# UBI® SARS-CoV-2 ELISA
# INSTRUCTIONS FOR USE

## FOR *IN VITRO* DIAGNOSTIC USE ONLY

## FOR EMERGENCY USE AUTHORIZATION ONLY

## FOR PRESCRIPTION USE ONLY

### INTENDED USE

The UBI® SARS-CoV-2 ELISA is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma (sodium heparin or dipotassium (K2) EDTA). The UBI® SARS-CoV-2 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 UBI® SARS-CoV-2 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 testing.

Results are for the detection of IgG 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 the UBI® SARS-CoV-2 ELISA 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 with the UBI SARS-CoV-2 ELISA 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 IgG antibody assay.

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

The UBI® SARS-CoV-2 ELISA is only for use under the Food and Drug Administration's Emergency Use Authorization.

### SUMMARY AND EXPLANATION OF THE TEST

The UBI® SARS-CoV-2 ELISA is an immunoassay that employs synthetic peptides derived from the Matrix(M), Spike(S) and Nucleocapsid(N) proteins of SARS-CoV-2 for the detection of antibodies to SARS-CoV-2 in human sera or plasma. These synthetic peptides, free from cellular or *E. coli*-derived impurities which the recombinant viral proteins are produced from, bind antibodies specific to highly antigenic segments of SARS-CoV-2 structural M, N and S proteins and constitute the solid phase antigenic immunosorbent. Specimens with absorbance values greater than or equal to the Cutoff Value (i.e. Signal to Cut-off ratio ≥ 1.00) are defined as positive.

## CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The UBI® SARS-CoV-2 ELISA employs an immunosorbent bound to the wells of the REACTION MICROPLATE consisting of synthetic peptides that capture antibodies with specificities for highly antigenic segments of the Spike (S), Matrix (M) and Nucleocapsid (N) proteins of SARS-CoV-2.
During the course of the assay, diluted negative controls and specimens are added to the REACTION MICROPLATE wells and incubated. SARS-CoV-2-specific antibodies, if present, will bind to the immunosorbent. After a thorough washing of the REACTION MICROPLATE wells to remove unbound antibodies and other serum/plasma components, a standardized preparation of Horseradish peroxidase-conjugated goat anti-human IgG antibodies specific for human IgG is added to each well. This conjugate preparation is then allowed to react with the captured antibodies. After another thorough washing of the wells to remove unbound horseradish peroxidase-conjugated antibody, a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) is added. A blue color develops in proportion to the amount of SARS-CoV-2-specific IgG antibodies present, if any, in most settings. Absorbance of each well is measured within 15 minutes at 450 nm by using a microplate reader such as a VersaMax™ by Molecular Devices® or equivalent.

## REAGENT COMPONENTS AND THEIR STORAGE CONDITIONS

| **UBI® SARS-CoV-2 ELISA** | **192 tests** |
|---|---|

**SARS-CoV-2 Reaction Microplates** **192 wells**
Each microplate well contains adsorbed SARS-CoV-2 synthetic peptides. Store at 2-8°C sealed with desiccant.

**Non-Reactive Control / Calibrator** **0.2 mL**
Inactivated normal human serum containing 0.1% sodium azide and 0.02% gentamicin as preservatives. Store at 2-8°C.

**Specimen Diluent (Buffer I)** **45 mL**
Phosphate buffered saline solution containing casein, gelatin and preservatives: 0.1% sodium azide and 0.02% gentamicin. Store at 2-8°C.

**Conjugate** **0.5 mL**
Horseradish peroxidase-conjugated goat anti-human IgG antibodies, with 0.02% gentamicin and 0.05% 4-dimethylaminoantipyrine. Store at 2-8°C.

**Conjugate Diluent (Buffer II)** **30 mL**
Phosphate buffered saline containing surfactant and heat-treated normal goat serum, with 0.02% gentamicin as a preservative. Store at 2-8°C.

**TMB Solution** **14 mL**
3,3',5,5'-tetramethylbenzidine (TMB) solution. Store at 2-8°C.

**Substrate Diluent** **14 mL**
Citrate buffer containing hydrogen peroxide. Store at 2-8°C.

**Stop Solution** **25 mL**
Diluted sulfuric acid solution (1.0M $H_2SO_4$). Store at 2-30°C.

**Wash Buffer Concentrate** **150 mL**
A 25-fold concentrate of phosphate buffered saline with surfactant. Store at 2-30°C.

