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Immunoassay

REF **CMC0502**

100 tests

Anti-HBc CLIA Microparticles

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

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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Contact your local dealer for all product-related questions in your local language.

Introduction

Hepatitis B core antigen (HBcAg) is a particle from the core of the hepatitis B virion (HCV), sometimes referred to as the Dane particle. The detection of antibody to hepatitis B core antigen (Anti-HBc) is used to monitor the progress of HBV infections[1]. Anti-HBc is found in serum shortly after the appearance of hepatitis B surface antigen (HBsAg) and, in acute hepatitis B, will persist after the disappearance of HBsAg and before the appearance of detectable antibody to HBsAg (Anti-HBs). Accordingly, they are an indicator of existing or past hepatitis B infection[2].

Due to the long persistence of Anti-HBc following a hepatitis B viral infection, screening for Anti-HBc provides the best information on the prevalence of hepatitis B in a particular group of persons[3]. During active infection both immunoglobulin M (IgM) and immunoglobulin G (IgG) anti-HBc are usually present. Therefore, in the absence of HBsAg and anti-HBs, Anti-HBc may be the only serological markers of HBV infection during the 'window period' when HBsAg has cleared but before antibodies to HBsAg are detectable [4, 5].

Measurement Principle

This assay uses a one-step competitive method. The sample, hepatitis B core antigen coated paramagnetic microparticles and HRP labeled Hepatitis B Core Antibody as enzyme conjugate are combined. During the incubation, HRP labeled Hepatitis B Core Antibody and Anti-HBc present in the sample compete for binding to hepatitis B core antigen coated microparticles. After washing, a complex is generated among the solid phase, Anti-HBc in the sample or enzyme-linked Anti-HBc by immunological reactions. After washing, the substrates are 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 Anti-HBc in the samples.

Materials provided

1. Calibrators

6 vials each containing 1.0 ml of calibrator A through F with corresponding approximate Anti-HBc concentrations showed in the following table. The matrix is Tris-NaCl containing heat-inactivated human plasma positive for Anti-HBc and BSA (bovine serum albumin). Contains ProClin 300® preservative.

Calibrators provided ready to use.

Calibrator	Anti-HBc Concentration (PEI U/ml)
A	0
B	0.7
C	3.5
D	9
E	22
F	45

2. Reagent pack

Reagent pack provided ready to use.

- Enzyme Conjugate

1 vial containing 5.5 ml of horseradish-peroxidase labeled Hepatitis B Core Antibody in Tris-NaCl containing casein. Contains ProClin 300® preservative.

- Microparticles Solution

1 vial containing 2.3 ml of recombined HBcAg coated microparticles in PBS (phosphate buffered saline) containing BSA (bovine serum albumin). Contains ProClin 300® and Sodium azide preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000
- AutoLumo A2000 Plus

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

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 procudure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The product calibrators are manufactured using pure grade Anti-HBc and signal matched to our working calibrators, which are also signal matched to a calibrator purchased from PEI (Paul-Ehrlich-Institute, Germany) at each concentration level.

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: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. It is recommended that all materials of human origin be considered potentially infectious. 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. 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 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 remaining calibrators to 2-8 ℃, under which conditions the stability will be retained for 1 month, 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 tubes or cups on the sample holder, 50 μl of samples and calibrators 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 holder 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 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 Anti-HBc 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 tubes or cups and place them on the sample holder. Conduct duplicate detection on the system.
- Load the sample holder 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 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 an Anti-HBc value exceeding 45 PEI U/ml may be diluted with the automated dilution method. Diluent Universal is used to dilute the samples. After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

- The concentration of the sample after dilution should not be less than 0.7 PEI U/ml.

Measurement Results

The sample test results are determined automatically by the system software utilizing a 4 parameter logistic curve fit data reduction method. The amount of Anti-HBc in the samples is determined from the measured light production by means of the stored calibration data. Sample test results can be reviewed using the appropriate screen. Refer to the Assay Analyzer's operation manual on reviewing sample results.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels. When a control value is out of the specified range, 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. This test measures concentrations within the range of 0.6- 45 PEI U/ml. If Anti-HBc concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:30 of this test, allowing samples to be quantitated up approximately to 1350 PEI U/ml.

