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Immunoassay

REF **CMU0401 / CMU0402**

50 tests / 100 tests

SARS-CoV-2 IgM II CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the qualitative detection of SARS-CoV-2 IgM (IgM 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. SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS coronavirus 2 or SARS-CoV-2)[1], a virus closely related to the SARS virus, causes an infectious disease named COVID-19 (Coronavirus disease 2019). Those affected may develop a fever, dry cough, fatigue and shortness of breath.[2] Cases can progress to pneumonia and multi-organ failure, particularly in the most vulnerable. The infection can also be diagnosed from a combination of symptoms, risk factors, and a chest CT scan showing features of pneumonia. The results of this test may vary by apparent disease periods by time after symptom onset.

This product is used for the qualitative detection of SARS-CoV-2 IgM antibodies in human serum or plasma samples *in vitro*. It is used as a supplementary indicator for nucleic acid detection of SARS-CoV-2. It cannot be used as the standard for the diagnosis and exclusion of pneumonitis caused by SARS-CoV-2.

Measurement Principle

This assay is based upon the two-step capture method. In the first step, sample and mouse anti-human IgM coated microparticles are combined. During the incubation, the IgM antibodies in the sample bind to the anti-human IgM coated on the microparticles. After the washing, in the second step, Enzyme Conjugate is added to the reaction mixture. During the incubation, the HPR-conjugated SARS-CoV-2 antigen in the Enzyme Conjugate is allowed to react with the SARS-CoV-2 IgM already bound to the solid phase in the first step. A complex is generated among the solid phase, antibodies in the sample and enzyme-linked antigens by immunological reactions. After a second washing, 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 IgM in the sample.

Materials provided

1. Positive Control

1 vial (1.0 mL). SARS-CoV-2 IgM recombinant antibody in Tris buffer containing ProClin 300® and Bronidox preservatives. Reagent provided ready to use.

2. Negative Control

1 vial (1.0 mL). Tris buffer contains ProClin 300® and Bronidox preservatives. Reagent provided ready to use.

3. Quality Control

1 vial (2.0 mL). SARS-CoV-2 IgM recombinant antibody in Tris buffer containing ProClin 300® and Bronidox 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	5.5mL	11.0mL
Sample Diluent	5.5mL	11.0mL

- Microparticles Solution

Mouse anti-human IgM coated microparticles in PBS buffer containing Casein. Contains ProClin 300® and sodium azide preservatives.

- Enzyme Conjugate

Horseradish peroxidase labeled SARS-CoV-2 antigens in a Tris buffer containing bovine serum and Casein. Contains ProClin 300® and Bronidox preservatives.

- Sample Diluent

Sample Diluent containing Tris buffer. Contains ProClin 300® and sodium azide 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. 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.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
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. Do not smoke, drink, eat or use cosmetics in the working area.
7. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of having COVID-19. Wash hands after operations.
8. 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).
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the controls, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. 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 Diluent Universal and sample into the reaction vessel
  - Aspirates and transfer diluted sample into the reaction vessel
  - Adds Microparticles Solution and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine SARS-CoV-2 IgM 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 IgM II 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 Positive Controls and duplicate for Negative Controls) at the beginning of each batch. The Assay Analyzer system will not generate results when controls values do not meet specifications. This may indicate either deterioration or contamination of reagents, or analyzer failure.
- Load the sample rack and input Negative & 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 measurements
  - A reagent kit and 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. Quality control

For quality control of the SARS-CoV-2 IgM II assay, use the SARS-CoV-2 IgM II QC 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 IgM II 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 IgM II CLIA Microparticles assay Cut-off value using the following formula:

1. Cut-off Values = Positive Control mean RLU Value x 0.25
2. S/CO = Sample RLU/Cut-off Value

- **Interpretation of Results**

Samples with S/CO values <1.00 are considered nonreactive (NR).

Samples with S/CO values ≥1.00 are considered reactive (R).

