【Version No】 2.0
【Issued Date】 March 02, 2010
【Intended Use】

The ReLIA Human Heart Fatty Acid Binding Protein (H-FABP) test is an in vitro diagnostic test which is intended to measure the concentration of H-FABP in human whole blood/serum/plasma, as an aid in the diagnosis of Acute Myocardial Infarction (AMI).

【Summary and Explanation】

Heart Fatty Acid Binding Protein (H-FABP) is an abundant cytosolic protein (~15Kd) located in normal cardiac muscle cells. Ischemic myocardium releases H-FABP into blood circulation. The concentration of H-FABP in the blood is increased rapidly. The half life of H-FABP is about 20min. H-FABP in blood is cleared mainly via the kidneys. In recent years, with the improvement of in-vitro diagnostic technology, the clinical value of H-FABP levels in blood and urine in the diagnosis of AMI has been recognized. Clinical studies have shown that the concentration of H-FABP in blood dramatically increases at the early stage of AMI, especially within 1-3h after the onset of infarction. H-FABP levels peaks at around 6-8h, and then returns to normal levels within 24-30h. Therefore, H-FABP indicates damage of cardiac muscle cells earlier than other cardiac markers including Myoglobin, cTnl and CK-MB, and has become an effective diagnostic tool of AMI.

【Principle of the Test】

The ReLIA H-FABP test is a bi-directional flow immunoassay utilizing a double-antibody sandwich format. During the assay, the buffer-A is added to port 1 to pre-wet the strip. When sample is added to port 2, analyte in the sample reacts with an anti-H-FABP immunogold conjugate on the conjugate pad and forms an immune complex, which then flows onto the nitrocellulose. When the immunogold complex reaches the test band, it will react with the anti-H-FABP antibody pre-coated on the nitrocellulose and will be fixed on the test band of the nitrocellulose. The higher the H-FABP level in the blood, the more immunogold complex binds to the test band.

During the assay the control conjugate, colloid gold labeled DNP–BSA, will also flow onto the nitrocellulose with sample by chromatography. The DNP–BSA gold will react with the anti-DNP antibody coated on the nitrocellulose in the locations of control band 1 and control band 2, then be fixed to the control bands. As the colloidal gold are colored red, the immune complex of DNP–BSA gold bound to the anti-DNP in the control band 1 and control band 2 on the nitrocellulose will show as red color. In the absence of H-FABP, only the control band 1 and control band 2 will be present. In the presence of H-FABP, a red test band will also be present.

When the reaction is complete, ReLIA Immunoassay Diagnostic Instrument (SSJ-2) will analyze the DR (density of reflectance) of the bands, and calculate RI (relative intensity of the test and control bands). Then the instrument will then determine the concentration of analyte based on the standard curve.
programmed into the instrument and display the result (ng/mL).

【Supplied Kit Components】

<table>
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<tr>
<th>Item</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Test Cassettes</td>
<td>20</td>
</tr>
<tr>
<td>Buffer-A</td>
<td>1 (3.5mL)</td>
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<td>Product Insert</td>
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</table>

The buffer-A is a non-hazard chemical reagent.

【Materials Required But Not Provided】

1. Precision pipettor for volumes between 40µL and 120µL with disposable polypropylene pipet tips.
2. ReLIA Immunoassay Diagnostic Instrument (SSJ-2).

【Warnings and Precautions】

1. This test is for in vitro diagnostic use.
2. This test is for professional use only.
3. Samples with extensive hemolysis or lipoemia or serious jaundice are not allowed to be used.
4. Do not use cassettes which are damaged, or have an unclear label or past expiration date.
5. Operator must follow this product insert and the onscreen instructions of the SSJ-2 instrument. Do not stop the assay once it starts. Otherwise, the assay cannot be resumed. If the assay has to be restarted, a new cassette must be used.
6. Samples with invalid results must be retested.
7. Must update assay table for each lot of product. Otherwise the cassettes from the new lot will not be recognized by the instrument.
8. The cassette is for one time use only. Used cassettes and samples should be treated as potentially bio-hazardous materials.
9. Do not eat the desiccant in the foil pouch.
10. Use disposable pipette tips and sample vials for each specimen. The pipet tips and sample vials should be for single use.
11. The ReLIA H-FABP test result should not be used as absolute evidence of elevated probability of AMI. The H-FABP level should be interpreted along with clinical findings and other diagnostic tests.
【Kit Storage and Product Shelf Life】
1. Store at room temperature (15-30°C or 59 – 86°F) in a dry place. Avoid direct sunlight.
2. 18 months of shelf life (date of manufacture to expiration date).

