EMERGENCY USE AUTHORIZATION (EUA) SUMMARY COVID-19 SELF-COLLECTED ANTIBODY TEST SYSTEM

(Symbiotica, Inc.)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only
For use with samples that are self-collected by an individual age 18 years or older, or that are collected by an adult from an individual 5 years of age and older.

(Testing of specimens collected using the COVID-19 Self-Collected Antibody Test System will be performed at Symbiotica, Inc., Vacaville, CA, a laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a that meets the requirements to perform high complexity tests as per the Standard Operating Procedure (SOP) that was reviewed by the FDA under this EUA.)

INTENDED USE

The COVID-19 Self-Collected Antibody Test System is an enzyme-linked immunosorbent assay (ELISA) and a blood collection kit intended for qualitative detection of IgG antibodies to SARS-CoV-2 in human fingerstick blood dried blood spot (DBS) samples that are self-collected at home by an individual age 18 years or older or that are collected by an adult from an individual 5 years of age or older using the COVID-19 Self-Collected Antibody Test System Collection Kit when determined to be appropriate by a healthcare provider. The COVID-19 Self-Collected Antibody Test System is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The COVID-19 Self-Collected Antibody Test System should not be used to diagnose or exclude acute SARS-CoV-2 infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity.

The COVID-19 Self-Collected Antibody Test System includes the COVID-19 Self-Collected Antibody Test System Collection Kit for use to self-collect fingerstick dried-blood spot specimens at home by individuals described above when determined to be appropriate by a healthcare provider.

Testing is limited to Symbiotica, Inc., located at 1350 Burton Drive, Ste 210
Vacaville, CA 95687, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests.

Results are for the detection of SARS-CoV-2 antibodies. 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 seroconversion.

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

The sensitivity of COVID-19 Self-Collected Antibody Test System 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 COVID-19 Self-Collected Antibody Test System may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The COVID-19 Self-Collected Antibody Test System is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The COVID-19 Self-Collected Antibody Test System comprises a collection kit, the COVID-19 Self-Collected Antibody Test System Collection Kit, which is delivered to individuals for home collection, and an ELISA assay, which is performed using the self-collected samples. The assay, part of the COVID-19 Self-Collected Antibody Test System, is referred to as the COVID-19 eSTAD. The COVID-19 eSTAD is based on the EUROIMMUN Anti-SARS-CoV-2 Assay (EUA200523) and provides qualitative in vitro determination of human antibodies of the immunoglobulin class IgG against SARS-CoV-2. The collection kit, part of the COVID-19 Self-Collected Antibody Test System, includes the necessary components for individuals to self-collect a sample for testing using the assay. The COVID-19 Self-Collected Antibody Test System Collection Kit consists of an instruction sheet, one PerkinElmer DBS card (product number GRNK 11022; product code ZV 9956-01100), two safety lancets, two sterile gauze pads, two alcohol prep pads, two Band Aids, and a return mailer with a prepaid label.

Medical oversight of the process is provided by independent third-party healthcare professionals. Individuals may request the COVID-19 Self-Collected Antibody Test System on an *online* platform or through their physician visit. Physicians who are interested in ordering the COVID-19 Self-Collected Antibody Test System for their patients will be provided with Symbiotica's test requisition form, which can be returned to Symbiotica via Symbiotica's HIPAA-compliant fax system. An HCP, licensed in the state where the participant is located, will determine eligibility based on a COVID-19 health history questionnaire, and will order testing when appropriate. Collection kits, part of the COVID-19 Self-Collected Antibody Test System, will only be sent to participants meeting the inclusion criteria based on the information provided by the HCP from the COVID-19 health history questionnaire and prescribed by HCPs. Kits will be shipped directly to the patients' home, who will collect their sample at home. Patients will return their sample using the provided mailer and return label. When results are ready, Symbiotica will make the reports ready using an agreed upon method, which includes, but not limited to, an online platform, HIPAA-compliant fax, or mailed to the physician's office. The HCP will attempt to contact the patient as soon as possible to deliver results and provide education via a telemedicine visit or phone consultation. Test results are made available upon completion of HCP contact protocols.