**Dilution Microplates** **192 wells**
Blank, yellow microplates for predilution of specimens. Store at 2° to 30°C.

**Plate Covers** **6 sheets**
Clear, plastic adhesive sheets to be used to cover the Reaction Microplate wells during each incubation. Plastic sheets may be cut, before removing the paper backing, whenever less than a full plate of Reaction Microplate wells is being assayed. Alternatively, standard microplate lids may be used.

## MATERIALS REQUIRED - NOT PROVIDED

1. **Anti-SARS-CoV-2 Positive Control 0.2 mL**
   Inactivated human plasma containing SARS-CoV-2 IgG antibodies. Store at ≤ -20°C. It may be purchased separately as Anti-SARS-CoV-2 Positive Control (PN 200238) for UBI SARS-CoV-2 ELISA.
2. Manual or automatic multi-channel- 8 or 12 channel pipettors (50 μL to 300 μL).
3. Manual or automatic variable pipettors (From 1 μL to 200 μL).
4. Incubator (37 ± 2°C).
5. Polypropylene or glass containers (25 mL capacity), with a cap.
6. Sodium hypochlorite solution, 5.25% (liquid household bleach).
7. A microplate reader capable of transmitting light at a wavelength of 450 ± 2 nm.
8. Automatic or manual aspiration-wash system capable of dispensing and aspirating 250-350 μL.
9. Pipettor troughs or boats.
10. Reagent grade (or better) water.
11. Disposable gloves.
12. Timer.
13. Absorbent tissue.
14. Biohazardous waste containers.
15. Pipettor tips.

## WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE ONLY
FOR PRESCRIPTION USE ONLY
FOR EMERGENCY USE AUTHORIZATION ONLY

1. This test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an 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 high complexity tests.
2. The emergency use of this test has been authorized only for detecting IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens.
3. The emergency use of 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 Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner
4. HANDLE ASSAY SPECIMENS, POSITIVE AND NON-REACTIVE CONTROLS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT. Wear disposable gloves throughout the test procedure. Dispose of gloves as biohazardous waste. Wash hands thoroughly afterwards.
5. DO NOT SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER. CONJUGATE and REACTION MICROPLATES are matched for optimal performance. Use only the reagents supplied by manufacturer.
6. Do not use kit components beyond their expiration date.
7. The NON-REACTIVE CONTROL / CALIBRATOR should be assayed in triplicate on each plate with each run of specimens and should be diluted in the same manner as the specimen.
8. Use only reagent grade quality water to dilute the WASH BUFFER CONCENTRATE.
9. Allow all kit reagents and materials to reach room temperature (15 to 30°C) before use.
10. Do not remove MICROPLATE from the storage bag until needed. Unused strips should be stored at 2 to 8°C securely sealed in its foil pouch with the desiccant provided.
11. Caution: STOP SOLUTION (1 mol/L $H_2SO_4$) causes burns. Never add water to this product. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
12. Avoid contact of the 1 mol/L SULFURIC ACID (Stop Solution) with any oxidizing agent or metal.
13. Follow the installation, operation, calibration and maintenance instructions provided by the instrument manufacturers for both microplate reader and automatic microplate washer.
14. Spills should be cleaned thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Iodophor Disinfectant: should be used at a dilution providing at least 100 ppm available iodine.

Sodium Hypochlorite:
**a.** Non acid-containing spills should be wiped up thoroughly with a 5.25% sodium hypochlorite solution (liquid household bleach).
**b.** Acid-containing spills should be wiped dry. Spill areas should then be wiped with a 5.25% sodium hypochlorite solution.

15. This product contains sodium azide as a preservative. Sodium azide may form lead or copper azides in laboratory plumbing. These azides may explode on percussion, such as hammering. To prevent formation of lead or copper azide, thoroughly flush drains with water after disposing of waste solutions. To remove suspected of azide accumulation, the National Institute for Occupational Safety and Health (USA) recommends: (1) siphon liquid from drain trap using a hose, (2) fill with 10% sodium hydroxide solution, (3) allow to stand for 16 hours, and (4) flush well with water.

## WASTE DISPOSAL

Dispose of all specimens and materials used to perform the test as if they contain infectious agents. Autoclaving at 121°C or higher is recommended prior to incineration.
Liquid wastes NOT CONTAINING ACID may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite. Allow at least 30 minutes at room temperatures for decontamination to be completed. The liquid may then be disposed in accordance with local ordinances.

