTION

All samples and reagents containing human-derived substances should be regarded as potentially infectious and handled properly in accordance with appropriate regulations.

WARNING

Sulfuric acid in the stop solution is a hazardous substance and can cause severe burns and eye damage, so care must be taken when using it.

This product contains sodium azide, which generates toxic gas when it reacts with acidic substances and highly explosive metal azides when it reacts with lead or copper, so care must be taken when using it.

When handling samples and reagents, take care not to let them get in contact with the skin, eyes, or mucous membranes by wearing safety glasses, protective clothing and gloves.

When contact is made, immediately wash with a large amount of water. Wash your hands thoroughly after each analysis. After the analysis, use purified water to clean the surface of the experiment table used, and disinfect with freshly prepared 0.5% sodium hypochlorite.

[HANDLING PRECAUTIONS]

Do not smoke or eat food while handling samples and test reagents.

Do not pipette reagents by mouth, and it is recommended to use sterile disposable pipettes or disposable pipette tips.

Do not reuse disposable items (tips, experimental gloves, tubes etc.) and microwell plates.

Reagents other than stop solutions and concentrated detergents should not be used in combination with reagents from other batches.

Take care not to splatter aerosols when handling all samples and reagents. Dispose of in accordance with appropriate waste regulations.

If the product packaging is damaged, or there is a leak in the reagent bottle, it should not be used because it can lead to misjudgment of results due to contamination or degradation.

Take care not to mix it with other test reagents or to place a cap of another solution on it.

[LIMITATION]

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 antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, the antibodies that are detected are not present during the stage of disease in which a sample is collected or if the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody used in the test.

False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

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.

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.

Not for the screening of donated blood.

It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to infection.

Use of AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA is limited to laboratory personnel who have been trained. Not for home use.

This assay has not been evaluated with fingerstick specimens. This test is not authorized for use with fingerstick whole blood.

The performance of this test has not been established in individuals who have received a COVID-19 vaccine. The clinical significance of a positive or negative antibody result following COVID-19 vaccination has not been established, and the result from this test should not be interpreted as an indication or degree of protection from infection after vaccination.

The performance of this test was established based on the evaluation of a limited number of clinical specimens. The samples for the positive percent agreement study were collected between April 2020 and February 2021 in South Korea. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

This device should not be used to diagnose or exclude acute SARS-CoV-2 infection. Direct testing for SARS-CoV-2 with a molecular assay should be performed to evaluate acute infection in symptomatic individuals.

Performance has only been established with the specimen types listed in the *Intended Use*. Other specimen types have not been evaluated and should not be used with this assay.

The performance of this device has not been established in samples collected from individuals less than 15 days following the onset of symptoms. Samples should be collected from individuals greater than 14 days following the onset of symptoms. Samples should not be tested if collected from individuals less than 15 days post symptom onset.

Results are not intended to be used as the sole basis for patient management decisions. Test results should be interpreted in conjunction with clinical observations, patient history, epidemiological information, and other laboratory findings.

The presence of 500 mg/dL and higher concentrations of hemoglobin may result in potential false positive results.

The presence of 250 mg/dL and higher concentrations of total cholesterol may result in potential false negative results.

The presence of 20 mg/dL and higher concentrations of unconjugated bilirubin at may result in potential false negative results.

The presence of 350 mg/dL and higher concentrations of IgA may result in potential false negative results.

The presence of 3 mg/dL and higher concentrations of IgD may result in potential false negative results.

Conditions of Authorization for the Laboratory

The AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA Letter of Authorization, along with the Authorized Fact Sheet for Healthcare Providers, the Authorized Fact Sheet for Recipients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

Authorized laboratories using the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

1. Authorized laboratories* using the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating Fact Sheets may be used, which may include mass media.

2. Authorized laboratories using the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA must use the product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

3. Authorized laboratories that receive the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

4. Authorized laboratories using the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

5. Authorized laboratories must collect information on the performance of the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and LG Chem, Ltd. (kellysp@lgchem.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of which they become aware.

6. All laboratory personnel using the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use this product 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 product.

7. LG Chem, Ltd., authorized distributors, and authorized laboratories using the AdvanSure™ SARS-CoV-2 IgG(RBD) ELISA must ensure that 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.

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

[SYMBOLS]

Following symbols may appear on the packaging and labeling

Symbols	Definitions
	**Caution**
	**Contains sufficient for <*n*> tests**
	**Use-by date**
	***In vitro* Diagnostic medical device**
	**Batch code**
	**Catalogue number**
	**Temperature limit**
	**Manufacturer**
	**Date of manufacture**
	**Authorized representative in the European Community**
	**This way up**
	**Consult instruction for use**
	**Corrosive substance (GHS)**