

## COVID-19 ELISA pan-Ig Antibody Test

### **EMERGENCY USE AUTHORIZATION (EUA) SUMMARY COVID-19 ELISA PAN-IG ANTIBODY TEST (UNIVERSITY OF ARIZONA GENETICS CORE FOR CLINICAL SERVICES)**

For *In vitro* Diagnostic Use  
Rx Only  
For use under Emergency Use Authorization (EUA) only

**(The COVID-19 ELISA pan-Ig Antibody Test will be performed at the University of Arizona Genetics Core for Clinical Services, located at 1657 E. Helen Street, Tucson, AZ 85721, 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 as per University of Arizona Genetics Core for Clinical Services SOPs that were reviewed by the FDA under this EUA)**

#### **INTENDED USE**

The COVID-19 ELISA pan-Ig Antibody Test is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for the qualitative detection of total antibodies (including IgA, IgG, and IgM) to SARS-CoV-2 in human serum. The COVID-19 ELISA pan-Ig Antibody Test 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 COVID-19 ELISA pan-Ig Antibody Test should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to University of Arizona Genetics Core for Clinical Services, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meets requirements to perform high-complexity tests.

Results are for the detection of SARS CoV-2 antibodies. 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 positive results to the appropriate public health authorities.

The sensitivity of the COVID-19 ELISA pan-Ig Antibody Test 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 the COVID-19 ELISA pan-Ig Antibody Test may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The COVID-19 ELISA pan-Ig Antibody Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

## **DEVICE DESCRIPTION AND TEST PRINCIPLE**

The COVID-19 ELISA pan-Ig Antibody Test is an ELISA-based assay utilizing a polystyrene testing plate containing sample wells with a hydrophilic high binding surface that binds and immobilizes polar proteins and peptides. The plates are coated with SARS-CoV-2 Spike protein (for the RBD test) or with SARS-CoV-2 Spike S2 Protein (for the S2 test). If present in patient serum, antibodies to SARS-CoV-2 bind to the immobilized antigens and subsequently to the secondary antibody, which is conjugated with horseradish peroxidase. After addition of a substrate, the color that develops is read at both 630nm and 450nm wavelengths.

All ELISA preparation steps are performed using an automated method on a liquid-handling robot; the ELISA plate is then read on a plate reader.

## **COMPONENTS SPECIFIC TO THE TEST**

The following components are specific to the COVID-19 ELISA pan-Ig Antibody Test:

- SARS-CoV-2 Spike protein
- SARS-CoV-2 Spike S2 protein
- Positive controls (monoclonal antibodies and samples derived from clinical specimens)

## **COMPONENTS REQUIRED BUT NOT SPECIFIC TO THE TEST**

- 1x PBS
- Tween 20
- NF Powdered Milk
- Anti-hIgG (Peroxidase-conjugated AffiniPure Goat Anti-Human IgA + IgG + IgM (H+L))
- 1x TMB
- 1x 2N H<sub>2</sub>SO<sub>4</sub> Sulfuric Acid
- ELISA Coating Buffer Solution

## **CONTROLS TO BE USED WITH THE COVID-19 ELISA PAN-IG ANTIBODY TEST**

The COVID-19 ELISA pan-Ig Antibody Test contains the following controls that are run with each plate:

- Negative Control – Derived from previously tested serum RBD and S2 negative samples.
- Positive Control – Derived from previously tested, pooled serum RBD and S2 positive samples. These consist of both a high absorbance pool (> 1.0 OD<sub>450</sub> for both RBD and S2) and a medium absorbance pool (between 0.7-0.8 OD<sub>450</sub> for both RBD and S2).
- Positive Control for RBD assay only – Human monoclonal antibody against SARS-CoV-2 spike protein S1.
- Negative Template Control – HPLC water.

## INTERPRETATION OF RESULTS

Assessment of COVID-19 ELISA pan-Ig Antibody Test 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.

The assay first screens samples for Spike RBD following negative (NEG) call threshold  $\leq 0.120$  and positive (POS) call threshold of  $\geq 0.389$ , with indeterminate (INDT) samples falling between these thresholds. POS and INDT specimens then undergo secondary confirmation using the S2 protein assay with a negative call threshold of  $\leq 0.350$ . Interpretation for final result follows reporting algorithm presented in **Table 1**.

