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Immunoassay

REF **CMC0901/CMC0902/ CMC0903/ CMC0904**

50 tests/100 tests/200 tests/500 tests

Anti-HBc IgM CLIA Microparticles

This assay is based on chemiluminescent microparticle immunoassay (CLIA Microparticles) for the qualitative determination of Anti-HBc IgM (IgM Antibody to hepatitis B core antigen) in human serum or plasma (EDTA, heparin or sodium citrate).

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

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IVD

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Introduction

IgM antibody to HBc (IgM anti-HBc) rise rapidly in patients with acute infection and persists for several weeks to months[1]. High anti-HBc IgM titers can be found in acute HBV infection and in attacks during chronic hepatitis B. The level of anti-HBc IgM decreases throughout the course of infection. Anti-HBc IgM may also be present in patients with chronic hepatitis B viral infection[2, 3]. The concentrations are generally lower than those associated with acute infections and may rise and fall with exacerbation of the disease[4]. Anti-HBc IgM can aid to distinguish acute hepatitis illness due to HBV versus superimposed infections by other possible agents such as hepatitis A, hepatitis C, or delta virus[5].

Measurement Principle

This assay is based upon the two-step capture method. In the first step, sample and mouse monoclonal anti-human IgM coated microparticles are combined. During the incubation, the antibodies present in the sample bind to the anti-human IgM coated on the microparticles. After the washing, in the second step, Hepatitis B Core antigen solution and HRP labeled Hepatitis B Core antibody as Enzyme Conjugate are added to the reaction mixture. During the incubation, a complex is generated among the solid phase, anti-HBc IgM in the sample, HBcAg solution and enzyme-linked anti-HBc by immunological reactions. 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 proportional to the amount of anti-HBc IgM in the samples.

Materials provided

1. Positive Control

1 vial (1.0ml) of purified HBc IgM antibody in PBS buffer containing BSA and ProClin 300® preservative. The Positive Control is nonreactive for HIV-1 and HIV-2, HCV and Syphilis.
Reagent provided ready to use.

2. Negative Control

1 vial (1.0ml) of PBS buffer containing BSA and ProClin 300® preservative. The Negative Control is nonreactive for HBsAg, HIV-1 and HIV-2, HCV and Syphilis.
Reagent provided ready to use.

3. Reagent pack

Reagent pack provided ready to use.

Σ	50	100	200	500
Microparticles Solution	1.2ml	2.3ml	4.3ml	11.5ml
Enzyme Conjugate	3.0ml	5.5ml	10.5ml	27.5ml
Antigen Solution	3.0ml	5.5ml	10.5ml	27.5ml
Sample Diluent	5.5ml	11.0ml	21.0ml	55.0ml

- Microparticles Solution

Mouse monoclonal anti-human IgM coated microparticles in PBS (phosphate buffered saline) buffer containing casein. Contains ProClin 300® preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled Hepatitis B Core Antibody in Tris-HCl buffer containing BSA (bovine serum albumin). Contains ProClin 300® preservative.

- Antigen Solution

HBcAg solution in PBS (phosphate buffered saline) buffer containing casein. Contains ProClin 300® preservative.

- Sample Diluent

PBS buffer containing BSA. Contains ProClin 300® 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 tube(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needle
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

Warnings and Precautions

1. For professional use only.
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. 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. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. 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 or label.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
11. Do not use reagents beyond the labeled expiry date.
12. Do not mix or use components from kits with different batch codes.
13. When storing the calibrators, be certain the vials are securely sealed.
14. Ensure the microparticles are resuspended before loading it on the analyzer.
15. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
16. Do not substitute any reagent in this kit from other manufacturers or other lots.
17. 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.
2. Refrigerate the reagent pack at 2-10℃ for a minimum of 2 hours prior to use.
3. Store the reagents pack upright at 2-10℃ on the analyzer. They may be stored on the analyzer 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 2-8℃ in an upright position. For reagents stored off the analyzer, it is recommended that they be stored in their original trays and boxes to ensure they remain upright.

4. Once the reagent pack is open, it can be stored at 2-8℃ for 1 month.
5. Seal and return the remaining positive or negative control to 2-8℃, under which conditions the stability will be retained for 2 months, for longer use, store opened calibrators in aliquots and freeze at -20℃. Avoid multiple freeze-thaw cycles.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. 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.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

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. 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 tube(s) or cup(s) on the sample rack, 4 μl of serum or plasma samples will be automatically diluted 1:99 with 396 μl of Diluent Universal by the Assay Analyzer before 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 10 μl of the 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 and Antigen Solution to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine Anti-HBc 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 required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the Anti-HBc IgM CLIA Microparticles positive and negative controls into the sample tube(s) or cup(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 a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

Measurement Results

- Calculation

The Assay Analyzer system calculates the Anti-HBc IgM CLIA

Microparticles assay Cut-off value using the following formula:

1. Cut-off Values = Positive Control mean RLU Value x 0.13
2. S/CO = Sample RLU/Cut-off Value

Assay analyzer calculates a result based on the samples RLU to the cut-off value for each sample and control.

- **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).

Note: All samples that are initially reactive must be centrifuged and retested in duplicate.