A reactive result indicates the possibility of SARS-CoV-2 infection, but it still needs to be diagnosed in combination with clinical symptoms or other tests.

A nonreactive result indicates no infection of SARS-CoV-2 or there is not enough detectable antibodies. Therefore the SARS-CoV-2 infection cannot be ruled out under this situation.

For those equivocal results near cut-off value (S/CO=0.80-1.20), it is recommended to perform a second test and dynamic observation or use other method.

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.

Combined methods of nucleic acid, antibody and antigen can shorten the window period and detection rate.

The test result indicates the reaction of SARS-CoV-2, however, it should not be solely used for the clinical diagnosis of SARS-CoV-2. Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results. As with all diagnostic assays, all results must be interpreted together with other clinical information available to the physician.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result. A negative result does not preclude the possibility of SARS-CoV-2 infection. Any positive result should be confirmed by chest CT, NAT or other clinical diagnosis result. If symptoms persist and the result from the SARS-CoV-2 IgM II CLIA Microparticles is negative or non-reactive, it is recommended to re-sample the patient a few days later or test with an alternative test device.
3. This kit DOES NOT produce an actual test report and the reporting laboratory MUST include this information in the test report. The results of this antibody detection kit should NOT BE SOLELY USED as the basis for diagnosis or exclusion. All testing results shall be judged in combination with epidemiological, and clinical signs, imaging, nucleic acid detection and other evidence.
4. When diagnostic testing is non-reactive, the possibility of a false negative result should be considered in the context of a patient's recent exposures and the presence of clinical signs and symptoms consistent with COVID-19. This is especially important if the patient has had recent exposure to COVID-19, or clinical presentation indicates that COVID- 19 is likely and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. Direct testing for virus (e.g., PCR testing) should always be performed in any patient suspected of COVID-19, regardless of the SARS-CoV-2 IgM II CLIA Microparticles.
5. Results from antibody testing should NOT be used as the SOLE basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
6. This test kit is for the detection of SARS-CoV-2 IgM in human serum or plasma samples. Quantitative value in SARS-CoV-2 IgM cannot be determined by this qualitative test.
7. Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product, the product is used outside of the control of the Manufacturer and Distributor and the result may accordingly be affected by environmental factors and/or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.
8. Antibodies are produced gradually by the immune response system after the pathogens invade the human body for a period of time. The sensitivity of antibody detection is directly related to the time after infection when blood samples are collected. Generally, IgM antibodies are gradually generated within one week of the appearance of symptoms during which the positive rate will be low. It increases rapidly after one week and reaches a peak in 2-3 weeks, and then begins to decline. IgG antibodies begin to generate after one week, reach a peak in 3-4 weeks, and last for a long period of time. The results of this test may vary during different infection periods.
9. All laboratories using this test must follow standard confirmatory testing and reporting guidelines according to their appropriate public health authorities.
10. As a newly emerging virus, extensive research is lacking for SARS-CoV-2. This assay is established mainly based on a Chinese population. Clinical samples haven't been studied on regions other than China. The results of this assay in different regions may be different due to regional cultures, procedures, epidemic strain, subtype, and serotype differences, virus mutation or other factors.
11. Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229.
12. For some patients with autoimmune diseases, the presence of autoantibodies may interfere with the detection of the reagents, resulting in false positive test results.
13. False positive test results may occur for some dialysis patients.
14. For individuals with immune system disorders, such as some immunosuppressed or excessive immune response, false positive or false negative results may occur.
15. The specificity of different populations may vary. False positive results may occur among the elderly hospitalized patients and severe ICU patients.
16. This kit has no studies for vaccination population.
17. It is reported that IgM antibodies against viruses usually persist for a long time in some people, but there are few studies on the persistence of SARS-CoV-2 IgM. The IgM antibodies can still be detected in some convalescent population by this assay. Under this situation, it is recommended to make a comprehensive judgment based on the changes of IgG antibody, clinical nucleic acid test results, contact history and clinical symptoms.
18. The positive predictive value and negative predictive value of this assay may different in different regions, the positive predictive value is low in the low infection rate area. Large-scale use in these areas is not

recommended. The positive result in the low infection rate area shall be analyzed in combination with clinical history and detection.

