Aurora HBsAg Assay

CARBONTECHNOLOGIES

REF	Σ
AU242005	50

Intended Use

The Aurora HBsAg Assay is used to quantitatively detect the hepatitis B surface antigen (HBsAg) in human serum and plasma.

Summary

Hepatitis B virus infection has become a common and important public health problem worldwide. There are 350-400 million people with chronic HBV infection in the world, and 2 billion people were infected with HBV, accounting for about 6% of global population. Hepatitis B surface antigen (HBsAg) is a marker of hepatitis B virus infection. With the highest titer, HBsAg appears first in the patients with HBV, and is an important indicator for early diagnosis of hepatitis B. A positive HBsAg result indicates the presence of hepatitis B virus infection. The quantitative measurement of HBsAg is a new tool for monitoring and predicting post- treatment response to hepatitis B virus infection, thereby being of great significance both in the diagnosis of HBV infection and in the monitoring of antiviral efficacy. There is evidence that the HBsAg level combined with the HBV DNA level is helpful to identify patients without the need of treatment.

Test Principle

This reagent is based on the double-antibody sandwich principle. Magnetic particles are coated with 4 anti-HBs murine monoclonal antibodies. The sample, reaction diluent, and magnetic microparticles are mixed for reaction, followed by washing. Then, the anti-HBs polyclonal antibody-acridinium ester-labeled complex is added so that the HBsAg in the sample and the anti-HBs antibody and anti-HBs polyclonal antibody-acridinium ester on magnetic microparticles form a complex of "magnetic microparticle anti-HBs antibody- HBsAg-antibody acridinium ester". Then, the substances that fail to bind to magnetic microparticles are removed by washing, and pre-trigger solution and trigger solution are added to the reaction mixture. Then, the results of the chemiluminescent reaction are measured, which are expressed as relative luminous intensity (RLU). The concentration of HBsAg in the sample is positively correlated to the RLU value measured by the analyzer. The HBsAg content in the sample is calculated from the calibration curve.

Reagents

R1	Anti-HBs monoclonal antibody coated magnetic microparticle prepared in TRIS buffer, containing preservative ProClin300.
R2	Acridinium ester-labeled anti-HBs polyclonal antibody, prepared in TRIS buffer containing protein stabilizer, containing preservative ProClin300.
R3	TRIS buffer containing protein stabilizer, containing preservative ProClin300.
CAL 1	Inactivated human serum or plasma without HBsAg and HBsAb, containing ProClin300 preservative.
CAL 2	0.05 IU/mL HBsAg diluted in inactivated human serum or plasma without HBsAg and HBsAb, containing preservative ProClin300.
CAL 3	50.00 IU/mL HBsAg diluted in inactivated human serum or plasma without HBsAg and HBsAb, containing preservative ProClin300.
CON	Contains 2 levels, 1.0 mL each level.

Note: The reagents and calibrators in different batches of kits cannot be used interchangeably.

Required Materials

- Pre-Trigger Solution: Hydrogen peroxide solution.
- Trigger Solution: Sodium hydroxide solution.
- Wash Buffer: Phosphate buffered saline solution with 0.05% ProClin 300.

Storage Condition

1- Store at 2-8℃ with a validity period of 12months.

2- The kit should be stored upright, not upside down or horizontally. The reagent could be used in the analyzer immediately after being taken out from 2-8℃, which could be stored on board the analyzer for a maximum of 28 days. After 28 days, the analyzer will automatically

remind that the kit has expired.
3- After each use, return the calibrator to 2-8℃ storage with a validity period of 4 weeks.
4-See label for manufacture date and expiration date.

Applicable Analyzer
Automatic Chemiluminescence Immunoassay Analyzer (model: Aurora S-01).

