**i-CHROMA™ D-Dimer**

ImmunoAssay for Quantitative Measurement of D-Dimer in Human Plasma with i-CHROMA™ Reader System.

**INTENDED USE**

i-CHROMA D-Dimer Test along with the i-CHROMA™ Reader is a fluorescence immunoassay that quantifies the total D-Dimer concentration in plasma. The test is used as an aid in the post therapeutic evaluation of thromboembolic disease patients.

**INTRODUCTION**

D-dimer, a degradation product of cross-linked fibrin formed during activation of the coagulation system, is commonly used to exclude thromboembolic disease in outpatients suspected of having deep venous thrombosis (DVT) and pulmonary embolism (PE).[1] DVT and PE is relatively common and can cause sudden, fatal embolic events in the pulmonary arteries and other regions.[2-3] Measurement of the D-Dimer level in plasma has been used as a screening strategy for subclinical DVT. A systematic review reported that a normal range of a highly sensitive D-dimer level accurately ruled out DVT in patients classified as having a low or moderate clinical probability of DVT. The DVT is a high-risk factor for the stroke because of advanced age, hemiplegia, and coagulation disorders, and DVT can cause paradoxical embolic stroke via a right-to-left shunt. Thus, it is important to monitor the level of D-Dimer the incidence and characteristics of DVT in acute stroke patients.[4-7] The Plasma D-dimer level has proven to be useful for DVT screening in chronic stroke patients undergoing rehabilitation.[8-10] National and international scientific organizations have suggested the use of these markers when implementing new diagnostic strategies in patients with coronary syndrome. Since D-Dimer is well known to be an important prognostic indicator of heart diseases, its most definitive role is on monitoring post-treatment clinical status and the post therapeutic evaluation of patients.

The i-CHROMA™ D-Dimer Test measures quantitative D-Dimer concentration in human plasma.

**PRINCIPLE**

The test uses the sandwich immunodetection method, such that the detection antibody in binder binds to D-Dimer in the plasma sample and antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocellulose matrix. The more D-Dimer antigen in the plasma, the more antigen-antibody complexes are accumulated on test strip. Signal intensity of fluorescence on detection antibody reflects amount of antigen captured and is processed by i-CHROMA™ Reader to show D-Dimer concentration in the specimen. The working range of i-CHROMA™ D-Dimer test is 50 – 10,000 ng/mL.

* Reference Value : 300 ng/mL, (DDU : D-Dimer Unit)

**COMPOSITION OF REAGENTS**

The i-CHROMA™ D-Dimer Test consists of Test Device, an ID Chip, and Detection Buffers. Test Device is individually sealed with a desiccant in an aluminum pouch, and detection buffer is together dispensed individually in a tube and the bottle containing the predisposed tubes is delivered separately from test device in a Styrofoam box filled with ice pack.

- The test device contains a test strip in which anti-D-Dimer antibody and streptavidin have been immobilized on the test and on the control line of strip, respectively.
- The detection buffer, predisposed in a tube, contains fluorescence-labeled anti D-Dimer antibody, fluorescence-biotin labeled BSA, BSA as a stabilizer, and sodium azide as a preservative in PBS.

**WARNINGS AND PRECAUTIONS**

- IVD For In Vitro Diagnostic Use.
- Carefully follow the instructions and procedures described in this insert. REF Catalog No. CFPC-25
- Don’t use the test device if its lot number should not match with an ID chip number that is inserted onto the instrument.
- The i-CHROMA™ D-Dimer is only operational in the i-CHROMA™ Reader. And tests should be applied by trained staff working in the laboratories where the sample(s) is taken by qualified medical personnel.
- LOT Neither interchange materials from different product lots nor use beyond the expiration date. The use of medical device beyond expiration date may after the test result.
- The i-CHROMA™ D-Dimer Device should remain in its original sealed pouch until ready to use. Do not use the Test Device if the pouch were damaged or the seal broken. Discard after single use.
- The i-CHROMA™ D-Dimer Device and Reader should be used away from vibration and magnetic field. During normal usage, i-CHROMA™ Reader may introduce minute vibration, which should be regarded normal.
- Use separate clean pipette tips and sample vials for different specimens. The pipette tips and sample vials should be used for one specimen only. Discard after single use.
- Urine specimens, used test devices, pipette tips and sample vials are potentially infectious. Proper laboratory safety techniques, handling and disposal methods should be followed in accordance with standard procedures and relevant regulations observed by microbiological hazard materials.
- The i-CHROMA™ D-Dimer should not be used as absolute evidence for thromboembolic disease. The results should be interpreted by the physician along with clinical findings and other laboratory test results.
- The test will be applied on a routine basis and not in emergency situations.
- Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.