## SPECIMEN COLLECTION AND PREPARATION

1. UBI® SARS-CoV-2 ELISA may be performed on human serum or plasma (anticoagulant sodium heparin or dipotassium EDTA). Specimens containing precipitates or particulate matter may give inconsistent test results. If necessary, specimens should be clarified by centrifugation prior to testing.
2. Specimens must not be heat-inactivated prior to assay
3. Specimens may be stored at 2 - 8 °C for up to 48 hours or at ≤ -20 °C for up to two months.
4. Specimens may be frozen and thawed once.

## PREPARATION OF REAGENTS

After removing assay reagents from the refrigerator, allow them to reach room temperature and mix thoroughly by gentle swirling before pipetting.

- **WASH BUFFER:**
  Prepare and load into plate washer prior to beginning ASSAY PROCEDURE. Dilute 1 volume of WASH BUFFER CONCENTRATE with 24 volumes of reagent grade water. Mix well. Once prepared, diluted WASH SOLUTION is stable for 3 months with occasional mixing. Store at 2 to 30°C. Do not use diluted WASH SOLUTION until it has reached room temperature (15 to 30°C), if it has been stored in the refrigerator.

- **WORKING CONJUGATE SOLUTION:**
  Prepare as step 6 of the ASSAY PROCEDURE. Dilute the conjugate 1:100 with the Conjugate Diluent. Refer to the chart below for the correct amount of Working Conjugate Solution to prepare. Mix well to ensure a homogenous solution.

WORKING CONJUGATE SOLUTION PREPARATION CHART

| Number of Strips | Number of Tests | Conjugate (μL) | Diluent (mL) |
|---|---|---|---|
| 1 to 2 | 8 to 24 | 25 | 2.5 |
| 3 to 6 | 25 to 48 | 50 | 5.0 |
| 7 to 9 | 49 to 72 | 75 | 7.5 |
| 10 to 12 | 73 to 96 | 100 | 10.0 |

- **TMB SUBSTRATE SOLUTION:**
  Prepare as step 8 of the ASSAY PROCEDURE. Mix the TMB Solution and Substrate Diluent in equal volumes. Refer to the chart below for the correct amount of TMB substrate solution to prepare. USE WITHIN 10 MINUTES OF PREPARATION, PROTECT FROM DIRECT SUNLIGHT.

TMB SUBSTRATE SOLUTION PREPARATION CHART

| Number of Tests | TMB Buffer (mL) | Substrate Diluent (mL) |
|---|---|---|
| 16 | 1.1 | 1.1 |
| 24 | 1.6 | 1.6 |
| 32 | 2.1 | 2.1 |
| 40 | 2.5 | 2.5 |
| 48 | 2.8 | 2.8 |
| 56 | 3.5 | 3.5 |
| 64 | 3.8 | 3.8 |
| 72 | 4.0 | 4.0 |
| 80 | 4.5 | 4.5 |
| 88 | 5.0 | 5.0 |
| 96 | 5.5 | 5.5 |

All materials should be used at room temperature (15 to 30°C). Liquid reagents should be thoroughly and gently mixed before use.

**STORAGE INSTRUCTIONS**

1. Store UBI® SARS-CoV-2 ELISA kit and its components at 2 to 8°C when not in use and use by the kit expiration date.
2. After opening, unused strips of the REACTION MICROPLATES must be stored at 2 to 8°C securely sealed in foil pouch with the desiccant provided. When kept in the closed pouch at 2 to 8°C, after opening once, the REACTION MICROPLATES are stable for 8 weeks.

**INDICATIONS OF INSTABILITY OR DETERIORATION**

1. Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials; do not use reagents which are visibly turbid.
2. The TMB Solution, Substrate Diluent and the prepared SUBSTRATE SOLUTION should be colorless to pale yellow in color for proper performance of the assay. Any other color may indicate deterioration of the TMB Solution and/or Substrate Solution.

## QUALITY CONTROL

The Anti-SARS-CoV-2 Positive Control is treated in the same manner as the test samples and is used to validate the test run. It is recommended that the Positive Control is run in a separate well, concurrently with patient specimens, in each run. The Positive Control absorbance value should be ≥ 0.5 and the Signal to Cutoff ratio should be >1.0. If either the Positive Control absorbance value or the Signal to Cut-off ratio falls outside the limits, the plate is invalid and the test must be repeated.