19. Due to limitation of methodology or immunological specificity and other reasons, the test results from different manufacturers' reagents for the same sample may be different, so such results should not be directly compared with each other, so as to avoid the wrong medical explanation. It is recommended that the characteristics of the different manufacturers' reagents should be indicated when reported to the clinician.
20. Samples of hemolysis, lipid or turbidity may result in incorrect results.
21. For samples containing sediment, centrifuge for 5-10 minutes to remove the sediment before use.
22. This test should not be used for screening of donated blood.
23. The kits shall be transported in cold chain to ensure the performance.
24. Due to the difference of quality control and clinical positive samples, quality control can only be used to verify the effectiveness of reagents and instruments. It is recommended to refer to the clinical results when deviation appears between quality control and clinical samples.

Performance Characteristics

1. Measurement Precision

The CV is ≤10%.

2. Analytical Specificity

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

Category	Number of sample	Number Reactive with this assay	Number non-reactive with this assay
Influenza A H1N1 IgM	3	0	3
Influenza A H3N2 IgM	3	0	3
Influenza B Yamagata IgM	3	0	3
Influenza B Victoria IgM	3	0	3
RSV IgM	5	0	5
Adenovirus IgM	5	0	5
Enterovirus 71 IgM	3	0	3
Coxsackie virus B IgM	5	0	5
Chlamydia pneumonia IgM	3	0	3
M. pneumoniae IgM	5	0	5
L. pneumophila IgM	3	0	3
Toxo IgM	5	0	5
Rubella IgM	5	0	5
CMV IgM	5	0	5
HSV IgM	5	0	5
Syphilis	94	0	94
Antinuclear antibody (ANA)	95	1	94
Rheumatoid factor (RF)	88	2	86
Total	338	3	335

The cross-reactivity of ANA 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 IgM II CLIA Microparticles was evaluated with endogenous substances, including bilirubin, haemoglobin and triglycerides.

Substance	Test concentration
Bilirubin	50 mg/ dL
Haemoglobin	1000 mg/dL
Triglycerides	6000 mg/dL

3. Sensitivity and Specificity

The clinical performance of the SARS-CoV-2 IgM II CLIA Microparticles was evaluated by testing a total of 1173 clinical samples from individual patients. A total of 106 confirmed positive samples and 1067 confirmed negative samples were tested with the SARS-CoV-2 IgM II CLIA Microparticles. The results of positive agreement and negative agreement are shown below.

Positive Agreement by days from Post-Symptom Onset

Days from Post-Symptom Onset	Number of samples	Reactive samples	Positive percent agreement (95% CI)
≤7	26	9	34.6% (19.4%-53.8%)
8-14	29	25	86.2% (69.4%-94.5%)
≥15	51	50	98.0% (89.7%-99.7%)

Negative Agreement by Category

Category	Number of samples	Non-reactive samples	Negative percent agreement (95% CI)
Healthy	704	700	99.4% (98.5%-99.8%)
Healthy elderly	278	276	99.3% (97.4%-99.8%)
Dialysis population	85	85	100.0% (95.7%-100.0%)
Total	1067	1061	99.4% (98.8%-99.7%)

Note: due to the difference in the selected infection period and the sample size in different infection periods, there were differences in the total clinical sensitivity of evaluation. The specificity may vary among different populations.

Literature References

1. "Naming the coronavirus disease (COVID-19) and the virus that causes it". World Health Organization. Archived from the original on 28 February 2020. Retrieved 28 February 2020.

2. "Coronavirus Disease 2019 (COVID-19) Symptoms". Centers for Disease Control and Prevention. United States. 10 February 2020. Archived from the original on 30 January 2020.