Sample Requirements
1-Collect samples in accordance with the general sampling techniques and process based on the standard operation steps.
2-The sample tested by this reagent is human serum or plasma. The samples containing anticoagulant of EDTA, heparin lithium or sodium citrate are applicable for the reagent and do not use the plasma sample with improper proportion of anticoagulant.
3-The analyzer does not provide the capacity to identify sample types. It is the responsibility of the operator to verify that the correct sample types are used.
4-You must be careful when manually processing the patient's sample under special condition, and it is recommended to use disposable pipettes or tips to avoid cross- contamination.
5-Do not use heat treatment samples; do not detect samples containing suspended fibrin or aggregates, grossly hemolyzed (hemoglobin content higher than 400 mg/dL); it's allowed to detect jaundice samples (bilirubin content lower than 100 mg/dL) or hyperlipidemia samples (triglyceride content lower than 10863mg/dL); in case that a sample contains macroscopic particles, fiber or red blood cell, the sample should be centrifuged before process. If the sample is covered with the lipid layer after centrifugation, it should be moved to a sample cup or new tube. Avoid absorbing the lipid layer.
6-The influences of microbial contaminated samples on the results have not been determined and such samples are not recommended for use.
7-For optimal results, check all the samples for bubbles. Remove the bubbles by using swabs prior to analysis. Use a new swab for each sample to avoid cross-contamination.
8-Ensure that the temperatures of the samples, calibrators, controls are balanced to room temperature prior to the initiation of the analyses.
9-Avoid the samples from repeated freezing-thawing, thaw the frozen samples before test, invert the sample 180 degree upright and repeat 10 times to mix well the thawed sample. Observe whether the sample is layered. If layering is observed, repeat the above- mentioned procedure until the sample is well distributed, and make use of it after centrifugation.
10-Samples without clots, serum separators or red blood cells can be stored at 2-8℃ up to 7 days; and it should be kept at -20℃ or lower for long-term storage.
11-Because of possible volatilization influence, the samples, calibrators and controls positioned on the analyzer should be tested within two hours.
12-When shipped, samples must be packaged and labeled in compliance with the regulations of the International Air Transport Association (IATA) or other relevant provisions and ensure that samples are transported at low temperature. Do not exceed the time range for storage specified in this package specification (this section). Before shipment, it is recommended that the samples be separated from blood clots, serum separating tubes or from red blood cells.

Assay Procedures
1-For the first time to load the Aurora HBsAg Assay for Antibody to Hepatitis B Virus Surface Antigen (Chemiluminescent Immunoassay) on the analyzer, it is needed to well mix the reagent in the Reagent 1 vial, and to resuspend the microparticles that have settled during the shipment.

- Invert the vial of Reagent 1 for 30 times and control the flip-flop speed to avoid generating bubbles.
- Visually inspect the vial of Reagent 1 to ensure microparticles are resuspended. If the microparticles remain adhered to the vial, continue inverting the vial until the microparticles have completely resuspended. If they do not resuspend, do not use.

2-Check the sample volume in the sample cup to ensure the volume maintaining above 360µL before each test.
3-Execute the calibration order when necessary: See Operation Manual of the analyzer for information about executing calibration order.
4-The calibrators should be balanced to room

temperature when being taken out from 2 ~ 8℃, it can be used. Prior to use, mix by gentle inversion (10 times). After each use, tightly close the cap and return the calibrator to 2-8℃ storage.

5-Load sample: See Operation Manual of the analyzer for information about sample loading.

6-Press Assay Start (run). The analyzer performs the following actions:

- Move the sample delivery unit to the sampling point;
- Load the reaction cup, suction and transfer the sample into the reaction cup;
- Move the reaction cup into specific position and add Reagent 3 and Reagent 1 to the reaction cup;
- Mix well, incubate and rinse the reactant mixture;
- Add Reagent 2 to the reaction cup;
- Mix well, incubate and rinse the reactant mixture;
- Add pre-trigger solution and trigger solution;
- Detect the relative luminous intensity (RLU) and calculate the contents of the substances to be measured in the sample;
- Pipette the content in the reaction cup to the waste liquid and discard the reaction cup into the solid waste bucket;
- Results display.

To perform calibration, test Calibrator 1, Calibrator 2 and Calibrator 3 in replicates of three, once the calibration is passed and stored, all subsequent samples can be detected without further calibration, unless under the conditions that diagnostic kit of new batch is used or the controls (QC) values are out of range. Meanwhile, the calibration result should be within the validity period (28 days).

The test results of the controls (purchased or self-prepared) should be within the specified range, when the values of the controls exceed the specified range, they may indicate reagent deterioration or technical problems. The relevant results may be invalid and it requires test again. Re-calibrate if necessary. See Operation Manual of the analyzer for information about trouble-shooting.

Reference Interval

This reagent was used to test the samples from 108 normal blood donors, and the HBsAg content measured by this reagent was less than 0.05 IU/mL. It is recommended that each laboratory establishes its own range of values due to such differences as location, race, sex and age.