COMPONENTS SPECIFIC TO THE KIT

The COVID-19 Self-Collected Antibody Test System is comprised of the following:

1. COVID-19 Self-Collected Antibody Test System Collection Kit: The collection kit consists of an instruction sheet, one PerkinElmer DBS card (product number GRNK 11022; product code ZV 9956-01100), two safety lancets, two sterile gauze pads, two alcohol prep pads, two Band Aids, and a return mailer with a prepaid label.

2. COVID-19 eSTAD assay: The test kit contains microplate strips each with 8 break-off reagent wells coated with recombinant structural protein of SARS-CoV-2. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a color reaction.

Table 1. Components of the COVID-19 eSTAD assay kit

1	**Microplate wells coated with antigens** 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use
2	**Calibrator** (IgG, human), ready for use
3	**Positive control** (IgG, human), ready for use
4	**Negative control** (IgG, human), ready for use
5	**Enzyme conjugate** peroxidase-labelled anti-human IgG, ready for use
6	**Sample buffer** ready for use
7	**Wash buffer** 10x concentrate
8	**Chromogen/substrate solution** TMB/H_2O_2, ready for use
9	**Stop solution** 0.5 M sulphuric acid, ready for use

CONTROLS TO BE USED WITH THE COVID-19 DRIED BLOOD SPOT COLLECTION KIT

The controls and calibrator included in the test kit must be used with each run. Results cannot be validated if the control values deviate from the expected values stated on the quality control certificate. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated. The positive control and negative control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay cut-off.

INTERPRETATION OF RESULTS

Assessment of the COVID-19 Self-Collected Antibody Test System results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Results are evaluated qualitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator. The ratio is calculated according to the following formula:

$$Extinction\ of\ the\ control\ or\ patient\ sample \div Extinction\ of\ calibrator = Ratio$$

The cut-off for negative samples is ratio <0.8.

The positive threshold is ratio ≥1.1.

The indeterminate range is ratio ≥0.8 to <1.1.

PERFORMANCE EVALUATION

1) *Analytical Specificity*

Cross reactivity
Potential cross-reactivity with antibodies to commonly identified pathogens in the general community was evaluated with the Euroimmun Anti-SARS-CoV-2 ELISA (IgG), which was EUA authorized on 04 May 2020 (EUA200523). The details of the performance of the authorized Euroimmun Anti-SARS-CoV-2 ELISA (IgG) can be found in the Euroimmun Anti-SARS-CoV-2 ELISA (IgG) Instructions for Use. Euroimmun granted Right of Reference to Symbiotica for this data.

Class Specificity
Class specificity was evaluated with the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG), which was EUA authorized on 04 May 2020. The details of the performance of the authorized Euroimmun Anti-SARS-CoV-2 ELISA (IgG) can be found in the Euroimmun Anti-SARS-CoV-2 ELISA (IgG) Instructions for Use. Euroimmun granted Right of Reference to Symbiotica for this data.

2) *Clinical Performance*

Clinical Sensitivity

The positive percent agreement (PPA) of the COVID-19 Self-Collected Antibody Test System was evaluated in 32 unique DBS samples from symptomatic patients who were confirmed as SARS-CoV-2 positive via RT-PCR at least 8 days but no more than 45 days prior to sampling. Individuals self-collected specimens in at-home settings. Samples were collected in the US between July 2020 and January 2021. Dried blood spots were tested using the COVID-19 eSTAD. The results are presented in the following table, stratified by days post-symptom onset.

Table 2. Results of the clinical study by days from RT-PCR result to DBS collection–positive specimens.

Days post-RT-PCR (positive)	Number of Subjects Tested	IgG Positive results	IgG PPA	95% CI
0–7 days	0	n/a	n/a	n/a
8–14 days	4	4	100%	51.02%–100%
≥15 days	28	28	100%	87.94%–100%
Total Subjects	32	32	100%	89.28%–100%

Clinical Specificity

The negative percent agreement (NPA) of the COVID-19 Self-Collected Antibody Test System was evaluated in 51 negative samples from subjects who were screened/assessed as negative based on no recent exposure to SARS-CoV-2 (within the prior 14 days) and not feeling ill or having a fever within the prior 3 days. Samples were collected in the US between May 2020 and June 2020. Dried blood spots were tested using the COVID-19 eSTAD. The results are presented in the following table.

