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Immunoassay

REF **CMU0501 / CMU0502**

50 tests / 100 tests

Anti-SARS-CoV-2 RBD CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of antibodies (including IgG) to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spike (S) protein receptor binding domain (RBD) in human serum and plasma.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		date of manufacture

EC REP

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CE IVD

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Introduction

Coronaviruses are a large family of single-stranded RNA viruses that infect mammals and birds, causing respiratory infection. SARS-CoV-2 is mainly composed of four structural proteins including nuclear protein (N), viral envelop (E), matrix protein (M) and spike protein (S). S protein is a trimer transmembrane glycoprotein, which is composed of S1 and S2 subunits. The receptor binding domain (RBD) on S1 is directly involved in the recognition of host receptors. Angiotensin converting enzyme-2 (ACE2) is the main specific receptor for virus invasion into host cells. It is found that the RBD of the SARS-CoV-2 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells.

Upon infection with SARS-CoV-2, the host mounts an immune response against the virus, typically including production of specific antibodies against viral antigens. It is found that the antibodies against SARS-CoV-2 with strong neutralizing capacity, especially potent if directed against the RBD. Numerous vaccines for COVID-19 are in development, many of which focus on eliciting an immune response to the RBD.

This kit is intended as an aid to assess the adaptive humoral immune response to the SARS-CoV-2 S protein. This kit uses a recombinant protein representing the RBD of the S antigen to quantitative determine the high affinity antibodies against SARS-CoV-2.

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, SARS-CoV-2 RBD antigen coated microparticles and HRP labeled SARS-CoV-2 antigen are combined. During the incubation, specified SARS-CoV-2 RBD antibodies present in the sample are allowed to react simultaneously with the two antigens, resulting in the RBD antibodies being sandwiched between the solid phase and enzyme-linked antigens. After washing, a complex is generated among the solid phase, RBD antibodies in the sample and enzyme-linked antigens by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of SARS-CoV-2 antibodies in the sample.

Materials Provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. Contains a selection of preservatives. Calibrators provided ready to use.

2. Quality Control

1 vial (1.0 mL). SARS-CoV-2 specific antibody in the buffer containing a selection of preservatives. Reagent provided ready to use.

3. Reagent pack

Reagent pack provided ready to use.

Σ	50	100
Microparticles Solution	1.2mL	2.3mL
Enzyme Conjugate	3.0mL	5.5mL

- Microparticles Solution

Recombinant SARS-CoV-2 RBD antigen coated microparticles in the buffer containing Casein. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish peroxidase labeled SARS-CoV-2 RBD antigens in the buffer containing bovine serum and Casein. Contains a selection of preservative.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or Autolumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled water or deionized water

Warnings and Precautions

1. For professional use only. For *In Vitro* Diagnostic use.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Handle the potentially contaminated materials and wastes safely according to local requirement.
4. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
5. Do not smoke, drink, eat or use cosmetics in the working area.
6. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of having COVID-19. Wash hands after operations.
7. Avoid testing in harsh environments (such as those containing 84 disinfectants, sodium hypochlorite, acid, alkaline, or acetaldehyde and other high concentration corrosive gases and dust).
8. Do not use reagents beyond the labeled expiry date.
9. Do not mix or use components from kits with different batch codes.
10. When storing the controls, be certain the vials are securely sealed.
11. Ensure the microparticles are resuspended before loading it on the analyzer.
12. Avoid foam formation in all reagents and sample types (samples and controls).
13. Do not substitute any reagent in this kit from other manufacturers or other lots.
14. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10 ℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 2 months.

