HBsAg Quantitative

Porrima

Hepatitis B Surface Antigen(CLIA)

REF: PM097701 **100**

Intended use

The Porrima HBsAg assay is a Chemiluminescent Immunoassay (CLIA) for the quantitative determination of Hepatitis B Surface Antigen (HBsAg) in **Human Serum and Plasma**. For professional use only.

Summary

References[1-7]

The hepatitis B surface antigen (HBsAg), a polypeptide of varying size, is a component of the external envelope of the hepatitis B virus (HBV) particle. In addition to the intact infectious viral particles, the blood of persons infected with HBV contains large amounts of non-infectious particles which consist only of an outer coat containing HBsAg. After infection, HBsAg is the first immunological marker detectable in serum and is usually present weeks to months before the onset of clinical symptoms and the appearance of other biochemical markers. In the case of acute HBV infection with recovery, HBsAg is detectable in serum for up to 6 months after its appearance. If HBsAg persists for more than 6 months after acute hepatitis, the presence of chronic hepatitis B (CHB) infection must be assumed. Classifying the stage of CHB infection is essential for identifying patients who require treatment and monitoring, as well as assessing the likelihood of responding to treatment and risk of progression to more severe liver disease.

Test principle

Sandwich principle. Total duration of assay: **20 minutes**

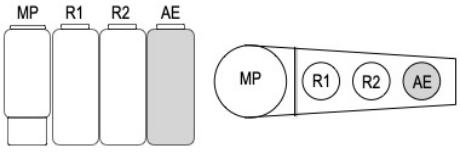
In the first step, sample, paramagnetic microparticles coated with monoclonal anti-HBsAg antibody and monoclonal anti-HBsAg antibody-acridinium labeled conjugate are added into a reaction vessel. After incubation, HBsAg present in the sample binds to both anti-HBsAg antibody coated microparticle and anti-HBsAg antibody acridinium-labeled conjugate to form a sandwich complex. Microparticle is magnetically captured while other unbound substances are removed by washing.

In the second step, Pre-Trigger and Trigger Solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the Porrima system.

Materials provided

MP	1 x 5.0 mL, anti-HBsAg antibody (mouse, monoclonal) coated Microparticles in TRIS buffer with protein (bovine) stabilizers with preservative.
AE	1 x 10 mL, monoclonal anti-HBsAg antibody (mouse)-acridinium ester labeled conjugate in MES buffer with preservative.
C0	Calibrator, 1 vial, 1.0 mL, ready to use.
C1	Calibrator, 1 vial, 1.0 mL, ready to use.
C2	Calibrator, 1 vial, 1.0 mL, ready to use.

The position of each reagent component is shown in the figure below (front view on the left and top view on the right):



Materials required (but not provided)

- Porrima Chemiluminescent Immunoassay Analyzer
- CT2201 DiaSino ControlSet: 2 levels, L and H
- TR7701 Porrima TriggerPack: ①Pre-Trigger Solution, ②Trigger Solution
- WB7701 Porrima Wash Buffer (20X)
- RV7701 Porrima Reaction Vessels
- PW7701 Porrima Probe Wash Buffer
- SC7701 Porrima Sample Tubes (D13x75mm, D13x100mm or D16x100mm can be acceptable on Porrima system)

Precautions and warnings

- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/ face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

- Store at 2-8°C. Do not freeze.
- Store the Porrima reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.
- The Porrima HBsAg reagent kit can be stored onboard and used for a maximum of 28 days after opening at 2-8°C.

Specimen collection and preparation

- Human serum or plasma is recommended for this assay.
- Serum collected using standard sampling tubes or tubes containing separating gel.
- Li-heparin, Na-heparin, K2-EDTA and Na-citrate plasma.
- Centrifuge samples containing precipitates before performing the assay.
- Do not use heat-inactivated samples.
- Do not use samples and controls stabilized with azide.
- For optimal results, serum and plasma specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at least 10,000 RCF (Relative Centrifugal Force) for 10 minutes.
- Ensure the samples are at 20-25°C prior to measurement.
- Due to possible evaporation effects, samples and calibrators on the analyzer should be analyzed/measured within 2 hours.

Assay procedure

- For optimal performance of this assay, operators should read the Porrima CLIA system operation manual carefully, to get sufficient information such as operation instructions, sample preservation and management, safety precaution, and maintenance. Prepare all required materials for the assay as well.
- Before loading the reagent kit on the machine for the first time, unopened reagent bottle should be inverted gently for at least 30 times to resuspend the microparticles that have settled during shipment or storage.
- Visually inspect the bottle to ensure the microparticles have been resuspended. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely resuspended.
- If the microparticles cannot be resuspended, **DO NOT USE**. Contact DiaSino Technical Support for help. Do not invert opened reagent bottle.
- This assay requires 100 µL of sample for a single test. This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirement of the assay to determine the minimum sample volume.

