en

Immunoassay

REF **CMU0601 / CMU0602**

50 tests / 100 tests

SARS-CoV-2 Neutralization Antibody CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the qualitative detection of SARS-CoV-2 neutralization antibodies (antibodies to Severe Acute Respiratory Syndrome Coronavirus 2) 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.

Infection with SARS-CoV-2 initiates an immune response, the body produces specific antibodies, including IgG and IgM, which can be detected in the blood several days after the initial infection. But not all antibodies can block cellular infiltration and replication of the SARS-CoV-2 virus. The antibodies that prevent virus infection and replication are named neutralizing antibodies. It is unknown how long it takes for producing of neutralizing antibodies, and if they are always produced after SARS-CoV-2 infection. Not all patients can produce neutralizing antibodies.

This kit is intended to specifically detect neutralizing antibodies.

Measurement Principle

The specific binding of ACE2 and RBD protein can be blocked by SARS-CoV-2 neutralizing antibody. This assay is based upon the one-step competitive method. SARS-CoV-2 specific neutralizing antibodies in the sample bind to the HRP labeled RBD antigen, which block the combination of ACE2 coated on the Microparticles and the RBD antigen. The HRP labeled RBD antigen not neutralized by the SARS-CoV-2 specific neutralizing antibodies forms a complex with ACE2 on the Microparticles. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is inversely proportional to the amount of SARS-CoV-2 neutralizing antibody in the samples. This test is similar to conventional Virus Neutralization Test (VNT).

Materials provided

1. Positive Control

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

2. Negative Control

1 vial (1.0 mL). Buffer containing a selection of preservatives. Reagent provided ready to use.

3. Quality Control

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

4. Reagent pack

Reagent pack provided ready to use.

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

- Microparticles Solution

ACE2 coated microparticles in the buffer and contains BSA and a selection of ppreservatives.

- Enzyme Conjugate

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

- Sample Diluent

Buffer contain Casein and a selection of preservatives.

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. Chemiluminescent Substrate
5. System Wash for washing the pipetting needles
6. Wash Buffer used in the washing procedure
7. 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.
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 and show this container.
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.

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 (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. 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 cup(s) or tube(s) on the sample rack, 20 μL of 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 sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution, Sample Diluent and Enzyme Conjugate into the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine SARS-CoV-2 neutralizing antibodies in the sample
  - Discards the used reaction vessel
  - Calculates the result.
- Refer to the Assay Analyzer's operation manual.

4. Calibration

- Analyzer can read the bar code on the reagent pack automatically to obtain the essential information for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the SARS-CoV-2 Neutralization Antibody CLIA Microparticles Positive and Negative Controls into the sample cup(s) or tube(s) and place them on the sample rack. They are automatically tested in triplicate or duplicate (triplicate for Negative Controls and duplicate for Positive Controls) at the beginning of each batch.
- Load the sample rack and input Negative and Positive Control information on the system software interface.
- Select "run" to start the test, calibration is required every 28 days.
- Once the control results is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measures
  - A reagent kit or Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of calibration
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

Control Procedure

For quality control of the SARS-CoV-2 Neutralization Antibody 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 SARS-CoV-2 Neutralization Antibody 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

- **Calculation**

The Assay Analyzer system calculates the SARS-CoV-2 Neutralization Antibody CLIA Microparticles assay Cut-off value using the following formula:

Percent Signal Inhibition = (1-sample RLU / Negative Control mean RLU Value) x 100%

- **Interpretation of Results**

Samples with Inhibition rate ≥ 30% are considered positive.

Samples with Inhibition rate < 30% are considered negative.

A positive result indicates the possibility presence of SARS-CoV-2 neutralizing antibody.

A negative result indicates no SARS-CoV-2 neutralizing antibody was detected in the samples.