Sample

1. Serum and plasma samples may be used in this assay. The anticoagulants EDTA, sodium heparin and sodium citrate have been tested and may be used with this assay. The correct sample type must be used in the assay.
2. Do not use samples with the following conditions:
   - pooled
   - grossly hemolyzed
   - obvious microbial contamination
   - cadaver samples or any other body fluids
   - sodium azide preservative
   - grossly lipemic
   - sediments or suspended solids
   - multiple freeze-thaw cycles
3. Collect samples in accordance with correct medical practices. After the blood collection, please follow the tube manufacturer's processing instructions for serum or plasma collection tubes.
4. Ensure complete clot formation in serum samples before centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
5. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
6. For optimal results, inspect all samples for bubbles. Remove bubbles with a pipette tip prior to analysis. Use a new tip for each sample to prevent cross contamination.
7. Samples must be separated from clots or red blood cells using centrifugation as recommended by the tube manufacturer. Gravity separation is not sufficient for sample preparation.
8. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
9. Samples may be stored at 2-8℃ up to 7 days. Or freeze the samples that need to be stored for more than 7 days at -20℃ or colder. Avoid multiple freeze-thaw cycles. Store the samples at 18-25℃ for no more than 8 hours. Prior to use, it is recommended the frozen samples be reconstituted, mixed well and centrifuged if sediments was observed.
10. Uncontrolled transport conditions (in terms of temperature and time) can cause inaccurate analytical results.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (un-punctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tubes on the sample rack, 20 μL of serum or plasma sample for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the samples to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of SARS-CoV-2 RBD antibodies in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. They are automatically tested.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date (28 days) of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a value exceeding 1000 AU/mL may be diluted manually. Diluent Universal is used to dilute the samples. After dilution, multiply the result by the dilution factor.

Note: Antibodies to SARS-CoV-2 RBD are heterogeneous. In some isolated cases, this may lead to non-linear dilution behavior.

Control Procedure

For quality control of the Anti-SARS-CoV-2 RBD CLIA Microparticles assay, use the quality controls at least once during each day that samples are analyzed. Use the quality control procedure on the system, which could be performed automatically.

- Analyzer can read the bar code automatically to obtain the essential information for the test.
- Transfer the Anti-SARS-CoV-2 RBD CLIA Microparticles Quality Control into the sample cup(s) or tube(s) and place them on the sample rack.
- Load the sample rack
- Select quality control procedure on the system software interface and click "run" to start the test

The Quality Control must be tested using quality control procedure, otherwise it will result in incorrect results. The quality control should be

re-established if the control and/or reagent lot is changed. Different batches of quality control should not be cross-used. When the controls fail to fall within the expected control interval, associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory develops its suitable quality control program complying with applicable government regulations and local guidelines.

Measurement Results

The sample test results are determined automatically by the system software. The amount of SARS-CoV-2 RBD antibodies in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample results.

Interpretation of Results

Results obtained with the Anti-SARS-CoV-2 RBD CLIA Microparticles can be interpreted as follows:
Non-reactive: <8 AU/mL
Reactive: ≥ 8 AU/mL
A nonreactive result indicates no infection of SARS-CoV-2 or there is not enough detectable antibodies. Therefore the production of SARS-CoV-2 RBD antibodies do not be excluded.
A reactive result indicates the present of SARS-CoV-2 RBD antibodies.
Due to the methodology, immunologic specificity or epidemiology, the different results of the same sample may be acquired with different reagents. Therefore, the results of different reagents should not be directly compared with each other to avoid the wrong medical interpretation.

Limitations of the Procedure

1. Negative results do not rule out SARS-CoV-2 infection, particularly those who have been in contacted with the virus. Direct testing with molecular diagnostic should be performed to evaluate for acute SARS-CoV-2 infection in symptomatic individuals.
2. Possible cross-reaction with other coronavirals, such as HKU1, NL63, OC43, 229E and SARS-CoV.
3. The diagnosis and exclusion of SARS-CoV-2 infection and infection status cannot be made solely on the basis of this assay results.
4. A reactive 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. It is unknown at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
6. This kit should not be used for blood screening.
7. At present, the time of production and duration of SARS-CoV-2 RBD antibodies after SARS-CoV-2 infection or vaccine injection are uncertain, and the time of production and duration of different individuals vary greatly. There are also differences in the timing of antibody production after injection of different types of vaccines.
8. This assay has not been studied in a large number of the recovery population and different types of vaccine injection populations. The reference interval of this assay may not be completely applicable to the above population, it is recommended that each laboratories refer to the results of local laboratories for confirmation.
9. The infection rate of SARS-CoV-2 varies in different countries and regions. People in different countries and regions may be injected different types of vaccines, and there are also differences in antibody production after injection. The reference interval suitable for the laboratory should be established according to the local actual situation.
10. There may be cross-react with other pathogens, such as respiratory pathogens.
11. There may be interference in elderly patients, or in patients with autoimmune abnormalities such as ICU patients, leading to false positive test results.
12. It is reported that no specified SARS-CoV-2 RBD antibodies production in some patients with confirmed infection. In some patients, the antibodies disappear a few months after infection.
13. The samples need to be diluted if beyond the detection range.
14. For citrated plasma, the dilution effect must be taken into account.
15. Due to the diversity of the antibodies, the measured anti-SARS-CoV-2 RBD value can vary depending on the testing procedure used and the applied standard. Results obtained from a single sample using tests from different manufacturers can therefore differ. If there is a change in the assay procedure used during the monitoring of antibody titers, then the anti-SARS-CoV-2 RBD values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.
16. This test measures concentrations within the range of 3-1000AU/mL. If Anti-SARS-CoV-2 RBD concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal. The recommended dilution is 1:9 of this test.