Calibration

The specific information of master calibration curve of HBsAg CLIA reagent kit is stored in the barcode attached in the reagent pack. It's used together with calibrators for the calibration of the specific reagent lot. When performing the calibration, scan the information of master calibration curve from the barcode into the system first, and then use the calibrators at two levels. Valid calibration curve is required before any HBsAg test. Recalibration is recommended every 28 days, or when a new reagent lot is used, or the quality controls are out of specified range. For detailed instruction of calibration, refer to the system operations manual.

IVD

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Quality control
For quality control, use DiaSino ControlSet. In addition, other suitable control material can be used.
Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
Quality control results should be within the acceptable ranges. If a control is out of its specified range, the associated test results are invalid and the samples must be retested. Recalibration may be required. Examine the assay system referring to the system operation manual. If the quality control results are still out of the specified range, please contact DiaSino Technical Support for help.

Calculation
The analyzer automatically calculates the analyte concentration of each sample in IU/mL.

Limitations - interference
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. Additional clinical or diagnostic information may be required to determine patient status.
- If the HBsAg results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute or chronic infection.
- Samples containing particulate matter or red blood cells must be centrifuged prior to running the assay.
- Do not use heat-inactivated specimens.
- Specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the specimen prior to heparin therapy.

Measuring range
0.05-250 IU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.05IU/mL. Values above the measuring range are reported as > 250 IU/mL

Lower detection limit
0.05 IU/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n = 21).

Expected values
- Specimens with concentration values < 0.05 IU/mL are considered nonreactive by the criteria of Porrima HBsAg.
- Specimens with concentration values ≥ 0.05 IU/mL are considered reactive by the criteria of Porrima HBsAg.
- All initially reactive specimens should be retested in duplicate. If both retest values are nonreactive, the specimen must be considered nonreactive for HBsAg. If either of the retest values is reactive, the specimen must be considered repeatedly reactive for HBsAg by the criteria of the Porrima HBsAg.
- Repeatedly reactive samples should be tested by a neutralizing confirmatory test. Samples which are confirmed by neutralization with human anti-HBs must be considered positive for HBsAg.

Specific performance data
Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

		Repeatability*		Intermediate precision	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human Serum 1	0.02	0.001	5.21	0.001	6.85
Human Serum 2	14.57	0.635	4.36	0.761	5.22
Human Serum 3	79.26	3.797	4.79	4.502	5.68
ControlSet 1	5.45	0.298	5.46	0.280	5.13
ControlSet 2	28.33	1.337	4.72	1.598	5.64

*Repeatability= within-run precision

Method comparison
A comparison of the Porrima HBsAg assay (y) with the Abbott ARCHITECT HBsAg assay (x) using 210 clinical samples gave the following correlations:
Linear regression
y = 1.074x - 0.004
r = 0.9788
The sample concentrations were between approx. 0 and 250 IU/mL.

Analytical specificity
No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.

Functional sensitivity
0.085 IU/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

1. Seeger C, Zoulim F, Mason WS. Hepadnaviruses. In: Field's Virology, Knipe DM, Howley RM (eds), 2007 5th edition, Lippincott Williams and Wilkins, Philadelphia, USA. Chapter 76, pp2977–3029.
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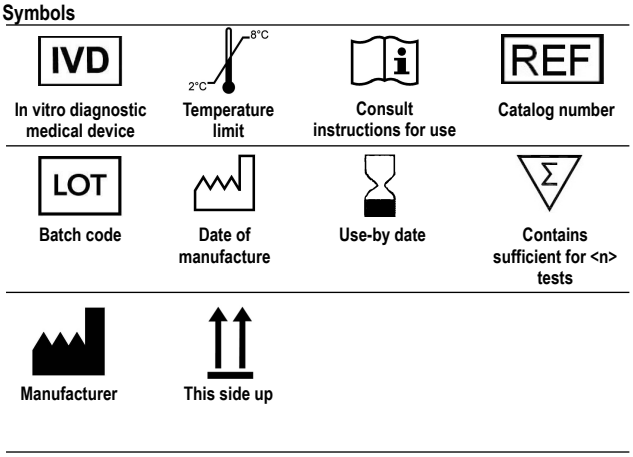
3. Liaw YF. Clinical utility of hepatitis B surface antigen quantification in patients with chronic hepatitis B: a review. Hepatology 2011;54:E1-E9.
4. Liaw YF, Chu CM. Hepatitis B infection. Lancet 2009;373:582-592.
5. European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol 2012;57:167-185.
6. Liaw YF, Kao JH, Piratvisuth T, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int 2012;6:531-561.
7. Lok ASF, McMahon B. Chronic hepatitis B: update 2009. AASLD Practice Guidelines. Available at: http://www.aasld. org/practiceguidelines/Documents/Bookmarked%20Practice%20Guidelines/Chronic_Hep_B_Update_2009%208_24_2009.pdf (accessed January 2013).

Symbols



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