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Immunoassay

REF **CMC0102**

100 tests

HBsAg CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of HBsAg (Hepatitis B Surface 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 is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). Hepatitis B infection can be spread through contact with the blood, semen, vaginal fluids, or other body fluids of someone who already has hepatitis B infection. The HBV can be passed to an infant during childbirth if the mother is infected.[1]It is a major global health problem and the most serious type of viral hepatitis. It can cause chronic liver disease and puts people at high risk of death from cirrhosis of the liver and liver cancer.[2] It used to be widely believed that the HBV is completely cleared by antiviral antibodies and specific cytotoxic T lymphocytes (CTLs) during acute viral hepatitis. It has been demonstrated however that traces of HBV are often detectable in the blood for many years after clinical recovery from acute hepatitis, despite the presence of serum antibodies and HBV-specific CTLs, which can be present at acute stage levels.[3]The global prevalence of HBV carriers varies greatly and countries can be defined as having a high, intermediate and low prevalence of HBV infection based on a prevalence of HBsAg carriers of ≥8%, 2%-7%, and <2% respectively.[4,5]

Measurement Principle

This assay uses a two-step sandwich method. In the first step, the sample is added to the anti-HBs-coated microparticles. During incubation, HBsAg present in the sample bind to the antibodies that coat the microparticles. After washing, in the second step, an anti-HBs antibody-enzyme conjugate is added. During this incubation step, HBsAg bound to the solid-phase are allowed to react with the enzyme-labelled antibodies, resulting in the HBsAg 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 HBsAg 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 HBsAg in the sample.

Materials provided

1. Calibrators

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

Calibrator	HBsAg Concentration (IU/ml)
A	0
B	0.05
C	0.4
D	4
E	40
F	250

2. Reagent pack

Reagent pack provided ready to use.

- Enzyme Conjugate

1 vial containing 5.5 ml of horseradish-peroxidase labeled goat polyclonal anti-HBs in PBS (phosphate buffered saline) buffer containing casein and BSA (bovine serum albumin). Contains ProClin 300® preservative.

- Microparticles Solution

1 vial containing 2.3 ml of mouse monoclonal anti-HBs 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 cup(s) or tube(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 HBsAg and signal matched to our working calibrators, which are also signal matched to a calibrator purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products, China) 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 waste safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HIV-1 and HIV-2, HCV and syphilis, and reactive for HBsAg. 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 vortexing 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 cups or tubes on the sample holder, Calibrators and 150 μl of samples were added. 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 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 the quantity of HBsAg 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 cups or tubes 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 instrument are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with the HBsAg value exceeding 250 IU/ml may be diluted with manual dilution method or 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.

- When dilute the samples with Diluent Universal manually, multiply the detection result by the dilution factor to obtain the final result.
- The recommended dilution of this test is 1:500.
- The concentration of the sample after dilution should not be less than 0.05 IU/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 HBsAg 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. Although the association of infectivity and the presence of HBsAg is strong, it is recognized that presently available methods for HBsAg detection are not sensitive enough to detect all potentially infectious units of blood or possible cases of HBV infection.
7. This test measures concentrations within the range of 0.03-250 IU/ml. If HBsAg concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:500 of this test, allowing samples to be quantitated up approximately to 125000 IU/ml.

Biological Reference Interval

A normal range of less than 0.05 IU/ml (central 95% interval) was obtained by testing samples from 1040 individuals defined as normal by clinician.

Samples with concentration between 0.03-0.08 IU/ml are considered borderline and must be retested in duplicate to confirm the initial result. After retesting, if both retest values are $<$0.05 IU/ml, the sample is considered nonreactive for HBsAg. If either of the retest values is $\geqslant$0.05 IU/ml, the sample must be considered reactive for HBsAg 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.

Performance Characteristics

1. Measurement Precision

This assay is designed to have a within-run precision of $<$10%. 2 pooled human plasma 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	2.44	0.10	4.10
2	1	10	0.14	0.01	7.14

This assay is designed to have a between-run precision of $<$15%. 2 pooled human plasma based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 2, 10 times 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	2.31	0.14	6.06
2	1	30	0.14	0.01	7.14

2. Analytical Sensitivity

LoB, defined as the concentration corresponding to the mean RLUs of 20 replicates of calibrator A (zero calibrator) plus 2 standard deviations, is 0.01 IU/ml.

LoD, 0.02 IU/ml, is determined based on the limit of blank and the standard deviation of low concentration samples. The limit of detection corresponds to the lowest analyte concentration which can be detected (value above the limit of blank with a probability of 95%).

Functional sensitivity, 0.03 IU/ml, is determined using human serum pools, performed with one instrument and during three calibration cycles, with 3 lots of reagents, generating 3 replicates per assay, over 20 runs, the functional sensitivity of 20% between run CV was obtained.

3. Analytical Specificity

Cross reaction: This assay was evaluated for potential cross-reactivity for samples from individuals with medical conditions unrelated to HBsAg infection. 42 potentially cross-reacting samples from patients were evaluated. All these samples were non-reactive with this assay and reference assay. The data are summarized in the following table.

Category	No.	HBsAg CLIA Microparticles assay	
		Reactive	Nonreactive
HCV-IgG	8	0	8
HIV-IgG	8	0	8
HEV-IgM	8	0	8
HAV-IgM	8	0	8

Interference: No interference with 2.5 mg/ml of hemoglobin, 200 mg/l of Bilirubin, 20 g/l of Intralipid.

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a microparticle based HBsAg test which was already available in the market. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	300	18.446	0.7494	0.8138

5. Clinical Sensitivity

Sensitivity was determined by testing samples that were found positive in a reference assay and tested in this HBsAg assay. A total of 1298 serum and plasma samples know to be positive for HBsAg were tested. The sensitivity on this population was 99.77%. The results of the study are shown below.

Sample Category	Number of Samples Tested	Number of Reactive Samples Tested	Sensitivity
HBsAg Reactive	1298	1295	99.77%

6. Clinical Specificity

Specificity was determined by testing samples that were found negative in a reference assay and tested in this HBsAg assay. All samples that were found to be repeatedly reactive in this assay were run in another HBsAg assay. In one study, a total of 5630 samples were tested. These samples were comprised of volunteer blood donors from different sites. The specificity on the volunteer blood donor population was 99.91%. The results of this study are shown below.

Sample Category	Number of Samples Tested	Number of Repeated Reactive Samples	Specificity
HBsAg non-reactive	5630	5	99.91%

7. Seroconversion panels

7 seroconversion panels were analyzed with this assay. In all panels this assay shows a significant increase in concentration upon seroconversion correlated to the shift as it is detectable in qualitative assays.

Literature References

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