The Non-Reactive Control / Calibrator is tested as described in the section Assay Procedure.

Expected results for the Non-Reactive Control / Calibrator are provided in the section Assay Validation.

## ASSAY PROCEDURE

1. To the DILUTION MICROPLATE:
   A. Dispense 200 µL of SPECIMEN DILUENT (Buffer I) into all wells.
   B. Use well A1 as reagent blank.
   C. Add 10 µL of Non-Reactive Control / Calibrator to wells B1, C1, D1
   D. Add 10 µL of Anti-SARS-CoV-2 Positive Control to the appropriate well.
   E. Add 10 µL of TEST SPECIMEN to the appropriate wells.
2. Ensure that the contents of the wells are thoroughly mixed. Manual mixing with a pipette or gently vibrating the plate is acceptable.
3. Open the foil pouch and remove the REACTION MICROPLATE. When not using the complete REACTION MICROPLATE, remove excess strips from the frame and return them to the storage pouch provided and securely seal. It may be necessary to insert alternate strips, depending on the washing system used.
4. Transfer 100 µL of Reagent Blanks, Non-Reactive Control / Calibrator and Diluted Specimens from each well of the DILUTION MICROPLATE to its corresponding well in the REACTION MICROPLATE.
5. Cover and incubate 60 ± 2 minutes at 37 ± 2°C.
6. Prepare the WORKING CONJUGATE SOLUTION (1:101) as described in PREPARATION OF REAGENTS prior to washing the REACTION MICROPLATES.
7. Wash the MICROPLATE with WASH BUFFER as described in PREPARATION of REAGENTS.
   Automatic Microplate Washer - Use six (6) washes with at least 300 µL/well/wash.
   Manual Microplate Washer or Pipettor (8 or 12 channel) - wash six (6) times, using at least 300 µL/well/wash. Fill the entire plate, then aspirate in the same order.
8. Make sure that the rest volume is minimal, e.g. by blotting dry by tapping plate onto absorbent paper.
9. Add 100 µL of the prepared WORKING CONJUGATE SOLUTION (1:101) to all wells of the REACTION MICROPLATE. Cover and incubate for 30 ± 1 minute at 37 ± 2°C.
10. Prepare TMB SUBSTRATE SOLUTION during the incubation prior to use according to the PREPARATION OF REAGENTS. Shield the solution from direct light.
11. Repeat the wash procedure as in step 7 and step 8.
12. Add 100 µL of the prepared TMB SUBSTRATE SOLUTION to each well of the REACTION MICROPLATE.
13. Cover and incubate for 15 ± 1 minute at 37 ± 2°C.
14. Add 100 µL of STOP-SOLUTION to each well of the REACTION MICROPLATE. Mix, e.g., by gently tapping or vibrating the plate.
15. Read the absorbance at 450 ± 2 nm with air blank. NOTE: Absorbance should be read within 15 minutes of the addition of the STOP SOLUTION to the REACTION MICROPLATE.

## ASSAY VALIDATION and CALCULATION of RESULTS

The presence or absence of antibody specific for SARS-CoV-2 is determined by relating the absorbance of the specimens to the Cutoff Value.

## ASSAY VALIDATION

For the assay to be valid:

- The Reagent Blank absorbance values should be less than 0.150. If it is outside the limit, the plate is invalid and the test must be repeated.
- Individual Non-Reactive Control / Calibrator absorbance values should be less than 0.200 and greater than the Reagent Blank. If one of the three Non-Reactive Control / Calibrator values is outside either of these limits, recalculate the Non-Reactive / Calibrator mean based upon the two acceptable control values. If two or more of the three control values are outside either of the limits (Less than 0.200 and greater than the reagent blank), the plate is invalid and the test must be repeated.
- The Anti-SARS-CoV-2 Positive Control absorbance value should be ≥ 0.5 and the Signal to Cutoff ratio should be >1.0. If either the Positive Control absorbance value or the Signal to Cut-off ratio falls outside the limits, the plate is invalid and the test must be repeated.