All initially reactive samples should be retested in duplicate. If both retest values are nonreactive, the sample must be considered nonreactive for Anti-HBc IgM. If either of the retest values is reactive, the sample must be considered repeatedly reactive for Anti-HBc IgM by the criteria of this assay.

Repeatedly reactive result is recommended dynamic observation or review by other approach.

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.

Control Procedure

The recommended control requirement for this assay involves using positive and negative controls to verify assay performance. The result is valid if the control results meet assigned specifications (Mean PC RLU/ Mean NC RLU＞10; CV% of each mean PC RLU should be less than 10%). When a control fails to meet assigned specifications, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

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.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Performance of this test has not been established with neonatal samples.
5. This assay was designed and validated for use with human serum or plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
6. Serious hemolysis, lipids, jaundice or turbid samples may result in incorrect test results.
7. In the early infection, pathogen-specific IgM are not produced or low titer. It may lead to negative results. If the patient is suspicious of pathogen infection, suggest that the patient should be rechecking in 7 to 14 days. This sample should be detected simultaneously with the first sample under the same conditions to determine whether there was a primary infection or increased pathogen-specific IgM titers.
8. High titer pathogen-specific IgG antibodies compete with specific IgM antibodies for antigen-binding sites, which may reduce the sensitivity of the assay and may result in false or negative results.
9. Patients with impaired immune function or undergoing immunosuppressive therapy, such as HIV patients or patients undergoing immunosuppression after organ transplantation. The reference value of serological IgM antibody testing is limited and may lead to erroneous medical explanations.
10. Anti-HBc IgM is not only occurred in acute hepatitis B infection, may also occurred in the acute onset of chronic hepatitis B infection.

Performance Characteristics

1. Measurement Precision

Within-run precision is designed to test 3 quality controls (Q1, Q2 and Q3), using 1 batch of reagents, in replicates of 20. Data from this study are summarized in the following table.

No.	Batch	n	Mean	Within-run Precision	
				SD	%CV
Q1	1	20	1.71	0.15	8.77
Q2	1	20	4.05	0.28	6.91
Q3	1	20	8.80	0.57	6.48

Between-run precision is designed to test 3 quality controls (Q1, Q2 and Q3), using 1 batch of reagents on 5 different analyzers and test in replicates of 4 per analyzer. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Between-run Precision	
				SD	%CV
Q1	1	20	1.88	0.12	6.38
Q2	1	20	4.00	0.19	4.75
Q3	1	20	7.98	0.53	6.64

2. Analytical Specificity

Cross reaction: this assay was evaluated for potential cross-reactivity for Anti-HBc IgM analogue samples (e.g. HIV, HEV, HAV, Anti-HBs, HCV, TP, EB, and TORCH). 72 potentially cross-reacting samples from patients were evaluated. All these samples were non-reactive with this assay. The data are summarized in the following table.

Substances	No.	Anti-HBc IgM CLIA Microparticles assay	
		Reactive	Nonreactive
Anti-HIV	8	0	8
Anti-TP	5	0	5
HCV-IgG	5	0	5
EB-IgG	5	0	5
HAV-IgM	5	0	5
HEV-IgM	5	0	5
HEV-IgG	5	0	5
TOX-IgG	2	0	2
TOX-IgM	5	0	5
RV-IgG	2	0	2
RV-IgM	5	0	5
CMV-IgG	2	0	2
CMV-IgM	5	0	5
HSV-1-IgG	2	0	2

HSV-1-IgM	2	0	2
HSV-2-IgG	2	0	2
HSV-2-IgM	2	0	2
Anti-HBs	5	0	5

Interference: No interference with 500mg/dl of hemoglobin, 40 mg/dl of Bilirubin, 3000 mg/dl of Triglycerides.

3. Clinical Coincidence Rate

A comparison study was performed where samples were tested using Anti-HBc IgM CLIA Microparticle assay and a reference assay which was already CE marked. Data and results are summarized in the following table.

		Reference Assay		
		Positive	Negative	Total
This Assay	Positive	221	8	229
	Negative	4	658	662
	Total	225	666	891
Sensitivity		98.22% (221/225)		
Specificity		98.80% (658/666)		
Total Coincidence Rate		98.65% (879/891)		

Literature References

1. Lindsay KL, Nizze JA, Koretz R, et al. (1986) Diagnostic usefulness of testing for anti-HBc IgM in acute hepatitis B. Hepatol;6:1325-8.

2. Decker RH (1993). Diagnosis. In: Zuckerman AJ, Thomas HC, eds. viral hepatitis - Scientific basis and clinical management. New York: Churchill Livingstone, 165-84.

3. Hollinger FB. (1996) Hepatitis B Virus. In: Fields BN, Knipe DM, Howley PM, et al., eds. Fields virology. Third ed. Philadelphia: Lippincott Raven Publishers, 2752-7.

4. Colloredo G, Bellati G, Leandro G, et al. (1996) Quantitative analysis of IgM anti-HBc in chronic hepatitis B patients using a new “grayzone” for the evaluation of “borderline” values. J Hepatol 25:644-8.

5. Kiyosawa K, Sodeyama T, Franca STM, et al. (1988) Serial assay for IgM anti-HBc in patients with anti-HBe-positive chronic hepatitis and its significance for long-term prognosis. J Med Virol 24:241-50.