【Specimen Requirements】
1. Only serum or heparinized and EDTA.K₂ plasma/whole blood can be tested.
2. Samples should be collected in an approved blood collection device. Contaminated samples should not be used.
3. Samples are recommended to be tested immediately after being collected. Serum/plasma samples should be separated as soon as possible after collection to avoid hemolysis. Extensively hemolyzed samples should not to be used.
4. Whole blood samples may be stored at 2-8 °C for up to 24 hours if not tested immediately.
5. Serum/plasma samples may be stored for 3 days if kept refrigerated at 2-8°C. For longer storage, place serum/plasma samples below –20°C. Samples should be subjected to a minimum number of freeze thaw cycles, since this causes analyte deteriorate.
6. Allow samples to warm to room temperature (20-26°C) before use.
7. Samples with extensive hemolysis or lipoemia or high levels of bilirubin are not allowed to be used.

【Assay Procedure】
Caution: Please carefully read the product insert of the test and the Instruction For Use of ReLIA Immunoassay Diagnostic Instrument (SSJ-2).
1. Warm stored samples to room temperature before starting use.
2. Turn on the computer linked to the ReLIA instrument (SSJ-2), then turn on the instrument and start the ReLIA™ software as described in the Instruction For Use.
3. Remove a ReLIA H-FABP test cassette from the foil pouch. Click on the module icon on the computer screen (or press the button of the module) in which you wish to run the test to eject the cassette tray. Then, insert the cassette into the tray.
   (Caution: Keep the ReLIA H-FABP test cassettes sealed in the protective foil pouch until just before use. The cassette should be used within 1 hour once it is opened. If the temperature is higher than 30°C or under conditions of high humidity, it should be used immediately once the foil pouch is opened)
4. Follow the software prompts to input information about the sample, then click “confirm”.
   (Caution: If test the whole blood, choose the option of “Whole Blood” on the computer screen.)
5. When the cassette tray pops out automatically, the computer will prompt the operator to add 40µL of buffer-A to port 1.
6. After 40 seconds, the cassette tray will then pop out of the instrument and the computer will prompt the operator to add 100µL of serum/plasma or 120µL of whole blood sample to port 2. The cassette tray is then withdrawn into the instrument for 20 minutes of incubation time.
7. When the assay is finished, the instrument will interpret the result automatically and display it on the screen.

[Disposal]
Dispose of bio-hazardous materials should follow the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all federal, state, and local requirements.

[Expected Values]
Detection Range: 0 – 60 ng/mL.
The 98.3rd percentile of H-FABP concentration in 175 healthy subjects is < 10 ng/mL.

[Clinical Significance]
The ReLIA H-FABP diagnostic cutoff for AMI patients was recommended to be 10ng/mL. Same as other *in vitro* diagnostic assays, each laboratory is recommended to determine its own reference range for the diagnostic evaluation of patient’s H-FABP results.

[Interpretation of Results]
1. ReLIA Immunoassay Diagnostic Instrument (SSJ-2) calculates the H-FABP level automatically and displays the H-FABP concentration on the screen as “ng/mL”.
2. When the test exceeds its expiration date, the instrument will report “Assay Expired” directly and the cassette will be rejected.
3. When the control band 1 and control band 2 results do not meet the product specifications, the instrument will report “Invalid”.

[Limitations of the Procedure]
1. High concentrations of triglycerides/cholesterol, bilirubin or hemoglobin (caused by hemolysis) may affect the chromatography of sample and colloidal gold conjugate on the nitrocellulose and may cause an erroneous result. Therefore, samples with serious lipemia (concentration of triglycerides > 15mmol/L or concentration of cholesterol > 4 mg/mL) or extensive hemolysis (concentration of hemoglobin > 6g/L) or serious jaundice (concentration of bilirubin > 4 mg/mL) are not allowed to be tested.
2. Immunoassays employing antibody-antigen reactions have certain limitations. The result of this assay indicates only the concentration of H-FABP in whole blood/serum/plasma, and should not be used as the only criteria for clinical diagnosis.

[Performance Characteristics]
1. Sensitivity: Functional sensitivity of the ReLIA H-FABP test, as calculated based on the results of 20 replicates per lot and three lots of product, is 2ng/mL.
2. Specificity: The cross-reactivity of cTnI (5 ng/mL), CK-MB (50 ng/mL), NT-proBNP (1 ng/mL), and Myoglobin (250 ng/mL) were tested. The detectable H-FABP levels of above substances were all < 2 ng/mL.
3. Precision: Within lot precision, 10 tests per concentration.

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<th>H-FABP (ng/mL)</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
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<tr>
<td>5</td>
<td>5.49</td>
<td>0.42</td>
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</table>
4. Linearity: An H-FABP standard was serially diluted from 60 to 2ng/mL. Each concentration was tested in triplicate. The correlation coefficient of the regression line was $\geq 0.99$.

[References]

2. Alhadi HA and Fox KAA. Do we need additional markers of myocyte necrosis: the potential value of heart fatty acid-binding protein. QJM; 2004; 97: 187-198.


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