Aurora HCV Ab Assay

CARBONTECHNOLOGIES

REF	Σ
AU242004	50

Intended Use

The Aurora HCV Ab Assay is used to qualitatively detect the antibody to Hepatitis C Virus (HCV) in human serum and plasma.

Summary

Hepatitis C is a disease caused by the hepatitis C virus (HCV) and also one of the infectious diseases that seriously threaten human health. In 1974, a non-A and non-B hepatitis observed after blood transfusion was first reported by Golafield. In 1989, the gene clone of hepatitis C virus was obtained by Choc, et al. using molecular cloning technology, and the disease and its corresponding virus were named as hepatitis C and hepatitis C virus. HCV is classified into the Flaviviridae family due to its similar genome with human flaviviruses and blast viruses in structure and phenotypic characteristics. The sources of infection of hepatitis C mainly are acute clinical and asymptomatic subclinical patients, chronic patients, and virus carriers of HCV. Generally, the blood of patients is infectious 12 days before the onset, and the virus can exist in the patients for more than 12 years. HCV is mainly transmitted through blood; 30%-90% of post-transfusion hepatitis cases abroad are hepatitis C, and hepatitis C accounts for 1/3 of post-transfusion hepatitis cases in China. HCV can also be transmitted by vertical transmission from parent to infants, daily household contact, and sexual transmission.

Test Principle

This kit is designed with the chemiluminescent immunoassay based on double- antigen sandwich method to detect HCV antibodies in human serum and plasma. Firstly, the sample, the antigen-coated magnetic particles, and the recombinant HCV antigen-labeled biotin are mixed, and the HCV antibodies in the sample bind to the antigens on the magnetic microparticles and the biotin-labeled antigens. Recombinant HCV antigen is pre-coated on magnetic microparticles, and the sample to be tested and the recombinant HCV antigen- labeled biotin are added; as a result, the HCV antibody in the sample binds to the antigen on the magnetic microparticle and the biotin-labeled antigen to form a complex of "pre- coating antigen-HCV antibody-antigen-labeled biotin". After magnetic separation and washing, other substances that fail to bind to the magnetic microparticles are removed. Then, acridine ester-labeled streptavidin (SA) and acridine ester-labeled recombinant HCV antigen are added. After incubation and washing, other substances that fail to bind to the magnetic microparticles are removed. Finally, the pre-trigger solution and the trigger solution are added so that the complex emits the light signals that the analyzer can measure in RLU. The intensity of light signals is positively correlated with the amount of HCV antibodies in the sample. The S/CO (COI) value of HCV antibodies in the sample is calculated through the calibration results by the analyzer.

Reagents

R1	Recombinant HCV antigen-coated magnetic microparticles in buffer containing protein stabilizer and preservative ProClin300.
R2	Acridine ester-labeled streptavidin (SA) and acridine ester-labeled recombinant HCV antigen in buffer containing protein stabilizer and preservative ProClin300.
R3	HCV recombinant antigen-coated biotin in buffer containing protein stabilizer and preservative ProClin300.
CAL 1	Containing inactivated human serum or plasma positive to HCV Ab; reactive to HCV Ab.
CAL 2	Containing inactivated human serum or plasma negative to HCV Ab; non-reactive to HCV Ab.
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 12 months.

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

3-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 30 days. After 30 days, the analyzer will automatically remind that the kit has expired.

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 and plasma. The samples containing anticoagulant of EDTA, sodium citrate or heparin are applicable for the reagent and do not use the plasma sample with improper proportion of anticoagulant. Each laboratory is required to determine the applicability of the blood vessel and serum separation products it uses, which vary according to the manufacturer and batch.

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 grossly hemolyzed (hemoglobin content higher than 400 mg/dL); it's allowed to detect jaundice samples (bilirubin content less than 99 mg/dL) or hyperlipidemia samples (triglyceride content less than 10863mg/dL).

6-In case that a sample contains macroscopic particles, fiber or red blood cell, the sample should be centrifuged before process.

7-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.

8-The influences of microbial contaminated samples on the results have not been determined and such samples are not recommended for use.

9-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.

10-Ensure that the temperatures of the samples, calibrators, controls are balanced to room temperature prior to the initiation of the analyses.

11-Avoid the samples from repeated freezing-thawing, thaw the frozen samples before test to mix well the thawed sample. and make use of it after centrifugation.

12-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.

13-Because of possible volatilization influence, the samples, calibrators and controls positioned on the analyzer should be tested within two hours.

14-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 Procedure

1-For the first time to load the The Aurora HCV Ab Assay for Antibody to Hepatitis C Virus (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 gently for mixing reagent 1 and control the flip-flop speed to avoid generating bubbles.

- Visually inspect the vial 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-The calibrators should be balanced to room temperature when being taken out from 2 ~ 8℃ and mixed by gentle inversion prior to use. After each use, tightly close the cap and return the calibrator to 2-8℃ storage.

