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Immunoassay

REF **CMC0301/ CMC0302/ CMC0303/ CMC0304/ CMC0305**

50tests*1/ 100 tests*1/ 100tests*2/ 100tests*5/ 50tests*2

HBeAg CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of HBeAg (Hepatitis B e 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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Introduction

Chronic infection with the HBV (hepatitis B virus) is a major cause of chronic liver disease worldwide.[1] The virus was first discovered as "Australia antigen", later renamed HBsAg (for hepatitis B surface antigen), in patient blood.[2] HBeAg (hepatitis B e antigen) was identified several years later as a marker for patients at high risk for transmission of the disease.[3] It has been found that HBeAg, in addition to HBsAg, may be a useful marker of the risk of hepatocellular carcinoma. Persons considered to be at high risk because of positivity for HBeAg would be candidates for antiviral-drug treatment and close monitoring for the development of liver diseases associated with chronic HBV infection.[4]

There are mainly 6 immunologic markers of HBV: HBeAg, HBcAg, HBsAg and their respective antibodies.[5] HBeAg however is detected only in HBsAg positive sera.[6] Its presence coincides with the rapid propagation of HBV and high infectivity. It is also a marker of questionable prognosis including the development of chronic hepatitis. On the contrary, Anti-HBe represents minimum viral replication and greatly reduced infectivity. When a patient changes from HBeAg to its antibody, he or she is likely to enter convalescent stage.[7] But it is also possible that patients with Anti-HBe are long-term carriers of HBV. Nevertheless, patients with Anti-HBe generally have optimistic prognosis.[8]

Measurement Principle

This assay uses a one-step sandwich method. The sample and enzyme conjugate are added to the anti-HBe-coated microparticles. During incubation, HBeAg present in the sample are allowed to react simultaneously with the two antibodies, resulting in the HBeAg being sandwiched between the solid phase and enzyme-linked antibodies. After washing, a complex is therefore generated by immunological reactions among the solid-phase antibody, the HBeAg that were present in the sample and the antibody in the enzyme conjugate. 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 HBeAg in the sample.

Materials provided

1. Calibrators

6 vials each containing 1 mL of Calibrator A through F. The matrix is PBS buffer containing recombinant HBeAg and BSA (bovine serum albumin). Contains ProClin 300® preservative.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

- Enzyme Conjugate

Containing horseradish-peroxidase labeled mouse monoclonal anti-HBe in Tris-Nacl buffer containing casein. Contains ProClin 300® preservative.

- Microparticles Solution

Contains mouse monoclonal anti-HBe coated microparticles in PBS (phosphate buffered saline) buffer containing BSA (bovine serum albumin). 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 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

Metrological Traceability of Calibrators

The product calibrators are manufactured using pure grade HBeAg and signal matched to our working calibrators, which are also signal matched to WHO 129097/12.

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.
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. 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.
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 calibrators, 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, calibrators 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.
4. Seal and return the remaining calibrators at 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 1 month.

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 rack, 50 μL of samples 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 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 HBeAg 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 rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrator's 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 the HBeAg value exceeding 210 IU/mL may be diluted via the program of the analyzer. Diluent Universal is used to dilute the samples. The software takes the dilution into account when reporting the result.

- The concentration of the sample after dilution should not be less than 0.1 IU/mL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of HBeAg 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.

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 acceptable concentration ranges. 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. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
6. 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.
7. This test measures concentrations within the range of 0.1-210 IU/mL. If HBeAg concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:49 of this test, allowing samples to be quantitated up approximately to 10500 IU/mL.

Biological Reference Interval

A normal range of less than 0.1 IU/mL (central 95% interval) was obtained by testing samples from 500 HBV infected patients and 500 individuals defined as normal by clinician. 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.

Performance Characteristics

1. Measurement Precision

A study based on guidance from Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) document EP5-A2 was performed for the Autobio HBeAg assay. Three internal control members (Q1, Q2 and Q3) were assayed, using one lot of reagent, in replicates of two at two separate times per day for 20 testing days. Data from this study are summarized in the following table.*

Panel Member	Lot	n	Mean	Within-run	Total
				%CV	%CV
Q1	1	80	1.32	5.15	4.77
Q2	1	80	2.92	3.24	3.28
Q3	1	80	5.67	5.08	3.92

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Analytical Sensitivity, defined as LOB is ≤0.05 IU/mL.

3. Analytical Specificity

Cross reaction: This assay was evaluated for potential cross-reactivity for samples from individuals with medical conditions unrelated to HBeAg infection. 48 potentially cross-reacting samples from patients were evaluated. The data are summarized in the following table.

Category	No.	HBeAg CLIA Microparticles assay	
		Reactive	Nonreactive
HIV	5	0	5
HCV IgG	5	0	5
HAV	3	0	3
HEV IgG	3	0	3
RF	10	0	10
ANA	12	0	12
HAMA	10	0	10

Interference: No interference with 2.5 mg/mL of hemoglobin, 40 mg/dL of Bilirubin, 500mg/dL of triglyceride.

4. Hook effect

A sample spiked with HBeAg up to 1680 IU/mL was determined, the concentration result obtained was ≥210 IU/mL.

5. Clinical Sensitivity

Sensitivity was determined by testing samples that were found positive in a reference assay and tested in this HBeAg assay. A total of 864 serum and plasma samples know to be positive for HBeAg were tested. The sensitivity on this population was 96.88%. The results of the study are shown below.

Sample Category	Number of Samples Tested	Number of Reactive Samples Tested	Sensitivity
HBeAg Reactive	864	837	96.88%

6. Clinical Specificity

Specificity was determined by testing samples that were found negative in a reference assay and tested in this HBeAg assay. All samples that were found to be repeatedly reactive in this assay were run in another HBeAg assay. In one study, a total of 1360 samples were tested. These samples were comprised of volunteer blood donors from different sites. The specificity on the volunteer blood donor population was 98.16%. The results of this study are shown below.

Sample Category	Number of Samples Tested	Number of Repeated Reactive Samples	Specificity

HBeAg non-reactive	1360	25	98.16%

Literature References

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