IVD

HYALURONIC ACID CHEMILUMINESCENCE IMMUNOASSAY

Catalog No. CL0315-1/CL0315-2

48 tests/96 tests

INTENDED USE

The Autobio hyaluronic acid (HA) chemiluminescence immunoassay (CLIA) is intended for the quantitative determination of HA concentration in serum specimens, aiding in the diagnosis, monitoring and prognosis of hepatic fibrosis.

INTRODUCTION

The formation of hepatic fibrosis is resulting from the over produce or deficient degradation, or both, of the extracelular matrix, hence excessive connective tissue builds up in the liver, then fibrosis is in turn formed. Hepatic fibrosis is the common pathological basis for chronic liver disorders. Various chronic liver disorders might gradually progress to hepatic fibrosis.

Various methods exist for the diagnosis of hepatic fibrosis, such as liver biopsy, imaging tests and serologic marker assays. The most reliable means to examine the extent of fibrosis and its activity is still the liver biopsy method. Although it is the Gold standard in hepatic fibrosis diagnosis, liver biopsy has many disadvantages. e.g. it is an invasive test, hence patients are reluctant to take this test and repeated tests are not able to be conducted. It is not possible to monitor the progress of the recovery and effects of the therapy. Fibrosis is characterized by focal inflammation and fibroelastosis, so deviations exist in specimen collection. Consequently, liver biopsy is much limited in clinical practice. Modern medical imaging methods such as type B ultrasound, CT, MRI etc. could observe certain symptoms of hepatic fibrosis. However, these medical imaging methods are yet to be confirmatory and identifiable, not to mention the inability to accurately determine the extent of liver fibrosis. Serological tests are able to identify different stages of liver fibrosis with relative accuracy, which indicates various changes during the development of liver fibrosis. Additionally, the effects of anti-fibrosis therapies could be monitored.

HA is widely distributed in extracellular spaces. It is a thread shaped polymer composed of repeated disaccharide units, with the molecular weight being 10 – 10, 000 kD. HA is mainly synthesized by fiber mother cells in the tissue, enters into the blood stream through the lymph system and is soon eliminated by the liver organ. Liver disorders caused by various pathogens could lead to elevated HA levels in serum, due to decrease in the clearing functions of the liver and increase in the synthesis of fibrosing cells within the liver. During hepatic fibrosis, HA synthesis capability of fibrosing cells is greatly elevated while the HA clearance capability of endothelial cells decreases. These two factors lead to the elevated HA levels during hepatic fibrosis.

BIOLOGICAL PRINCIPLE OF THE PROCEDURE

This assay combines the competitive EIA method with the chemiluminescence technique. In this two step assay, HA derivatives are first pre-coated onto microtiter wells, then reference standards, specimens and binding protein solution are added, respectively. Then the plate is incubated. During incubation, HAs present in specimens or reference standards compete with pre-coated HA derivatives for combining with binding proteins. When this step is fully completed, wash the microtiter plate to remove unbound materials. Add enzyme conjugate reagent, which combines with binding proteins attached on microtiter wells in the previous step. After a second incubation, remove unbound enzyme conjugates by washing. Add chemiluminescent substrates, measure the RLU value for each well, construct a calibration curve with values obtained from reference standards, calculate the HA concentration in each specimen through the calibration curve. HA concentrations in specimens are inversely proportional to RLU values.

MATERIALS PROVIDED

1. Coated Wells: microplate with HA derivative coated wells (1 plate, 48 wells/96 wells)
2. Enzyme Conjugate Reagent: horseradish-peroxidase (HRP) labeled antibody to HA binding protein stabilizing buffer (1 vial, 6 mL/11 mL)
3. HA Binding Protein Solution: (1 vial, 3 mL/6 mL)
4. Reference Standards: 0, 60, 180, 360, and 1000 ng/mL HA in Stabilizing Buffer (5 vials, 0.5 mL/ea)
5. Substrate A: (1 vial, 3.5ml/6 mL)
6. Substrate B: (1 vial, 3.5ml/6 mL)
7. Wash Powder: (1 bag, 5 g/2 bags, 5 g/ea.)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes and multichannel micropipettes of appropriate volume (the use of accurate pipettes with disposable plastic tips is recommended)
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Incubator
6. Disposable reagent troughs
7. Instrumentation
    1. Automated microplate stripwasher
    2. Microplate reader

    or

    3. Fully automated microplate processor

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 – 8℃ upon receipt. The test kit may be used throughout the expiry date of the kit (6 months from the date of manufacture). Refer to the package label for the expiry date.
2. Reference Standards after first use could be stored at 2 – 8℃ for 2 months. For longer storage, store frozen at -20℃. Avoid multiple freeze-thaw cycles.
3. Microplate after first use should be kept in a sealed ziplock bag with desiccants to minimize exposure to damp air. Use within the expiry date.
4. Return other unused components to 2 – 8℃. Use within the expiry date.