## CALCULATION of RESULTS

1. Absorbance of the Reagent Blank (RB)

| | | |
|---|---|---|
| Example: | Reagent Blank | Absorbance |
| | Well A1 | 0.044 |

2. Determine the Mean of the NON-REACTIVE CONTROL / CALIBRATOR (NRC)

| | | |
|---|---|---|
| Example: | NRC | Absorbance |
| | Well B1 | 0.062 |
| | Well C1 | 0.066 |
| | Well D1 | 0.063 |
| | Total | 0.191 |
| | Mean | 0.191 ÷ 3 = 0.064 |

3. Calculation of the Cutoff Value:

| | | |
|---|---|---|
| | Cutoff Value | = Mean NRC + 0.2 |
| Example: | Mean NRC | = 0.064 |
| | Cutoff Value | = 0.064 + 0.2 = 0.264 |

4. Calculation of the Signal to Cutoff (S/C) ratio

| | | |
|---|---|---|
| | S/C ratio | = OD of sample ÷ Cutoff Value |
| Example: | Sample OD | = 0.542 |
| | Cutoff Value | = 0.264 |
| | S/C ratio | = 0.542 / 0.264 = 2.05 |

## INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than the Cut-off Value (i.e., Signal to Cutoff ratio < 1.00) are negative by the criteria of the UBI® SARS-CoV-2 ELISA and may be considered negative for IgG antibodies to SARS-CoV-2.
2. Specimens with absorbance values greater than or equal to the Cutoff Value (i.e., Signal to Cutoff ratio ≥ 1.00) are positive by the criteria of the UBI® SARS-CoV-2 ELISA and may be considered positive for antibodies to SARS-CoV-2.

Results of the UBI® SARS-CoV-2 ELISA are interpreted as follows:

| S/C ratio | Result | Interpretation |
|---|---|---|
| <1.00 | Negative | Negative for IgG antibodies to SARS-CoV-2 |
| ≥1.00 | Positive | Positive for IgG antibodies to SARS-CoV-2 |

The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present in the sample.

## LIMITATIONS OF THE PROCEDURE

- Use of the UBI SARS CoV-2 ELISA is limited to laboratory personnel who have been trained. Not for home use.
- The UBI® SARS-CoV-2 ELISA PROCEDURE and the INTERPRETATION OF RESULTS sections must be closely adhered to.
- 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.
- This assay has not been evaluated with fingerstick specimens. This test is not authorized for use with fingerstick whole blood.
- SARS-CoV-2 antibodies may be below detectable levels in samples collected from patients who have been exhibiting symptoms for less than 15 days. Samples should be collected from individuals that are ≥ 15 days post symptom onset. Samples should not be tested if collected from individuals less than 15 days post symptom onset.
- Assay results should be utilized in conjunction with other clinical and laboratory methods to assist the clinician in making individual patient decisions.
- Assay results should not be used to diagnose or exclude acute COVID-19 infection or to inform infection status. Direct viral nucleic acid detection or antigen detection methods should be performed if acute infection is suspected.
- False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
- 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. The sensitivity of this assay early after infection is unknown.
- 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.
- Pedigreed specimens with direct evidence of antibodies to non-SARS-CoV-2 coronavirus (common cold) strains such as HKU1, NL63, OC43, or 229E have not been evaluated with this assay.
- If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to infection.
- 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.
- The UBI® SARS-CoV-2 ELISA is authorized for use with a manual assay procedure. Assay performance has not been established for use on automated instrument platforms.
- Not for the screening of donated blood.

### Conditions of Authorization for the Laboratory

The UBI® SARS-CoV-2 ELISA Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, 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/vitro-diagnostics-euas

Authorized laboratories using the UBI® SARS-CoV-2 ELISA must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

- Authorized laboratories[a] using the UBI® SARS-CoV-2 ELISA must 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.

- Authorized laboratories must use the UBI® SARS-CoV-2 ELISA as outlined in the authorized labeling. 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 product are not permitted.

- Authorized laboratories that receive the UBI® SARS-CoV-2 ELISA must notify the relevant public health authorities of their intent to run the assay prior to initiating testing.
- Authorized laboratories using the UBI® SARS-CoV-2 ELISA must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of the UBI® SARS-CoV-2 ELISA and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH EUA-Reporting@fda.hhs.gov) and UBI Technical Support (http://www.unitedbiomedical.com/support.html) 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.
- All laboratory personnel using the UBI® SARS-CoV-2 ELISA must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the UBI® SARS-CoV-2 ELISA 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 the UBI® SARS-CoV-2 ELISA .
- United Biomedical Inc., authorized distributors, and authorized laboratories using the UBI® SARS-CoV-2 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.