Results

1-The master curve is generated by 4PLC fitting regression for the HBsAg testing reagent and corrected by the analyzer by using the mean value of the relative luminous intensity (RLU) obtained by testing calibrators for three times, and the RLU value of the sample to be tested is substituted into the curve equation to obtain the concentration of corresponding antigen.

2-Dynamic observation is recommended when the HBsAg level is higher than the reference value.

3-The effective linear range of this reagent is 0.05IU/mL~250.00IU/mL.

4-The following factors can lead to the non-repetitive responses:

- The sample to be tested is not in line with the sample requirements;
- The operations are not in accordance with the instructions and the analyzer requirements;
- The experimental environment or reagent is contaminated.

Limits

1. This reagent can only be used for the test of human serum or plasma samples, instead of saliva, urine or other bodily fluids.

2. The test results of this reagent must be determined based on the medical history, clinical symptoms and signs and other indicators.

3. Patients treated with mouse monoclonal antibodies or routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Heterophile antibodies in human serum can react with immunoglobulins in the reagent, interfering with in vitro immunoassay, causing abnormal detection results; additional information may be required for a definite diagnosis.

4. Due to the limitation of methodological principles, the

negative result only represents that HBsAg in sample did not reach the lowest limit of detection of the reagent but cannot be regarded as no HBsAg existed in the sample.

Specific Performance Characteristics

1-Meet appropriate national criteria when testing the national reference materials.

2-Meet the following criteria when testing the enterprise reference materials:

Accuracy

Use 8 accuracy reference materials for calibration, and the relative deviation between the measurement results and the theoretical concentration should be ≤ ± 20%.

- Coincidence rate of negative reference materials: Coincidence rate of 20 negative reference materials (-/-) should be 20/20.
- Coincidence rate of positive reference materials: Coincidence rate of 3 positive reference materials (+/+) should be 3/3.
- The lowest limit of detection: When testing 9 sensitivity reference materials, the minimum detectable concentration obtained from adr sub type should be no more than 0.1 IU/mL, that obtained from adw sub type should be no more than 0.1 IU/mL, and that obtained from ay sub type should be no more than 0.2 IU/mL.

Accuracy

CV (%) of the test results obtained when testing the precision reference materials should be no more than 10% (n=10).

Specificity

- No cross-reactions occur in testing HAV IgM, HIV Ab, HCV Ab, HTLV Ab, TP Ab, HEV IgG, HDV IgG, CMV IgG, EBV VCA IgA, HSV IgG, RV IgG, Tox IgG positive sample, HIV Ag, HCV Ag, HBeAg (recombinant antigen) HBcAg (recombinant antigen) and Alcohol liver, Hepatic adipose infiltration samples.
- Hemolysis (hemoglobin < 400 mg/dL), lipidemia (triglyceride < 10863 mg/dL), jaundice (bilirubin < 100 mg/dL), increased ALT and rheumatoid factors had no impact on the test results of this reagent.

Linearity

The reagent has clinically acceptable linearity in the range of 0.05 IU/mL-250.00 IU/mL.

- The reportable range of reagents is 0.04IU/mL~250000.00IU/mL.
- The reagent has the comparable detection capabilities with other commercially available HBsAg kits for the samples of different serotypes (adr, adw, and ay) and the samples of common mutant strains.
- The reagent has the comparable detection capabilities with other commercially available HBsAg kits for 8 sets of BBI seroconversion panels.

Lowest limit of detection

The lowest limit of detection of this reagent for adr, adw and ay serotypes is no more than 0.05 IU/mL.

Functional sensitivity

The functional sensitivity of this reagent for adr, adw and ay serotypes is less than 0.05 IU/mL.

- HBeAg positive samples were tested with this reagent, and negative results were obtained for all the samples, indicating that hepatitis B virus e antigen had no effect on the detection capabilities of this reagent.

Hook effect

The reaction mode of this product is a two-step method and theoretically there is no HOOK effect.

Warning

-H319: Causes serious eye irritation.

-H315: Causes skin irritation.

-H317: May cause an allergic skin reaction.

-H411: Toxic to aquatic life with long lasting effects.

-P280: Wear protective gloves/protective clothing/eye protection/face protection.

-P302 + P352: IF ON SKIN: Wash with plenty of water.

-P337 + P313: If eye irritation persists: Get medical advice/attention.

-P362: Take off contaminated clothing.