SPECIMEN COLLECTION, PREPARATION, TRANSPORT AND STORAGE

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Any turbidity and particulate matters might interfere with the test, hence must be removed by centrifugation before testing.
4. Allow samples to clot for 1 hour before centrifugation.
5. Ensure the specimens are not decayed prior to testing.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Store samples at room temperature. Samples not required for assay within 24 hours should be stored at 2 – 8℃. Samples to be transported or stored for more than 48 hours should be stored frozen at - 20℃ or colder. Avoid multiple freeze-thaw cycles. After thawing, ensure samples are thoroughly mixed and brought to room temperature before being assayed.
8. Do not add sodium azide into the specimen as a preservative.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. This package insert must be fully understood prior to operation. The operation must be stringently in accordance with the instruction for use.
3. The assay must be conducted away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as 84 disinfectant, sodium hypochlorite, acid, alkaline, acetaldehyde and so on, or containing dust.
4. Micropipette tips are not interchangeable to eliminate cross contamination.

5. Specimens added must be mixed thoroughly. The presence of bubbles must be eliminated.
6. The microplate must be washed completely. Each well must be fully injected with Wash Fluid. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, all wells must be dried. The microplate should be stroked onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate stripwasher.
7. The substrate reaction must be conducted away from strong light since the RLU would be elevated if the reaction is exposed to light.
8. Wear disposable gloves when dealing with specimens and reagents. Wash hands after operations. All specimens must be regarded as potentially infectious. Waste material must be disposed of safely according to relevant local and national requirements.
9. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18~25℃) prior to use.
2. Add the whole content of 1 bag of Wash Fluid Powder into 500 mL of deionised water.
3. Adjust the incubator to 37℃.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. Do no reuse the plate covers.
4. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all Reference Standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
5. Replace caps on reagents immediately. Do not switch caps.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Dispense 50 μL of Reference Standards, specimens into appropriate wells.
3. Dispense 50 μL of Binding Protein Solution into each well.
4. Thoroughly mix for 30 seconds on a vortex mixer. It is important to have complete mixing in this step.
5. Incubate at 37℃ for 30 minutes.
6. Wash the microplate for 5 times. Strike the plate sharply onto absorbent paper to remove residual water droplets at the end of the last wash cycle. The use of an automated microplate stripwasher is recommended.
7. Add 100 μL of Enzyme Conjugate Reagent into each well.
8. Repeat steps 4 and 5.
9. Dispense 50μl of Substrate A, then 50μl of Substrate B into each well. Gently mix for 10 seconds.
10. Put the microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. Construct the standard curve by the using log-logit curve fitting method.
3. Read the concentration for each sample on the standard curve.
4. Any diluted specimens must be corrected by the appropriate dilution factor.

REFERENCE NORMAL RANGE

Each laboratory should establish its own reference normal range. The following information is given only for guidance.

526 samples from a population of various ages, different genders and without any history of liver disorder were assayed. In statistical analysis, the percentile method with a 95% confidence intervall gave a reference normal value of 120 ng/mL.

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of 10 replicates of the 0 ng/mL Reference Standard minus 2 SD. Therefore, the analytical sensitivity of Autobio HA CLIA is lower than 30 ng/mL.

2. Specificity

No interference was detected with the performance of Autobio HA CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferent	Concentration
LN	1000 ng/mL
CIV	1000 ng/mL
PIIINP	100 ng/mL

3. Precision

The intra-assay precision is lower than 15%, whilst the inter-assay precision is lower than 20%.

4. Linearity

The correlation coefficient of the standard curve is higher than 0.9900.

LIMITATIONS

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.

SYMBOLS

LOT	**BATCH CODE**
	USE BY
	MANUFACTURER
Σ	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
8 °C 2 °C	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	CONSULT INSTRUCTIONS FOR USE

REFERENCES

1. Wang H., Gao C. *Journal of Clinical Medicine in Practice* **2005**, 9, 4.
2. Tian S. Wang S. *Journal of Shanxi Medical University* **2002,** 33, 178.

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