[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 high complexity tests” as “authorized laboratories".

## PERFORMANCE EVALUATION

### CROSS-REACTIVITY

The UBI® SARS-CoV-2 ELISA was evaluated in a clinical agreement study (described below) and demonstrated a negative percent agreement of 100% (154/154). In addition, cross-reactivity of non-SARS-CoV-2 specific antibodies were examined using sera with known antibodies against Respiratory Syncytial viruses (10) and ANA (6). No interference was observed.

### CLINICAL AGREEMENT STUDY

Studies were performed to determine the clinical performance of the UBI® SARS-CoV-2 ELISA assay.

To estimate the positive percent agreement (PPA) between the UBI® SARS-CoV-2 ELISA and the PCR comparator, 100 serum and 5 EDTA plasma specimens were collected from 95 subjects who tested positive for SARS-CoV-2 by a polymerase chain reaction (PCR) method and who also presented with COVID-19 symptoms. Each specimen was tested using the UBI® SARS-CoV-2 ELISA.

To estimate the negative percent agreement (NPA), 62 serum and 92 EDTA plasma specimens were collected from 154 subjects presumed to be negative for SARS-CoV-2. All of the 154 specimens were collected prior to COVID outbreak. Each specimen was tested using the UBI® SARS-CoV-2 ELISA. The results of both groups are presented in the following tables.

**Positive Agreement by Days Post-Symptom Onset**

| | | UBI SARS-CoV-2 ELISA | | | |
|---|---|---|---|---|---|
| **Days Post Symptom Onset** | **Number Tested** | **Number IgG Positive** | **Number IgG Negative** | **Positive Percent Agreement** | **95% Confidence Interval** |
| 0 - 7 days | 13 | 5 | 8 | 38.5% | (17.7%, 64.5%) |
| 8 - 14 days | 39 | 31 | 8 | 79.5% | (64.5%, 89.2%) |
| ≥15 days | 53 | 53 | 0 | 100.0% | (93.2%, 100%) |

**Negative Percent Agreement**

| | UBI SARS-CoV-2 ELISA | | | |
|---|---|---|---|---|
| **Number Tested** | **Negative** | **Positive** | **Negative Percent Agreement** | **95% Confidence Interval** |
| 154 | 154 | 0 | 100% | 97.6% - 100.0% |

**Independent Clinical Agreement Validation Study**

The UBI SARS-CoV-2 ELISA was tested on June 17 and September 1, 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 58 SARS-CoV-2 antibody-positive serum samples and 97 antibody-negative serum and plasma samples. Each of the 58 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 58 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the UBI SARS-CoV-2 ELISA. 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) Eighty-seven (87) 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 UBI SARS-CoV-2 ELISA. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).
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). Study results and summary statistics are presented in the following tables.

Summary results of independent evaluation

| **UBI SARS-CoV-2 ELISA** | | **Comparator Method** | | | |
|---|---|---|---|---|---|
| | | **Positive (IgG +)** | **Negative (IgG -)** | **Negative, HIV+** | **Total** |
| **Positive** | **IgG+** | 52 | 0 | 0 | 52 |
| **Negative** | **IgG-** | 6 | 87 | 10 | 103 |
| **Total** | | 58 | 87 | 10 | 155 |

Summary statistics of independent evaluation

| Measure | Estimate | 95% CI |
|---|---|---|
| **IgG Sensitivity** | 89.7% (52/58) | (79.2%; 95.2%) |
| **IgG Specificity** | 100% (97/97) | (96.2%; 100%) |
| **Combined PPV for prevalence = 5.0%** | 100% | (52.3%; 100%) |
| **Combined NPV for prevalence = 5.0%** | 99.5% | (98.9%; 99.7%) |
| **Cross-reactivity with HIV+** | 0.0% (0/10), not detected | |

Limitations of the 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.

## MATRIX EQUIVALENCY

The matrix equivalency study was conducted with patient-matched serum and plasma samples from five healthy donors. Plasma samples were drawn in vials containing sodium heparin or K2 EDTA as the anticoagulants. The matched samples were negative when tested with the UBI SARS-CoV-2 ELISA. Then the sample pairs were spiked with a sample positive for SARS-CoV-2 IgG to obtain three concentrations, and tested in duplicate. The results showed 100% agreement of positive and negative signal for each matrix, indicative of no effect of matrix-reactivity for the SARS-CoV-2 IgG detection in serum or plasma samples with UBI® SARS-CoV2 ELISA.
The study demonstrates that the performance of the UBI® SARS CoV-2 ELISA is equivalent with serum, sodium heparin plasma, and K2 EDTA plasma samples.