Biological Reference Interval

A normal range of less than 0.7 PEI U/ml (central 95% interval) was obtained by testing samples from 800 individuals defined as normal by clinician and 200 HBV patients.

Samples with concentration between 0.6-2.7 PEI U/ml are considered borderline and must be retested in duplicate to confirm the initial result. After retesting, if both retest values are $<$0.7 PEI U/ml, the sample is considered nonreactive for Anti-HBc. If either of the retest values is $\geqslant$ 0.7 PEI U/ml, the sample must be considered reactive for Anti-HBc by the criteria of this assay. Repeatedly reactive result is recommended dynamic observation or review by other approach.

It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

1PEI U/ ml$\approx$0.5IU/ml[6]

Performance Characteristics

1. Measurement Precision

This assay is designed to have a within-run precision of $<$15%. 2 pooled human serum based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 10. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision	
				SD	%CV
1	1	10	3.78	0.14	3.70
2	1	10	1.22	0.05	4.10

This assay is designed to have a between-run precision of $<$20%. 2 pooled human serum based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 10, once per day across 3 testing days. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Between-run Precision	
				SD	%CV
1	1	30	3.74	0.21	5.61
2	1	30	1.16	0.13	11.20

2. Analytical Sensitivity

Analytical sensitivity, defined as the concentration corresponding to the mean RLUs of 20 replicates of calibrator A (zero calibrator) minus 2 standard deviations, is 0.4 PEI U/ml.

3. Analytical Specificity

Cross reaction: this assay was evaluated for potential cross-reactivity for samples from individuals with medical conditions unrelated to Anti-HBc infection. 27 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.

Category	No.	Anti-HBc CLIA Microparticles assay	
		Reactive	Nonreactive
HIV-IgG	2	0	2
HEV-IgG	10	0	10
HAV-IgM	5	0	5
RF	6	0	6
ANA (Antinuclear Antibodies)	4	0	4

Interference: No interference with 5 mg/ml of hemoglobin, 200 mg/l of Bilirubin, 20 mg/ml of Intralipid.

4. Clinical Sensitivity

Sensitivity was determined by testing samples that were found positive in a reference assay and tested in this Anti-HBc assay. A total of 526 serum and plasma samples know to be positive for Anti-HBc were tested. The sensitivity on this population was 97.34%. The results of the study are shown below.

Sample Category	Number of Samples Tested	Number of Reactive Samples Tested	Sensitivity
Anti-HBc Reactive	526	512	97.34%

5. Clinical Specificity

Specificity was determined by testing samples that were found negative in a reference assay and tested in this Anti-HBc assay. All samples that were found to be repeatedly reactive in this assay were run in another Anti-HBc assay. In one study, a total of 303 samples were tested. These samples were comprised of volunteer blood donors from different sites. The specificity on the volunteer blood donor population was 94.72%. The results of this study are shown below.

Sample Category	Number of Samples Tested	Number of Repeated Reactive Samples	Specificity
Anti-HBc non-reactive	303	16	94.72%

Literature References

1. Decker RH. (1993), Viral Hepatitis. Churchill Livingstone, Section 9, 165-184..

2. Hoofnagle JH. (1981). T ype B Hepatitis: Virology, Serology and ClinicalCourse. Seminars in Liver Disease: I;1:7-1 4..

3. Kumar S, Pound DC. (1992). Serologic diagnosis of viral hepatitis. Postgraduate Medicine; 92(4):55-65.

4. Gerlich WH, Caspari G, Uy A, Thomssen R. (1991). A critical appraisal of Anti-HBc, HBV DNA and anti-HCV in the diagnosis of viral hepatitis. Biotest Bulletin; 4:283-293.

5. Lemon S.M. et al. (1981). IgM antibody to hepatitis B core antigen as a diagnostic parameter of acute infection with hepatitis B virus. J. Infect. Dis. 143,803

6. First International Standard for anti-Hepatitis B core antigen (anti-HBc), plasma, human Instructions for use.