IVD

COLLAGEN TYPE Ⅳ CHEMILUMINESCENCE IMMUNOASSAY

Catalog No. CL0318-1/CL0318-2

48 tests/96 tests

INTENDED USE

The Autobio collagen type IV (CIV) chemiluminescence immunoassay (CLIA) is intended for the quantitative determination of CIV 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.

CIV is a major component of basal membrane. Unlike other fibrous collagens, it is composed of at least two different peptides, i.e. 1 (IV) and 2 (IV). Serum may contain 3 different major degradation fragments, i.e. amine terminal 7S collagen, carboxyl terminal NCL collagen and the main triple helix zone. Alterations in the concentrations of these substances indicate their metabolism within the body. In a normal liver, no basal membrane is present in the Disse gaps, type IV collagen concentration is very low. However, during the fibrosis process from hepatitis through to cirrhosis, the formation of basal membrane occurs in Disse gaps. Meanwhile, type IV collagen concentration in liver tissue and blood stream increases accordingly.

BIOLOGICAL PRINCIPLE OF THE PROCEDURE

This assay combines the sandwich EIA method with the chemiluminescence technique. In this two step assay, anti-CIV is first pre-coated onto microtiter wells, then reference standards, specimens are added, respectively. Then the plate is incubated. During incubation, CIVs present in specimens or reference standards bind to pre-coated anti-CIVs. When this step is fully completed, wash the micrplate to remove unbound materials. Add enzyme conjugate reagent, which combines with CIVs attached on microtiter wells in the previous step, forming a solid phase antibody-antigen-antibody-enzyme complex. After a second incubation, remove unbound enzyme conjugates by washing. Add chemiluminescent substrates, measure the RLU value for each well, construct a standard curve with values obtained from reference standards, calculate the CIV concentration in each specimen through the standard curve. CIV concentrations in specimens are directly proportional to RLU values.

MATERIALS PROVIDED

1. Coated Wells: microplate with anti-CIV coated wells (1 plate, 48 wells/96 wells)
2. Enzyme Conjugate Reagent: horseradish-peroxidase (HRP) labeled antibody to CIV in stabilizing buffer (1 vial, 6 mL/11 mL)
3. Reference Standards: 50, 100, 250, 500, and 1000 ng/mL CIV in Stabilizing Buffer (5 vials, 0.5 mL/ea.)
4. Substrate A: (1 vial, 3.5ml/6 mL)
5. Substrate B: (1 vial, 3.5ml/6 mL)
6. 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. luminometer

    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 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 identifica tion.
2. Dispense 100 μL of Reference Standards, specimens into appropriate wells.
3. Incubate at 37℃ for 30 minutes.
4. 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.
5. Add 100 μL of Enzyme Conjugate Reagent into each well.
6. Repeat steps 4 and 5.
7. Dispense 50μl of Substrate A, then 50μl of Substrate B into each well. Gently mix for 10 seconds.
8. 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-log curve fitting method, with concentrations on the x axis and RLU values on the y axis.
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 based on the particular population group it focuses on. The following information is given only for guidance.

546 samples from a population of various ages, different genders and with no history of liver disorder were assayed. In statistical analysis, the percentile method with a 95% confidence intervalg ave a reference nor-

mal value of 95 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 zero titer serum plus 2 SD. Therefore, the analytical sensitivity of Autobio HA CLIA is lower than 15 ng/mL

2. Specificity

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

Interferent	Concentration
HA	1000 ng/mL
LN	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%.

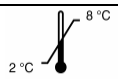
4. Linearity

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

	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**CONTAINS SUFFICIENT FOR <n> TESTS**
	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
	**TEMPERATURE LIMITATION**
	**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.
3. Chai M. and Chen J., et. al. *Shanghai Journal of Medical Laboratory Sciences* **1996,** 11, 231.

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