## CLASS SPECIFICITY

Eight serum samples positive for IgG and IgM antibodies to SARS-CoV-2 were tested with the UBI SARS CoV-2 ELISA. The samples were then treated with DTT to destroy the IgM antibodies and re-tested with the UBI SARS CoV-2 ELISA. Results for all eight samples were positive both before and after DTT treatment, demonstrating class-specific reactivity to human IgG isotypes. The UBI® SARS-CoV-2 ELISA assay demonstrates class-specific reactivity only to human IgG isotypes. No binding interactions were observed to human IgM.

**Manufacturer:**
**United Biomedical, Inc.**
**25 Davids Drive**
**Hauppauge, NY 11788**
**U.S.A.**

Effective: 01/xx/2021
Version 7.0

## Anti-SARS-CoV-2 Positive Control

For use under Emergency Use Authorization only
For In Vitro Diagnostic use only
For prescription use only

### INTENDED USE
The Anti-SARS-CoV-2 Positive Control (PC) is intended to be used in the quality control of the UBI SARS-CoV-2 ELISA kit.

### SUMMARY AND EXPLANATION OF THE POSITIVE CONTROL
The Anti-SARS-CoV-2 Positive Control is manufactured using heat inactivated human plasma containing IgG antibodies to SARS-CoV-2. The source plasma sample was tested negative or non-reactive for HBsAg, HIV and HCV. The Positive Control should be assayed in a separate well, concurrently with the specimens in each run.

### REAGENTS PROVIDED
Item P/N 200238 1 vial, 0.2 mL per vial

Inactivated human plasma containing SARS-CoV-2 IgG antibody.

### WARNINGS AND PRECAUTIONS
For *In Vitro* Diagnostic Use.
For use under Emergency Use Authorization only.
This product has not been FDA cleared or approved but has been authorized for emergency use 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 high complexity tests.
This product has been authorized only for detecting IgG antibodies against 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 diagnostic tests 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. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
Anti-SARS-CoV-2 Positive Control is a quality control reagent for use only with the UBI SARS-CoV-2 ELISA.
Use of control reagents manufactured by any other sources may not produce the required results, and therefore, will not meet the requirements for adequate quality control.

The handling and disposal of the Anti-SARS-CoV-2 Positive Control should follow the same procedure as the specimens used to perform the test.

CAUTION: handle the Anti-SARS-CoV-2 Positive Control as if capable of transmitting an infectious agent. The Positive Control is manufactured from human plasma nonreactive for HBsAg, HIV and HCV. Wear disposable gloves throughout the test procedure. Dispose of gloves as biohazardous waste. Wash hands thoroughly afterwards.

### WASTE DISPOSAL
Dispose of the Anti-SARS-CoV-2 Positive Control used to perform the test as if they contain infectious agents. Autoclaving at 121°C or higher is recommended prior to incineration.

### STORAGE
Store Anti-SARS-CoV-2 Positive Control at ≤ -20°C.
Do not use products beyond the expiration date printed on the product labeling.

### INSTRUCTION FOR USE
Allow the Anti-SARS-CoV-2 Positive Control to reach room temperature prior to use, and return the control to freezer storage after use. Gently mix the contents of the vial before use. The control should be included in a test run with UBI SARS-CoV-2 ELISA using exactly the same procedure as for the test specimens. Refer to the instructions for use of the UBI SARS-CoV-2 ELISA kit.

### EXPECTED RESULTS
The Anti-SARS-CoV-2 Positive Control absorbance value should be ≥0.5 and the Signal to Cutoff ratio should be >1.0. Calculation of the Signal to Cutoff ratio follows the instructions in the UBI SARS-CoV-2 ELISA kit insert. If either the Positive Control absorbance value or the Signal to Cut-off ratio falls outside the limits, the test run is invalid and must be repeated.

### LIMITATIONS OF THE PROCEDURE
The test procedures and interpretation of results provided in UBI SARS-CoV-2 ELISA kit insert must be followed.
Performance characteristics for the Anti-SARS-CoV-2 Positive Control have been established only for the UBI SARS-CoV-2 ELISA.

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**Effective: xx/xx/xxxx**

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