Table 3. Results of the clinical study–negative specimens.

Number of Samples Tested	IgG Negative Results	IgG NPA	95% CI
51[a]	50[b]	98.04%	89.70%–99.65%

[a]The negative percent agreement (NPA)/specificity of the Euroimmun Anti-SARS-CoV-2 ELISA (IgG), the assay on which the COVID-19 eSTAD assay is based, was previously demonstrated using human serum and plasma under EUA200523. Symbiotica, Inc. obtained a Right of Reference from Euroimmun US, Inc. for this information. Because the study performed with fingerstick DBS is supplemental to the referenced clinical agreement studies, a minimum of 30 negative fingerstick DBS samples was sufficient.
[b]One sample gave a borderline result. For the more conservative measure of NPA, this result was considered positive in the analysis.

*3) **Collection device stability***

Based on the results of initial real-time stability studies in which collection devices were stored up to 8 months and which tested 5 low positive samples over time, the initial shelf-life for the collection device is set at 7 months at room temperature (15–30°C).

*4) **Shipping Stability***

The shipping stability study was designed to simulate the following situations that occur during home collection and shipping:

- Storage of samples before shipment
- Sample sitting in the mailbox or drop box before pick up
- Shipping conditions after pick up when the sample is shipped to the testing laboratory

Samples included at least 10 negative, 20 low positive, and 10 moderate positive or high positive samples (see below). Each temperature profile was designed to replicate worst-case scenarios for an 8-hour period at the customer's house followed by a 48-hour shipping cycle. A Spring/Summer profile and a Winter profile were tested. Results of the shipping stability study demonstrate 100% agreement between expected results after shipment under both winter and summer shipping conditions.

Table 4. Shipping stability study results.

A. **Summer Profile**			
Temperature	**Cycle Period**	**Cycle Period (Hr)**	**Total Time (Hr)**
40 °C	1	8	8
22 °C	2	4	12
40 °C	3	2	14
30 °C	4	36	50
40 °C	5	6	56
	Samples Tested, n	**IgG Positive Results**	**IgG PPA**
LOW positive	22	22	100%
MODERATE/HIGH positive	12	12	100%
	Samples Tested, n	**IgG Negative Results**	**IgG NPA**
Negative	12	12	100%
B. **Winter Profile**			
Temperature	**Cycle Period**	**Cycle Period (Hr)**	**Total Time (Hr)**
-10 °C	1	8	8
18 °C	2	4	12
-10 °C	3	2	14
10 °C	4	36	50
-10 °C	5	6	56
	Samples Tested, n	**IgG Positive results**	**IgG PPA**
LOW positive	22	22	100%
MODERATE/HIGH positive	12	12	100%
	Samples Tested, n	**IgG Negative Results**	**IgG NPA**
Negative	12	12	100%

5) Self-Collection: Usability Evaluation

53 participants were enrolled in a self-collection study. Subjects were enrolled based on the following inclusion and exclusion criteria:

Inclusion Criteria:

- All genders
- Aged 5 and older
- Willing to conduct a self-collection DBS card collection to determine or confirm their presence or absence of Anti-SARS-CoV-2 (IgG) antibody

- Willing to reveal their test results
- Willing to provide a blood sample
- Willing to give informed consent for themselves and/or their child

Exclusion Criteria:

- Children <5 years of age
- Adults with clinical or medical background
- Adults with prior medical or laboratory training
- Participants who have prior experience with self-collection of blood samples
- Parents or adult caregivers with prior experience collecting blood samples for minors or other adults requiring assistance
- Has a medical condition that means that is not appropriate to give a blood sample
- Less than 10 days since being diagnosed with COVID-19
- Directly exposed to COVID-19 in the past 14 days
- Feeling sick or have had a fever higher than 100.5°F in the last 3 days

Collection occurred at home environments using one of the participant's homes on two different dates. Participants were provided the kit, including sample collection instructions, shipping instructions, shipping materials, and pre-prepared return labels. Each participant collected their sample or collected the sample of an individual > 5 years of age or older under observation but without assistance, and the observing healthcare provider noted any areas of difficulty. After collection, the participants wrote their names and the date on the cards, allowed the DBS cards to dry for at least 30 minutes, and then closed the card and packaged up the mailer. Participants were then given a questionnaire to indicate ease of use of the kit and sample collection as well as understanding of consequences if steps are not performed correctly. After completion, the users mailed their samples to the laboratory. The primary outcome measure was the participant-reported device usability, measured by the number of participants who record that the kit was easy to use.

