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Immunoassay

REF **CMU0701 / CMU0702**

50 tests / 100 tests

SARS-CoV-2 NAb Q CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination 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 neutralization antibodies (NAb).

Measurement Principle

The specific interaction of ACE2 and RBD protein can be neutralized by SARS-CoV-2 neutralizing antibodies. 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 neutralize 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.

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. Control 1

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

3. Control 2

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 PBS buffer and contains BSA and a selection of preservatives.

- Enzyme Conjugate

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

- Sample Diluent

Tris-NaCl buffer contains 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. Decontaminate and dispose of all samples, reaction kits, and potentially contaminated materials as if they were infectious waste, in a biohazard waste container.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. 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.
6. CAUTION: It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of having COVID-19. Wash hands after operations.
9. 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).
10. Do not use reagents beyond the labeled expiry date.
11. Do not mix or use components from kits with different batch codes.
12. When storing the controls, be certain the vials are securely sealed.
13. Ensure the microparticles are resuspended before loading it on the analyzer.
14. Avoid foam formation in all reagents and sample types (samples and controls).
15. Do not substitute any reagent in this kit from other manufacturers or other lots.
16. 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, 30 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 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, 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.

5. Dilute the sample

Samples with a value exceeding 3000 AU/mL may be diluted manually. Negative serum or plasma is used to dilute the samples. After dilution, multiply the result by the dilution factor.

Control Procedure

For quality control of the SARS-CoV-2 NAb Q CLIA Microparticles assay, use the 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 NAb Q CLIA Microparticles controls 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 Controls 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 neutralization 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 SARS-CoV-2 NAb Q CLIA Microparticles can be interpreted as follows:
Non-reactive: <30 AU/mL
Reactive: ≥ 30 AU/mL
It is recommended that each laboratory establish its own normal range depending upon its actual condition and contact population.
A nonreactive result indicates no detection of SARS-CoV-2 or there is not enough detectable antibodies. Therefore the production of SARS-CoV-2 neutralization antibodies do not be excluded.
A reactive result indicates the present of SARS-CoV-2 neutralization antibodies. But it still needs to be combined with clinical symptoms or other detection methods for comprehensive judgment.

Limitations of the Procedure

1. Conduct this assay in combination with clinical examination, patient's medical history and other test results.
2. The relationship between the concentration of SARS-CoV-2 neutralization antibody and its immunological protection is not clear yet.
3. Immune status cannot be assessed solely on the basis of results of this assay.
4. It is not known how long the SARS-CoV-2 neutralization antibody persists in the body.
5. 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.
6. There may be interference in elderly patients, or in patients with autoimmune abnormalities such as ICU patients, leading to false positive test results.
7. It is not clear the difference of neutralizing antibody production after injection of different types of COVID-19 vaccines. The neutralizing antibodies may not be produced after COVID-19 vaccine injection in very few people.
8. If SARS-CoV-2 neutralization antibody concentrations exceed 3000 AU/mL, it is recommended to dilute the samples with negative serum or plasma. The recommended dilution is 1:9 of this test.
9. The kits shall be transported in cold chain to ensure the performance.

Performance Characteristics

1. Measurement Precision

The CV was ≤15%.

2. Analytical Specificity

Cross reaction: Cross-reactivity of the SARS-CoV-2 NAb Q CLIA Microparticles was evaluated using samples containing antibodies to other pathogens. 2 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	0
ANA (anti-nuclear antibodies)	18	2
HIV	6	0
HBV	18	0
HCV	20	0
TP (Treponema pallidum)	28	0
Rubella IgM	5	0
Rubella IgG	5	0
CMV IgM	5	0
CMV IgG	5	0
Toxo IgM	5	0
Toxo IgG	5	0
B19 IgM	5	0
RSV IgM	3	0
Chlamydia pneumoniae IgM	5	0
Mycoplasma pneumoniae IgM	3	0
ADV IgM	5	0
Coxsackie B IgM	5	0
Influenza A IgM	5	0
Influenza B IgM	5	0
Legionella pneumophila IgM	5	0
Total	171	2

The cross-reactivity of ANA cannot be excluded.

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

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

3. Sensitivity and Specificity

A total of 176 samples were tested with this assay and a reference assay (CE marked), the positive agreement and the negative agreement are summarized in the following table:

		Reference assay		
		Positive	Negative	Total
this assay	Positive	94	0	94
	Negative	0	82	82
	Total	94	82	176
Positive agreement		100.00%		

Negative agreement	100.00%
Total agreement	100.00%

Relative Specificity: 680 samples from different populations were tested with SARS-CoV-2 NAb Q CLIA Microparticles assay, resulting in a total specificity of 99.71%. Among the 60 myocardial injury samples, 2 samples were tested positive with SARS-CoV-2 NAb Q CLIA Microparticles assay, with a lower specificity than other populations.

Category	N	Nonreactive	Specific
Children respiratory patients	278	278	100.00%
Healthy	184	184	100.00%
Elderly	94	94	100.00%
Dialysis population	64	64	100.00%
Myocardial injury population	60	58	96.67%
Total	680	678	99.71%

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