IMMY

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clarus SARS-CoV-2 Total Antibody EIA

For the Detection of SARS-CoV-2 Antibodies – REF COV105

CE IVD **QUALITATIVE**

INTENDED USE

The clarus SARS-CoV-2 Total Antibody EIA is an enzyme immunoassay intended for the qualitative detection of total antibodies (including IgM/IgA/IgG) to SARS-CoV-2 in human serum. The clarus SARS-CoV-2 Total Antibody EIA is intended for use as an aid in identifying patients 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 clarus SARS-CoV-2 Total Antibody EIA should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratory professionals.

Results are for the detection of SARS CoV-2 antibodies. Total antibodies (including IgG/IgA/IgG) 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.

The sensitivity of clarus SARS-CoV-2 Total Antibody EIA 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 clarus SARS-CoV-2 Total Antibody EIA may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

SUMMARY AND EXPLANATION OF THE TEST

SARS-CoV-2 is the virus responsible for COVID-19. SARS-CoV-2 is a coronavirus with significant sequence homology to SARS-CoV-1 and bat coronaviruses.[1] SARS-CoV-2 is the seventh coronavirus to cause disease in humans, emerging in late 2019 in Wuhan City, Hubei Province, China.[2] Current diagnosis is based upon Real-Time PCR (RT-PCR) detection of viral RNA.[1] Serology testing could serve as an adjunct to this method of diagnosis. Coronavirus serum antibodies begin to appear 5-17 days post disease onset, with a median time of 11 days.[3,4] Total antibody detection has been reported as the most sensitive and specific immunoglobulin detection method.[4,5] The detection of antibodies against this disease will be useful for the identification of prevalence and exposure from the disease in certain populations.

BIOLOGICAL PRINCIPLES

The clarus SARS-CoV-2 Total Ab EIA is a simultaneous sandwich antibody EIA utilizing conjugated antigen as the detection reagent. The clarus SARS-CoV-2 Total Ab EIA uses recombinant SARS-CoV-2 antigens pre-coated onto microwells. Patient specimens and controls are added to all wells, then Horse-Radish Peroxidase (HRP) conjugated antigen is dispensed into microwells. The sealed plate is incubated while shaking, washed and then substrate is added. The reaction is completed with the addition of Stop Solution to all microwells. The assay is then analyzed on a microplate reader capable of dual-wavelength reading (450 nm with a 620/630 nm reference wavelength). Results are interpreted by comparison to a calibrator. Specimen optical density (O.D) is divided by the average O.D. of the calibrator to determine EIA Unit values. This assay detects all classes of immunoglobulin and no differentiation is possible between isotypes.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use Only.
For prescription use only.

WARNINGS FOR USERS

1. Use of this kit with samples other than human serum is not recommended.
2. The Positive Control and Negative Control are manufactured from human serum that has tested negative for antibodies against HIV, HBV, HBsAg, HCV, CMV, Chagas, HTLV, Syphillis, West Nile and Zika. Both control reagents are of human origin and the amount of etiologic agents present in the controls is unknown. Controls are biohazardous and should be treated as infected patient material. All precautions and prevention measures for patient testing should be taken with these controls.
3. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves, and handle the kit reagents and patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
4. Maintain proper pipetting techniques and pattern throughout procedure to ensure optimal and reproducible results
5. Avoid splashing when dispensing or aspirating reagents or samples from the microwells as this causes errors.
6. Inadequate washing can cause excessive background in any EIA protocol.
7. Biological spills should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach, 70% ethanol, or 0.5% Wescodyne Plus™. Materials used to wipe up spills may require biohazardous waste disposal.
8. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all local, regional, and national regulations.
9. Refer to Hazards and Precautionary Information section for hazards associated with specific reagents. Safety Data Sheets are available upon request.