A total of 51 participants completed the study. A total of 9 participants collected samples from individuals 5 years of age or older, and one withdrew from the study. Based on survey results, 90.6% of users (n=49/53) responded with positive feedback for ease of use of the device. Details regarding the observers' findings during collection are shown in Table 5.

Table 5. Observation of Sample Collection by Healthcare Professionals.

Question (answer)	**N (%)**
Did packet content match what was listed on packet? (yes)	53 (100)
Did barcodes on DBS cards match? (yes)	53 (100)
Did you observe the user having difficulties? (no)	44 (83)
Of the 9 with difficulties, what was the observed difficulty?	
Slow blood flow	7 (77.8)
Lancet malfunction	1 (11.1)
Opted out	1 (11.1)
Did the participant ask any questions/ask for help? (no)	50 (94.3)
Of the 3 who were observed "yes":	
How to fill the blood sample card	1 (33.3)
Had difficulty filling the spots	1 (33.3)
Did not like the finger stick	1 (33.3)

Question (answer)	N (%)
Did you observe the participant having any difficulties with instructions/materials provided? (no)	40 (75.5)
Of the 13 that were observed "yes":	
Finger prick problem	1 (7.7)
Opening blue bag	9 (69.2)
Missed instructions	3 (23.1)
Was the participant able to complete the process entirely by themselves? (yes)	43 (81.1)
Of the 10 that were observed "no":	
Minor or adult requiring assistance	9 (90.0)
Participant opted not to continue	1 (10.0)
Any feedback, comments, or additional notes to document? (no)	45 (84.9)
Of the 8 that were observed with feedback:	
Pressed finger	4 (50.0)
Tore biohazard foil	3 (37.5)
Other, dizzy	1 (12.5)

Analytical Robustness Testing

Venous whole blood was collected in dipotassium EDTA tubes from 5 individuals who were found to be negative for SARS-CoV-2 by RT-PCR and 7 individuals who were confirmed to be positive for SARS-CoV-2 by RT-PCR. For RT-PCR-confirmed positive samples, the samples were obtained ≥14 days and no more than 2 months from the date of RT-PCR test. For negative samples, the samples were collected within 7 days of the date of the RT-PCR test. These samples were then used to generate DBS specimens for downstream robustness testing. The acceptance criterion for all robustness studies was 100% agreement with the expected qualitative results.

Drying time: Samples dropped onto DBS cards (prepared as described above) were allowed to dry for 8, 15, 23, 45, and 90 minutes (4x below to 3x above the recommended 30-minute drying time). The samples were then closed and placed into the sample biohazard bag with the desiccant.

Table 6. Drying time results.

Test Conditions	IgG Positive results	IgG PPA	IgG Negative Results	IgG NPA
8 mins	7/7	100.00%	5/5	100.00%
15 mins	7/7	100.00%	5/5	100.00%
23 mins	7/7	100.00%	5/5	100.00%
30 mins	(Recommended)			
45 mins	7/7	100.00%	5/5	100.00%
90 mins	7/7	100.00%	5/5	100.00%
STUDY SUMMARY	35/35	100.00%	25/25	100.00%

Temperature and Humidity: Whole blood, collected from 7 PCR-confirmed positive and 5 PCR-confirmed negative (or clinically assessed as negative), was spotted onto a DBS card and exposed to the following conditions overnight before (standard) testing:

- Incubator set at 50 °C
- Refrigerator (1–9 °C)
- Freezer (-40 to 10 °C)
- In a tub of water (18–25 °C)

Table 7. Temperature and humidity results.

