Product Insert

D-Dimer Test

(For In Vitro Diagnostic Use Only)

【Version No】 1.0
【Issued Date】 02/12/2009

【Intended Use】
The ReLIA™ D-Dimer test is an in vitro diagnostic test which is intended to measure the concentration of D-Dimer in human whole blood/serum/plasma, as an aid in the diagnosis of thrombotic disease such as deep vein thrombosis (DVT), pulmonary embolism (PE), venous thromboembolism (VTE) and the measurement of the D-Dimer index.

【Summary and Explanation】
During the coagulation process, the enzyme thrombin cleaves fibrinogen to form fibrinopeptides A and B, and converts fibrinogen to soluble fibrin. Soluble fibrin then spontaneously polymerizes in a process that involves covalent crosslink of the D regions by factor XIIIa11.

Following tissue repair, the clot is degraded through the fibrinolytic pathway12-15. During this process, plasmin cleaves chemical bonds of cross-linked fibrin and releases fibrin degradation products (FDPs) including D-Dimer. D-Dimer consists of two cross-linked fragment D molecules. The amount of D-Dimer in the circulation system has been shown to be elevated in patients with venous thromboembolism (VTE)6, pulmonary embolism (PE)1-4,7,8,10 and deep venous thrombosis (DVT)3,5,9.

【Principle of the Test】
The ReLIA™ D-Dimer test is a bi-directional lateral flow immunoassay utilizing a double-antibody sandwich format. During the assay, the blood sample is added to port 1 first. Then the sample flows past the test band, which the capture antibody is coated on nitrocellulose. If D-Dimer is present in the blood sample, it will react with the anti-D-Dimer antibody in the test band and form an immune complex. When the buffer-A is added to port 2, it dissolves anti-D-Dimer antibody labeled colloidal gold and DNP-BSA labeled colloidal gold coated on the conjugate pad. These two conjugates will then flow onto the nitrocellulose with the buffer. When the anti-D-Dimer-immunogold conjugate flows past the test band, it will bind to D-Dimer captured on the test band to form a red color.

During the assay the control conjugate, colloid gold labeled DNP–BSA binds to the anti-DNP antibody coated on the nitrocellulose in the locations of control band 1 and control band 2. Since the colloidal gold are in red color, the immune complex of DNP-BSA gold and anti-DNP in the control band 1 and control band 2 on the nitrocellulose will show a red color. In the absence of D-Dimer, only the control band 1 and control band 2 will be present. In the presence of D-Dimer, a red test band will also be present.

When the test 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).
Product Insert

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

【Supplied Kit Components】

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<tr>
<td>Test Cassettes</td>
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【Materials Required But Not Provided】

1. Precision pipettor for volumes between 70µL 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™ D-Dimer test should not be used as absolute evidence of thrombosis occurred. The D-Dimer 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 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™ D-Dimer 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™ D-Dimer 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 70µL of serum/plasma to port 1.
   (Caution: To perform a whole blood test, add 70µL of whole blood sample to the dilution tube containing 70µL buffer and mix gently, and then add 70µL of diluted sample to port 1)
6. The cassette pops out again after the instrument has counted down for 120 seconds. The computer
will then prompt the operator to add 120µL buffer-A to port 2. Then the instrument counts down 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: 500 – 5,000ng/mL.
The 97.5<sup>th</sup> percentile of D-Dimer concentration in 365 healthy subjects is < 1,500ng/mL.

【Clinical Significance】
The ReLIA<sup>TM</sup> D-Dimer diagnostic cutoff for VTE, PE or DVT patients was determined to be 1,500ng/mL.

Same as other in vitro diagnostic assays, each laboratory is recommended to determine its own reference range for the diagnostic significance of patient’s D-Dimer results.

【Interpretation of Results】
1. ReLIA Immunoassay Diagnostic Instrument (SSJ-2) calculates the D-Dimer level automatically and displays the D-Dimer concentration on the screen as “ng/mL”.
2. When the test past its expiration date, the instrument will report “Assay Expired” directly.
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 > 15 mmol/L or concentration of cholesterol > 4 mg/mL) or extensive hemolysis (concentration of hemoglobin > 6 g/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 D-Dimer 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 TM D-Dimer test, as calculated based on the results of 20 replicates per lot and three lots of product, is 500ng/mL.
2. Specificity: The cross-reactivity of fibrinogen (2mg/mL) was tested. The detectable D-Dimer level of fibrinogen was <1,000 ng/mL.
3. Precision: Within lot precision, 10 tests per concentration.
4. Linearity: A D-Dimer standard was serially diluted from 5,000 to 500ng/mL. Each concentration was tested in triplicate. The correlation coefficient of the regression line was ≥ 0.99.

**References**


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