REAGENT PRECAUTIONS

1. Specific standardization is necessary to produce our high-quality reagents and materials. The user assumes full responsibility for any modification to the procedures published herein.
2. Avoid contact with Stop Solution (methanesulfonic acid) (REF EIASS2). If exposed to skin or eyes, immediately flush with copious amounts of water.
3. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimens.
4. The SARS-CoV-2 Total Antibody EIA (REF COV105) is biohazardous after running specimens. Handle and dispose of accordingly.
5. Always wear gloves when handling reagents in this kit as some reagents are preserved with less than 0.1 % (w/w) sodium azide. Sodium azide should never be flushed down the drain as this chemical may react with lead or copper plumbing to form potentially explosive metal azides. Excess reagents should be discarded in an appropriate waste receptacle.
6. For facilities using automated washing systems, use a low dispense rate to avoid displacing reagents adsorbed to the well. If low signal is observed, lower the dispense pressure of the washing system.

REAGENTS

1. **Antigen-Coated Microwells (5 plates, 96 microwells/plate) (REF COVMW1) 1** – 96-polystyrene microwells featuring breakaway microwells coated with SARS-CoV-2 antigen
2. **Calibrator Cutoff (1.5 mL) (REF COVCC1) 2** - An Anti-SARS-CoV-2 antibody in a buffered solution with preservative that establishes cutoff signal for the calculation of EIA units
3. **Conjugate (28 mL) (REF COVDA1) 3** – HRP conjugated SARS-CoV-2 antigen in a buffered solution containing a preservative
4. **20X Wash Buffer (60 mL) (REF EIAWB1) 4** – 20X concentrated wash buffer containing a preservative
5. **TMB Substrate (55 mL) (REF EIATUS) 5** – Buffered solution containing tetramethylbenzidine (TMB) when storing, protect from light
6. **Stop Solution(55 mL) (REF EIASS2) 6** – Methanesulfonic acid
   *WARNING may be corrosive to metals. H290, P234, P390, P406
7. **Positive Control (1 mL) (REF COVPC1)** + – human serum containing anti-SARS-CoV-2 antibodies in a buffered solution containing a preservative
8. **Negative Control (1 mL) (REF COVNC1)** – – human serum not containing anti-SARS-CoV-2 antibodies in buffered solution containing a preservative
9. **Package insert**
10. **Plate seals (5/kit)**

MATERIALS NOT PROVIDED

1. Distilled or deionized water, for dilution of concentrated wash buffer
2. Disposable gloves
3. Absorbent paper
4. Timer
5. Graduated cylinder for diluting wash buffer
6. Pipettors capable of delivering ranges from 50-300 µL and disposable tips
7. Microplate shaker with a 3 mm radius (.12 inch) capable of reaching at least 300-400 RPM
8. Microplate washer or multichannel pipettor for washing
9. Microplate reader capable of reading absorbances at 450 and 620/630 nm
10. Biohazard receptacle

REAGENT PREPARATIONS

Prepare a 1X solution of Wash Buffer by mixing 19 parts deionized water with 1-part 20X wash buffer (REF EIAWB1). 1X Wash Buffer is stable for 1 month when stored at 2-8°C.

REAGENT STABILITY AND STORAGE

All reagents included in this kit should be stored at the stated temperature (2-8°C) until the expiration dates listed on the reagent labels.

Unused microwells should be returned immediately to the mylar bag and sealed immediately after opening and stored at 2-8°C. Care should be taken to ensure the desiccant pouch remains in the bag with the unused microwells.

The control reagents included in this kit should be stored at the stated temperature (2-8°C) until the expiration dates listed on the reagent labels.

SPECIMEN COLLECTION AND PREPARATION

Use established techniques by qualified personnel, collect samples aseptically. **Samples must be collected by a healthcare professional. Home collection is not allowed.** When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents. This assay has not been validated on specimens other than serum. For optimal results, sterile non-hemolyzed specimens should be used. Collect serum specimens aseptically following accepted procedures. If a delay is encountered in specimen processing, storage at 2-8°C for up to 5 days is permissible. Serum may be stored for longer periods at <-20°C, provided they are not repeatedly thawed and refrozen. Serum in transit should be maintained at 2-8°C or <-20°C.

PROCEDURE

REFER TO *REAGENTS* SECTION FOR A LIST OF MATERIALS PROVIDED.