Performance Characteristics

1. Measurement Precision

The CV should be ≤ 10%.

2. Analytical Sensitivity

Limit of Detection: 3AU/mL.

3. Analytical Specificity

Cross reaction: 247 potentially cross reacting samples were tested with the Anti-SARS-CoV-2 RBD CLIA Microparticles assay, no cross-reactivity was found. Results are shown in the following tables:

Substance	N	Reactive
RF (rheumatoid arthritis)	10	0
ANA (anti-nuclear antibodies)	18	0
HIV	6	0
HBV	30	0
HCV	50	0
TP (Treponema pallidum)	50	0
HSV-1 IgM	5	0
HSV-1 IgG	3	0
HSV-2 IgM	5	0
HSV-2 IgG	5	0
Rubella IgM	4	0
Rubella IgG	5	0
Toxo IgM	5	0
Toxo IgG	5	0
CMV IgM	2	0
CMV IgG	5	0
Mycoplasma pneumoniae IgM	3	0
Mycoplasma pneumoniae IgG	4	0

Influenza A IgM	5	0
Influenza B IgM	5	0
Chlamydia pneumoniae IgM	4	0
B19 IgM	3	0
ADV IgM	5	0
Legionella pneumophila IgM	5	0
Coxsackie B IgM	5	0
Total	247	0

Interference: bilirubin (up to 2000 mg/dL), hemoglobin (up to 1000 mg/dL), triglyceride (up to 50 mg/dL) have no inference with this assay.

4. Clinical Study

Relative Sensitivity: A total of 273 samples from symptomatic patients with a PCR confirmed SARS-CoV-2 infection were tested with the Anti-SARS-CoV-2 RBD CLIA Microparticles. A total of 123 samples were at acute phase (within 10 days from post-symptom onset), and a total of 150 samples were at recovery phase (days from post-symptom onset were >14), the sensitivity are summarized in the following table:

Phase	N	Reactive	Sensitivity
Acute phase	123	73	59.35%
Recovery phase	150	144	96.00%
Total	273	217	79.49%

Correlation of assay results to serum neutralization capacity

The Anti-SARS-CoV-2 RBD CLIA Microparticles was compared with a reference neutralization assay authorized by FDA EUA, the results are summarized in the following table:

		Neutralization assay		
		Positive	Negative	Total
Anti-SARS-CoV-2 RBD CLIA Microparticles	Positive	195	24	219
	Negative	0	54	54
	Total	195	78	273
Positive agreement rate		100.00%		
Negative agreement rate		69.23%		
Total agreement rate		91.21%		

Relative Specificity: 1334 samples from different populations were tested with Anti-SARS-CoV-2 RBD CLIA Microparticles assay, 3 samples were tested reactive, resulting in a total specificity of 99.8%.

Category	N	Nonreactive	Specific
Healthy	688	687	99.85%
Elderly	249	248	99.60%
Dialysis population	81	81	100.00%
Children	206	206	100.00%
Myocardial injury population	60	60	100.00%
Hypertension population	50	49	98.00%
Total	1334	1331	99.78%

Note: due to the complex disease background of clinical patients, there may be a large difference in specificity among different populations.