-P333 + P313: If skin irritation or a rash occurs: Get medical advice/attention.
-P362 + P364: Take off contaminated clothing and wash it before reuse.
-P273: Avoid release to the environment.
-P391: Collect spillage.

Cautions

1-The kit is only used for in vitro diagnosis, not for other purposes, the operations should be proceeded strictly in accordance with the instructions.
2-Calibrators may not be used immediately after removal from 2-8℃ storage and should be balanced to room temperature.
3-Do not use expired kit; do not pool reagents between kits; do not mix up with the kit from another manufacturer.
4-Due to the influence of test method, recognition site, specificity and interference factor, there may be different results for some specific samples. The laboratory technicians must indicate the experimental methodological information when issuing a test report to clinicians. The results obtained by different test methods cannot be directly compared, and direct cross-utilization may mislead the interpretation of their clinical significance. In the continuous monitoring of efficacy in patients, sufficient parallel experiments between the old and the new methods must be conducted and the feasibility must be validated prior to a halfway method change.
5-Pay special attention to the fact that all the samples, waste liquid and other materials, such as tubes, buffer and pipette tips, may contain infectious materials. During the operations, operators should wear coveralls and gloves, it is strictly prohibited to suction samples with mouth, and if contact the wound accidentally, seek medical advice in time. Use disinfectant for disinfection treatment immediately in case there is liquid overflow during the experiment. After trial, all the samples and experimental items used should be treated as medical wastes.
6-The kit contains certain component, such as ProClin300, which may lead to allergic reactions in a very few people. Avoid prolonged exposure to the skin, and wash hands completely after contact.
7-The manufacturer only guarantees the function for in vitro diagnosis in the specific range described in the instruction of the diagnostic kit when operating the test based on the product instruction, and the manufacturer assumes no responsibility for other warranty or suggestion, including other purposes of commercial value and range of application. The manufacturer only takes the responsibility for replacement or returning payment for goods and does not take the responsibility for any injury or property damage of customer purchasing product or the third party caused during the use process of the product.

Symbols

	Manufacturer
	Date of manufacture
	Use-by date
	Contains sufficient for <n> tests
	Consult instructions for use
	Biological risks
	Temperature limit
	CE Marking
EC REP	EU Representative
IVD	In Vitro diagnostic medical device
REF	Catalogue Number
LOT	Batch code
REAGENT	Reagent
R1	Microparticles
R2	Conjugate
R3	Assay auxiliary.

CAL	Calibrator
CON	Control

References

1. Yi Wei, Xiong Pian, Tian Deying. Sequence analysis and clinical significance for preS/S region of serum virus in patients with chronic HBV infection with simultaneous positive HBsAg and HBsAb[J]. Journal of Integrated Traditional Chinese and Western Medicine for Liver Diseases, 2011, 21(2): 77-79.
2. Wang Lei, Liu Hua, Zhang Li et al. Study on correlation for hepatitis B serum marker HBsAg and HBsAb positive mode[J]. Laboratory Medicine, 2008, 23(5): 530-534.
3. Zhu Haowen, LIiu Fengwei, Li Yong et al. Study on mechanism of production of hepatitis B virus surface antibody and double-positive samples[J]. Journal of Modern Laboratory Medicine, 2010, 25(5): 8-11.
4. CHEN Yu, ZHONG Buyun, XU Genyuan et al. Determination of low-level serum hepatitis B virus surface antigen and its clinical significance[J]. Chin. J Lab Med, 2001, 24(1): 39-41.
5. XIA Guoliang, JIA Zhiyuan. Characterization and distribution of hepatitis B virus genotypes and subtypes in 4 provinces of China[J]. Chinese Journal of Epidemiology, 2001, 22(5): 348-351.
6. Chunchen Wu, Xiaoyong Zhang, Yongjun Tian, et al. biological significance of amino acid substitutions in hepatitis B surface antigen (HBsAg) for glycosylation, secretion, antigenicity and immunogenicity of HBsAg and hepatitis B virus replication[J]. Journal of General Virology, 2010(91): 483-492.

Contact Information

EC REP Medunion S.L.
Carrer de Tapioles 33, 2-1, 08004,
Barcelona, SPAIN.

Carbon Technologies LLC
Innovation Park Muscat (IPM), P.O. Box 92, Al Khoudh 123, Muscat, OMAN.

24-hour service hotline: +968-97058350
After-sale Service Center: Carbon Technologies LLC

CE
IVD

Release Date: … … … Date of Manufacture: … … …