3-Execute the calibration command: execute the calibration command information, after the calibration shows that the calibration is valid, then continue to execute the follow-up procedure; if the calibration is invalid after the calibration, then re-execute the calibration command information.

4-Check the sample volume in the sample cup to ensure the volume maintaining 310μL before each test. When blood collection tube is used, make sure that the amount of fluid in the serum and plasma.

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 1 and Reagent 3 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 Calibrators 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 (30 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

The analyzer calculates the Cutoff (CO) using the mean value from three replicates of the relative luminous intensity (RLU) for calibrator 1 and calibrator 2.

CO = mean RLU value of calibrator 1×0.1+ mean RLU value of calibrator 2×1. S/CO=Sample RLU/Cutoff.

Results

1-When S/CO was less than 1.0, it is considered to be non-reactive for the detection of HCV Ab.

2-When S/CO was greater than or equal to 1.0, it is considered to be reactive for the detection of HCV Ab. HCV Ab reactive samples should be tested by two-well replicate experiments. If the repeated replicate test of the sample is non-reactive, it should be judged as non-reactive, on the contrary reactive.

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.

4-The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be comprehensively considered in combination with symptoms, signs, medical history, other laboratory tests, and therapeutic response.

Limits

1-This reagent is for qualitative test and cannot be used as a quantitative reagent.

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

3-This kit can be used for the test of human serum and plasma samples, while the reliability to detect the substance in saliva, urine or other bodily fluid samples has not been fully confirmed.

4-Positive results from the people who have received blood transfusion or other blood products in recent months should be considered carefully.

5-Due to the limitation of methodological principles, the negative result only represents that HCV Ab in sample fails to reach the minimum detectable concentration of the reagent, but the sample cannot be considered to contain no HCV Ab. A negative HCV Ab result may be obtained early in the acute infection of HCV; therefore, acute infection with HCV cannot be excluded by a negative HCV Ab result, which should be interpreted with reference to clinical symptoms or pathogen exposure and other diagnostic tests.

6-Patients treated with mouse monoclonal antibodies or routinely exposed to animals or animal serum products can be prone to this interference so that abnormal 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. Although the reagent already contains inhibitors to eliminate such interferences, there is still the possibility of false negative or false positive samples in theory; therefore, additional information should be referenced for a definite diagnosis.

Specific Performance Characteristics

1-Testing of national reference materials: The reference panel includes 30 positive samples, 30 negative samples, 4 sensitivity samples, and 1 precision sample. The test results of national reference materials met appropriate national criteria.

2-Testing of enterprise reference materials: The reference panel includes 30 positive samples, 30 negative samples, 4 sensitivity samples, and 1 precision sample. The test results of enterprise reference materials met appropriate following criteria:

- Negative reference materials: The coincidence rate of negative reference materials N1~N30 should be not less than 29/30.
- Positive reference materials: The coincidence rate of positive reference materials P1~P30 should be not less than 29/30.
- Sensitivity reference materials: Test with sensitivity references L1~L4, L1-L2 should be positive, L3 should be positive or negative, L4 should be negative.
- Precision reference materials: CV (%) of the precision reference materials should be no more than 10% (n=10).

Specificity

- There's no cross reaction in HAV IgM positive sample, HBsAg positive sample, HDV IgG positive sample, HEV IgM positive sample, HTLV antibody positive sample, TP antibody positive sample, HIV antibody positive sample, RV IgG positive sample, Tox IgM positive sample, CMV IgG positive sample, HSV-1 IgG positive sample, HSV-2 IgG positive sample, alcoholic liver sample and EBV VCA IgG positive sample.
- Hemolysis (hemoglobin < 400 mg/dL), lipidemia (triglyceride < 10863 mg/dL), jaundice (bilirubin < 99 mg/dL), increased ALT and rheumatoid factors had no impact on the test results of this reagent.

Sensitivity

145 pre-identified clinical samples were tested by this reagent, and the results suggested that the sensitivity of this reagent was 100%.

Hook effect

No hook effect exists in the detection of high concentration specific IgM antibodies.

Genotype detection

The HCV BBI genotype panel was tested, and the detection

rate of HCV genotypes 1-6 was 100%.

The lowest limit of detection

The lowest limit detection of this reagent is 0.003 NCU/mL.

Samples treated with different anticoagulants (EDTA, heparin, and sodium citrate) had no effect on the test results of this reagent.

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 mix use the kits from different batches; 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. 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-All the materials from human used in the process of preparation of this reagent have been tested and proved, with result of negative to HBsAg, HIV p24 Ag or HIV-1 RNA, HAV-IgM, HIV-1 Ab and HIV-2 Ab. As it's still not clear that the test method could guarantee the negative samples will not present HBV, HAV, HIV and other infectious virus, so all the materials from human body, in particular clinical samples should be processed as infectious samples and operated based on the local related laboratory practice and requirements.

6-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.

7-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.

8-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

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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

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