Limitations of the Procedure

1. This assay is designed for qualitative detection of SARS-CoV-2 neutralizing antibodies.
2. This assay has not been studied in a large number of different types of vaccine injection populations. The setting of reference interval and test situations for these populations should be determined based on actual testing in local laboratories.
3. 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.
4. Possible cross-reaction with other coronavirus, such as HKU1, NL63, OC43, 229E and SARS-CoV.
5. Results from this test should not be used to diagnose or to exclude acute SARS-CoV-2 infection or to inform infection status.
6. 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.
7. It is unknown at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
8. This kit should not be used for blood screening.
9. It is not clear how long it takes to produce SARS-CoV-2 neutralizing antibodies after infection.
10. There may be cross-react with other pathogens, such as respiratory pathogens.
11. False positive test results may be found in patients with abnormal ACE2 content, such as patients with myocardial injury, patients with hypertension, patients with abnormal thyroid function, and patients with pulmonary disease.
12. There may be interference in elderly patients, or in patients with autoimmune abnormalities such as ICU patients, leading to false positive test results.
13. The use of citrated plasma may cause high false positive rate.

Performance Characteristics

1. Measurement Precision

The CV is ≤10%.

2. Analytical Specificity

Cross reaction: Cross-reactivity of the SARS-CoV-2 Neutralization Antibody CLIA Microparticles was evaluated using samples containing antibodies to other pathogens. 3 positive results were observed with the potential cross reactants listed in the following table:

Category	Number of sample	Number Positive with this assay
RF (rheumatoid arthritis)	10	1
ANA (anti-nuclear antibodies)	18	1
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
Mycoplasma pneumoniae IgG	4	0
Rubella IgM	8	0
Rubella IgG	14	0
CMV IgM	7	0
CMV IgG	15	0
Toxo IgM	8	0
Toxo IgG	10	0
B19 IgM	5	0
RSV IgM	3	0
Chlamydia pneumoniae IgM	5	0
Mycoplasma pneumoniae IgM	5	0
ADV IgM	6	1
Coxsackie B IgM	10	0
Influenza A IgM	10	0
Influenza B IgM	10	0
Legionella pneumophila IgM	7	0
Total	309	3

The cross-reactivity of ANA, ADV IgM and RF cannot be excluded.

Interference: the impact of potentially interfering substances on the detection of SARS-CoV-2 antibodies with the SARS-CoV-2 Neutralization Antibody CLIA Microparticles was evaluated with endogenous substances, including bilirubin, haemoglobin and triglycerides, there is no interference at the following concentrations:

Substance	Test concentration
Bilirubin	50 mg/ dL
Haemoglobin	1000 mg/dL
Triglycerides	2000 mg/dL

3. Sensitivity and Specificity

A total of 273 samples from symptomatic patients with a PCR confirmed SARS-CoV-2 infection were tested with the SARS-CoV-2 Neutralization Antibody CLIA Microparticles and a reference neutralization assay authorized by FDA EUA. 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 results of agreement are shown below.

		Reference assay		
		Positive	Negative	Total
This assay	Positive	193	2	195
	Negative	2	76	78
	Total	195	78	273
Positive agreement		98.97%		
Negative agreement		97.44%		
Total agreement		98.53%		

Sensitivity by days from post-symptom onset

Days from Post-Symptom onset	N	This assay		Reference assay	
		Positive	Sensitivity	Positive	Sensitivity
Acute phase	123	51	41.46%	51	41.46%
Recovery phase	150	144	96.00%	144	96.00%
Total	273	195	73.01%	195	73.01%

Relative Specificity: 1621 samples from different populations were tested 5 samples were tested positive with SARS-CoV-2 Neutralization Antibody CLIA Microparticles assay, resulting in a total specificity of 99.7%. Among the 60 myocardial injury samples, 2 samples were tested positive with SARS-CoV-2 Neutralization Antibody CLIA Microparticles assay, with a lower specificity than other populations.

Category	N	Negative	Specificity
Healthy	921	918	99.7%
Adult respiratory patients	48	48	100.0%
Elderly	100	100	100.0%
Dialysis population	81	81	100.0%
Children	171	171	100.0%
Myocardial injury population	60	58	96.7%
Hypertension population	50	50	100.0%
Thyroid dysfunction population	190	190	100.0%
Total	**1621**	**1616**	**99.7%**

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