**Table 1.** Interpretation of results.

Assay	OD Value	Result	Assay	OD Value	Result	Final report
RBD	$\geq 0.389$	Positive	S2	$> 0.350$	Positive	Positive
RBD	$\geq 0.389$	Positive	S2	$\leq 0.35$	Negative	Negative
RBD	0.120 – 0.389	Indeterminate	S2	$> 0.350$	Positive	Indeterminate
RBD	0.120 – 0.389	Indeterminate	S2	$\leq 0.350$	Negative	Negative
RBD	$\leq 0.120$	Negative	N/A	N/A	N/A	Negative

## PERFORMANCE EVALUATION

### 1) Analytical Sensitivity:

There is no standard reference SARS-CoV-2 antigen material available; accordingly, absolute analytical sensitivity cannot be calculated.

### 2) Analytical Specificity

#### *Reactivity/Inclusivity*

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

#### *Cross reactivity*

Three hundred twenty serum specimens were obtained through the UAHS Biorepository. These specimens were treated as true negatives; all specimens were collected between 2014 and 2019, prior to the onset of SARS-CoV-2 in the human population. These specimens were collected under an IRB from the general population and presumed to have a high prevalence of vaccination against, and/or infection with expected cross-reactants. Three independent RBD ELISAs following the established protocol were performed for each of the 320 specimens.

Only the results of the first run were used to calculate specificity. Three of the 320 samples generated an indeterminate RBD result followed by a positive S2 result, ultimately interpreted as indeterminate. Therefore, the specificity of the COVID-19 ELISA pan-Ig Antibody Test is 99.06%.

## COVID-19 ELISA pan-Ig Antibody Test

### *Class Specificity*

Not applicable.

### *Matrix Equivalency*

Not applicable.

## **3) Clinical Performance**

### **Clinical Sensitivity**

UAGC-CS received 40 serum specimens collected at Banner University Medical Center in Tucson, AZ, from patients that were confirmed positive for SARS-CoV-2 by PCR 14–67 days prior to serum collection. The confirmatory PCR was the FDA-authorized assay for the Roche cobas 8800 Instrument performed on nasopharyngeal swabs at Banner University Medical Center. All samples were tested according to the SOPs, and 39 samples out of 40 generated a positive final result; one was reported as indeterminate. Positive percent agreement (PPA) is therefore 97.50% (39/40), and 95% CI is 87.12% - 99.56% (see **Table 2**).

**Table 2.** PPA of the COVID-19 ELISA pan-Ig Antibody Test.

<b>Days from onset of symptoms</b>	<b>Total PCR Positive Samples</b>	<b>COVID-19 ELISA pan-Ig Antibody Test Positive Results</b>	<b>PPA</b>	<b>95% CI</b>
<b>≤ 7</b>	0	0	N/A	N/A
<b>8 - 14</b>	0	0	N/A	N/A
<b>≥ 15</b>	40	39	97.50%	87.12% - 99.56%

### **Clinical Specificity**

Three hundred twenty negative serum specimens were obtained through the UAHS Biorepository. These specimens were collected between 2014 and 2019, prior to the onset of SARS-CoV-2, under an IRB from the general population, and presumed to be negative. Three of the 320 samples generated an indeterminate final result. Negative percent agreement (NPA) is therefore 99.06% (317/320), and 95% CI is 97.28% - 99.68% (see **Table 3**).

**Table 3.** NPA of the COVID-19 ELISA pan-Ig Antibody Test.

<b>Total Presumably Negative Samples</b>	<b>COVID-19 ELISA pan-Ig Antibody Test Negative Results</b>	<b>NPA (95% CI)</b>
320	317	99.06% (97.28% - 99.68%)

### ***Warnings***

- This test has not been cleared or approved by the US Food and Drug Administration.
- This test has been authorized by FDA under an emergency use authorization only for use by the authorized laboratory, University of Arizona Genetics Core for

## COVID-19 ELISA pan-Ig Antibody Test

Clinical Services, located at 1657 E. Helen Street, Tucson, AZ 85721, which is certified under CLIA, and meets requirements to perform high complexity tests.

- This test has been authorized only for the detection of total antibodies to 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.