Aurora HBsAb Assay

CARBONTECHNOLOGIES

REF	Σ
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Intended Use

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

Summary

In the early stage of hepatitis B infection, HBsAg appears first, followed by surface antibody (HBsAb). For neutralizing the antigen, an antigen-antibody immune complex is formed. Anti- HBs is an antibody against the antigen on the HBV shell surface, which can prevent HBV from crossing the cell membrane and entering new liver cells and neutralize HBV infection, thereby protecting the body. Therefore, the presence of hepatitis B surface antibody proves that immunity is developed in the body. Hepatitis B surface antibody may be produced after natural HBV infection or hepatitis B vaccination. The concentration of hepatitis B surface antibody reflects the antibody titer. In case of too low level of hepatitis B surface antibody titer (less than 10 mIU/mL), the antibody would lose its protective effect, followed by infection with hepatitis B virus. A titer of hepatitis B surface antibody greater than 10 mIU/mL indicates that there are protective antibodies in the body, which means the ability to prevent infection with hepatitis B virus. The higher the hepatitis B surface antibody titer, the better the protective effect, and the longer the protection lasts.

Test Principle

This reagent is based on the double-antigen sandwich principle. The magnetic microparticles are coated with recombinant hepatitis B surface antigen. With the sample to be tested, the reaction diluent and the magnetic microparticles pooled, if the sample contains anti-HBs, the HBsAb in the incubated sample specifically recognizes and binds to HBsAg to form a solidified complex of "magnetic microparticles-antigen-antibody". The unbound antibody is removed by washing, and then acridinium ester labeled HBsAg is added to form a sandwich complex of antigen-antibody-antigen with labeling; pre-trigger solution and trigger solution are added to the reaction complex, and acridinium ester catalyzes chemiluminescence to generate a light signal. The concentration of anti-HBs in the sample is proportional to the luminescence value measured by the luminometer. The HBsAb content in the sample is calculated by the calibration curve.

Reagents

R1	Recombinant HBsAg coated magnetic microparticles, prepared in TRIS buffer containing preservative ProClin300.
R2	Acridine ester-labeled HBsAg, prepared in TRIS buffer containing protein stabilizer and preservative ProClin300.
R3	TRIS buffer containing protein stabilizer and preservative ProClin300.
CAL 1	Inactivated human serum or plasma without HBsAg and HBsAb, containing ProClin300 preservative.
CAL 2	10.00 mIU/mL HBsAb positive serum diluted in inactivated human serum or plasma without HBsAg and HBsAb, containing preservative ProClin300.
CAL 3	398.00 mIU/mL HBsAb positive serum diluted in inactivated human serum or plasma without HBsAg and HBsAb, containing preservative ProClin300.
CON	Contains 2 levels, 1.0 mL each level.

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 12 months.

2-The Aurora HBsAb Assay should be stored upright, not upside down or horizontally.

3-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.

4-After each use, return the calibrator to 2-8℃ storage with a validity period of 4 weeks.

5-See label for manufacture date and expiration date.

Applicable Analyzer

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

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 HBsAb 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 Aurora HBsAb Assay 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.

Positive Determination Value

Studies showed that protective effects can be observed only when HBsAb concentration reached more than 10.00 IU/mL, and most people are vaccinated clinically; therefore, 10.00 mIU/mL is used as the reference value.

Results

1-The master curve is generated by 4PLC fitting regression for the HBsAb 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 antibody.

2-The calibration range of this reagent is 0.00mIU/mL~1000.00mIU/mL.

3-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-If the test results are inconsistent with the clinical evidence, a complementary test is recommended to validate the results.

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

3-The results of this reagent must be confirmed by other methods and analyzed in combination with patients' clinical information.

4-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.

5-Due to the limitation of methodological principles, the negative result only represents that HBsAb in sample did not reach the lowest limit of detection of the reagent but cannot be regarded as no HBsAb existed in the sample.

Specific Performance characteristics

Accuracy

Use 8 accuracy reference materials for calibration, and the relative deviation between the measurement results and the theoretical concentration should be within the range of ± 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 10 positive reference materials (+/+) should be 10/10.

The lowest limit of detection

Test with three sensitivity reference samples for calibration, and the lowest limit of detection should not higher than 10.00mIU/mL.

Accuracy

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

- The linear range of reagent detection is 3.00mIU/mL~1000.00mIU/mL.
- The reportable range of reagents is 2.00mIU/mL~1000.00mIU/mL.
- he lowest limit detection of the reagent is not higher than 10.00mIU/mL.

Specificity

- No cross-reactions occur in testing HCV Ab, HIV Ab, HTLV Ab, HAV IgM Ab, HEV IgM Ab, HDV IgG, CMV IgG, EBV VCA IgA, HSV IgG, TP Ab, RV IgG, Tox IgG and HBcAb, 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.
- The samples containing anticoagulant of EDTA, heparin lithium or sodium citrate had no impact on the test results of this reagent.

HOOK effect

No HOOK effect was observed by testing highly concentrated samples diluted gradiently by WHO standards.

Clinical trial

A comparative study was conducted between this reagent and the reference reagent, and the negative coincidence rate was 98.04%, the positive coincidence rate was 99.66%, and the total coincidence rate was 99.24%.

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 Aurora HBsAb Assay 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 Aurora HBsAb Assay 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.
The manufacturer only guarantees the function for in vitro diagnosis in the specific range described in the instruction of the Aurora HBsAb Assay 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.
7-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	Biotin conjugate.
R4	Assay auxiliary 1
R5	Assay auxiliary 2
CAL	Calibrator
CON	Control

References

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