1. Retrieve all needed reagents and allow to come to room temperature for at least 1 hour.
2. Snap off a sufficient number of antigen-coated microwells for patient samples and controls and insert into microwell holder, recording the position of each sample and control.
3. Add 50 µL of a Positive Control to a designated well.
4. Add 50 µL of a Negative control to a designated well.
5. Add 50 µL of Calibrator Cutoff reagent to **two** microwells.
6. Add 50 µL of patient specimens to microwells. This **must** be performed before addition of conjugate.
7. Add 50 µL of conjugate solution to all wells.
8. Seal the plate carefully with provided plate sealer. All microwells used must be sealed.
9. Shake hard for 30 minutes (+/- 1 minute) at 20-25°C at a minimum of 300-400 RPM. (See Onboarding Guide for guidance on selecting appropriate shaking parameters for your instrumentation).
10. Aspirate all contents from wells. If possible, manual removal of contents will allow for less frequent cleaning of automated plate washers.
11. Wash the plate **4X with 300 µL** of 1X Wash Buffer. If using a plate washer, ensure excessive force during dispensing and aspiration does not occur (See On-boarding Guide for washing guidance).
12. Smack against a clean paper towel to remove residual moisture.
13. Add 100 µL of TMB and incubate at 20-25°C for 20 minutes (+/- 1 minute) stationary.
14. Add 100 µL of Stop Solution
15. Read and record the results (See READING THE TEST).

READING THE TEST

1. Mix by gently tapping the side of the plate or shaking on the countertop for 1-5 seconds.
2. Carefully wipe the undersides of the microwells with a clean, lint-free tissue.
3. Read the optical density of each microwell at both 450 nm and 620/630 nm. Corrected OD values will be used for interpretation (see Interpretation of Results section below). Results must be read within 15 minutes of adding Stop Solution.
4. Discard any used assay materials as hazardous waste and retain microwell holder.
5. Disinfect the microwell holder with a disinfectant such as:
   a. A solution of 10% bleach
   b. 70% Ethanol
   c. 1% Lysol brand I.C. TM

NOTE: If using automation to run the assay, please contact the equipment manufacturer for further instructions.

QUALITY CONTROL

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

1. Average the corrected OD values of the Calibrator Cutoff (Ref COVCC1). Calibrator Cutoff should have a average corrected OD value greater than 0.06, but less than 0.12.
2. Divide all control and specimen wells by this averaged value. The Positive Control specifications are between 1.5 and 3.5 EIA units. The Negative Control specification is less than 1.25 EIA units
3. Positive and negative controls must be tested each time clarus SARS-CoV-2 Total Antibody EIA is performed to ensure proper assay function.

Reagent	QC specification
Calibrator Cutoff (COVCC1)	**Corrected O.D between 0.06 – 0.12**
Positive Control (COVPC1)	**EIA units between 1.5 -3.5**
Negative Control (COVNC1)	**< 1.25 EIA units**

INTERPRETATION OF RESULTS

1. Interpretation of clarus SARS-CoV-2 Total Antibody EIA results should be performed only after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, patient results cannot be interpreted.
2. The Corrected OD (also referred to as "Raw OD") is used for calculations. The Corrected OD is calculated by subtracting the sample OD read at 620/630nm from the sample OD read at 450nm. This calculation is typically performed using microplate reader software, but can also be performed manually (see equation and example below).

For manual Corrected OD calculation use the equation below:

Corrected OD of Sample 1 = OD (450nm) Sample 1 – OD (620/630nm) Sample 1

Example:
Corrected OD = $0.112_{(450\ Sample\ 1)} - 0.043_{(620\ Sample\ 1)}$
Corrected OD = 0.069

3. Calculate EIA Units by dividing the corrected OD of each specimen well by the average corrected OD value of the Calibrator Cutoff wells.

$$EIA\ Units = \frac{Corrected\ O.D.of\ Specimen}{Corrected\ O.D.of\ \text{Calibrator}\ Cutoff}$$

Interpretation Criteria

EIA Units	Interpretation
x < 1.25	Negative
x ≥ 1.25	Positive

Negative results do not rule out the presence of antibody. The specimen may be drawn before detectable antibody is present.