	IgG Positive results	IgG PPA	IgG Negative Results	IgG NPA
Overnight in Incubator (50°C)	7/7	100.00%	5/5	100.00%
0	(Recommended)			
Overnight in Refrigerator (4°C)	7/7	100.00%	5/5	100.00%
Overnight in Freezer (-20°C)	7/7	100.00%	5/5	100.00%
Overnight in water	7/7	100.00%	5/5	100.00%
STUDY SUMMARY	28/28	100.00%	20/20	100.00%

Unique test characteristics: The following scenarios were tested:

a. Finger touching DBS card: Each DBS card was opened, and each circle was dabbed 10 times by ungloved fingers to represent the scenario where patients touch the DBS and possibly contaminate it with finger oils. Seven confirmed positive and 5 confirmed negative DBS were tested.

Table 8. Finger touch results.

	IgG Positive results	IgG PPA	IgG Negative Results	IgG NPA
Finger touch DBS card	7/7	100.00%	5/5	100.00%
STUDY SUMMARY	7/7	100.00%	5/5	100.00%

b. Alcohol drying time on finger: To simulate a user wiping the subject's finger prior to obtaining the blood sample, the pipet tips used to apply blood to the DBS paper were wiped with alcohol prep pads and allowed to dry for the following times before pipetting the blood:
   - 4 seconds
   - 8 seconds
   - 12 seconds
   - 23 seconds
   - 45 seconds

After pipetting onto the DBS paper, the blood spots were allowed to dry per the SOP.

Table 9. Alcohol drying time results.

	IgG Positive results	IgG PPA	IgG Negative Results	IgG NPA
4 seconds	7/7	100.00%	5/5	100.00%
8 seconds	7/7	100.00%	5/5	100.00%
12 seconds	7/7	100.00%	5/5	100.00%
15 seconds	(Recommended)			
23 seconds	7/7	100.00%	5/5	100.00%
45 seconds	7/7	100.00%	5/5	100.00%
STUDY SUMMARY	35/35	100.00%	25/25	100.00%

WARNINGS

- This test system has not been FDA cleared or approved but has been authorized for emergency use by FDA under an Emergency Use Authorization for use by Symbiotica Inc., located at 1350 Burton Drive, Vacaville, CA 95687, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests.

- This test system has been authorized only for detecting IgG antibodies to SARS-CoV-2, not for any other viruses or pathogens.

- The emergency use of this test system 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.

LIMITATIONS

- Specimens submitted using the COVID-19 Self-Collected Antibody Test System Collection Kit must be tested using the COVID-19 eSTAD.

- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target antibodies.

- Results obtained with the COVID-19 Self-Collected Antibody Test System may not be used interchangeably with values obtained with different manufacturers' test methods.

- Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection or to inform infection status. An assay that directly detects the virus should be used to evaluate symptomatic patients for acute COVID-19.

- False negative results may occur for immune-compromised individuals or individuals who receive immunosuppressive therapy.

- 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 seroconversion.

- Negative results do not preclude acute SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. IgG antibodies may not be detected in the first few days of infection. The sensitivity of the COVID-19 eSTAD 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.

- A positive result may not indicate previous SARS-CoV-2 infection. False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Consider other information, including clinical history and local disease prevalence, in assessing the need for an alternative 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.

- 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 the antibodies that are detected are not present during the stage of disease in which a sample is collected.

- 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, different serology test to confirm an adaptive immune response.

- For assays that employ antibodies, the possibility exists for interference by heterophile antibodies in the test sample. Patients who are regularly exposed to animals, or are subjected to medical treatments that utilize immunoglobulins or immunoglobulin fragments, may produce human anti-animal antibodies, e.g. HAMA, that interfere with immunoassays. These interfering antibodies may cause erroneous results.

- The COVID-19 Self-Collected Antibody Test System's COVID-19 eSTAD assay results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.

- The COVID-19 Self-Collected Antibody Test System's COVID-29 eSTAD assay is intended for qualitative detection only. Test value itself cannot be used to determine the quantity of SARS-CoV-2 IgG antibodies.

- This test is not to be used for screening donated blood.

- The performance of this device has not been established in individuals that 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 results from this assay 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 negative agreement study were all collected in the United States between May 2020 and June 2020. The samples from the positive percent agreement were collected in the United States between July 2020 and January 2021. 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.