LIMITATIONS OF THE PROCEDURE

1. The detection of anti-SARS-CoV-2 antibodies is dependent on the presence of the analyte in the specimen. A negative result can occur if the quantity of antibodies for the SARS-CoV-2 present in the specimen is below the detection limit of the assay.
2. 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 the test early after infection is unknown. False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic determination is made.
3. A negative result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody used in the test.
4. 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.
5. The clarus SARS-CoV-2 Total Ab EIA test should not be used for the screening of donated blood.
6. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.
7. Correct performance of sample collection and storage is crucial for accurate test results.
8. The partial or complete adjustment of the test system to the use of instruments for automated sample processing or other liquid handling devices may result in differences between the results obtained with automated processing and those obtained with a manual procedure. It is the responsibility of the user to validate the instruments used so that they yield test results within the reliable range.
9. Immunocompromised individuals and patients undergoing immunosuppressive therapies may not develop a detectable immune response to the SARS-CoV-2 virus.
10. Following infection, it is unknown for how long antibody response may persist.
11. clarus SARS-CoV-2 Total Antibody EIA performance characteristics have not been established for samples other than serum.
12. The results obtained with this test should only be interpreted in conjunction with other laboratory tests, and clinical findings.

CROSS-REACTIVITY ANALYSIS

The clarus SARS-CoV-2 Total Antibody EIA has been evaluated for cross-reactivity to several potential cross-reactants (See table below). No cross-reactivity was observed. The following potential cross-reactive samples were not available and therefore not tested: *Haemophilus influenzae,* coronavirus 229E, and coronavirus HKU1.

Analyte	# of Samples Tested	Nonreactive	Reactive
anti-HCV	7	7	0
RSV (infection)	1	1	0
CMV (infection)	10	10	0
Corona – OC43 (infection)	1	1	0
Corona – NL63 (infection)	1	1	0
Flu A/B (infection)	10	10	0
HIV (infection)	14	14	0
anti-HBV (vaccinated)	17	17	0
Total	61	61	0

CLINICAL AGREEMENT STUDY

The purpose of this study was to establish performance characteristics for the clarus SARS-CoV-2 Total Antibody EIA by evaluating clinical agreement using human specimens from patients with microbiologically confirmed COVID-19 infection (qPCR+).

A total of 368 serum (72 positive/ 296 negative) were evaluated. PCR positive samples were collected from 3/31/2020 through 5/7/2020. Presumed negative samples were collected prior to December 2019. Sample preparation and testing procedures were performed based on the clarus SARS-CoV-2 Total Ab EIA IFU. The results of this comparison are shown in the table below:

clarus SARS-CoV-2 Total Antibody EIA	Comparator/Clinical Truth	
	Positive	Negative
Reactive	66	0
Non-reactive	6	296

**The above 2X2 table excludes patient specimens that were collected less than 8 days post symptom onset, and patients on immunosuppressive medications (when known).*

Senstivity and Specificity were 92% (95% CI: 83, 97%) and 100% (95% CI: 99, 100%), respectively.
Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 100% (95% CI: 95, 100) and 98% (95% CI: 96, 99), respectively.

Note: IMMY did not have access to patient history for some of the PCR+ samples, immune status (on immunosuppressants) or date of symptom onset. Therefore, false negatives cannot be fully resolved.

CLASS SPECIFICITY

All classes of antibody are detected by this assay.

HAZARDS AND PRECAUTIONARY INFORMATION

Refer to the product Safety Data Sheets (SDS) for Hazards and Precautionary Statements.

BIBLIOGRAPHY

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International Symbol Usage

	Storage 2-8 °C
	Manufactured by
	Expiration Date
	Protect from Humidity
	Lot Number
	Reference Number
	*In Vitro* Diagnostics
	Sufficient for "#" Tests
	Keep away from sunlight

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ON-BOARDING ASSISTANCE
To assist in bringing the clarus SARS-CoV-2 Total Antibody EIA into use, request IMMY's On-Boarding Guide by emailing techsupport@immy.com.