**STORAGE AND STABILITY**

- Store the detection buffer in a refrigerator at 2° - 8°C. The Detection Buffer is stable up to 20 months if stored in a 2°C refrigerator.
- Once removed from refrigerator, allow the detection buffer for 30 minutes to return to room temperature before testing.
• Store the test device at 4°C-30°C in its sealed pouch. The test device is stable for 20 months (while in the sealed pouch) if stored at 4°C-30°C.

• If stored in a refrigerator, allow a minimum of 30 minutes for the test device and detection buffer to reach room temperature while it is in the sealed pouch.

• Do not remove the device from the pouch until ready to use. The Test Device should be used immediately once opened.

• The storage and shipping of i-CHROMA™ D-Dimer Device should be compiled as indicated in manual. However, it is remotely possible that only part of i-CHROMA™ D-Dimer Device is affected by stability problems.

SAMPLE COLLECTION AND PREPARATION
The test can be performed with plasma.

• Preparing the Plasma specimen
Collect the blood in a tube treated with sodium citrate. Anticoagulants other than sodium citrate for plasma specimen have not been evaluated.

• If testing cannot be conducted within an hour after preparation of samples, the plasma should be stored at -20°C until tested.

• The specimen must be at room temperature and be homogeneous before testing. Frozen specimens must be completely thawed, homogenized, and brought to the room temperature prior to testing. If specimens are to be shipped, they should be packed in compliance with applicable regulations.

• For D-dimer analysis, venous blood must be collected in 5 ml vacuum tubes containing sodium citrate.

• For best results, it is strongly recommended that the sample be centrifuged at 3,000 rpm for 2–5 minutes to separate lipids.

• Also sample with excessive hemolysis should be avoided whenever possible.

• It is recommended to avoid using severely hemolyzed and hyperlipidemia specimens whenever possible. If the specimen appears to be severely hemolyzed, another specimen should be obtained and tested.

MATERIALS PROVIDED
Boditech Med Incorporated i-CHROMA™ D-Dimer
REF Catalog No. CFPC-25

Kit contains:
Test Devices 25 test/box
ID Chip 1ea/box
Detection Buffer 25 tubes/pouch

MATERIALS REQUIRED BUT NOT PROVIDED
i-CHROMA™ Reader REF Catalog No. FR-203
Thermal Printer
Transfer pipette (75μL size)

PROCEDURE
• Image of the test kit

1. Set a Test Device on a dust-free clean place.
2. Check/insert ID Chip onto the instrument. Make sure that the Test Device lot # matches with ID Chip #.
3. Take out one tube of Detection Buffer from refrigerator and leave it at room temperature.
4. Draw 75 μL of plasma or Control with a transfer pipette and add it to the tube containing Detection Buffer.
5. Mix well the specimen with Detection Buffer by tapping or inverting the tube.
6. Take the 75 μL of sample mixture and “immediately” load it onto the well of disposable Test Device. (Delays would introduce notically errors in the result.)
7. Leave the Test Device at room temperature for 5 min before inserting the device into the holder.
8. To start scanning, the insert test device onto the holder of i-CHROMA™ Reader and press “SELEcT” button.
9. The test devices should be pushed with the way into the holder.
10. The instrument will automatically start to scan the Test Device immediately.
11. Read the results on the display screen of i-CHROMA™ Reader.

Refer to i-CHROMA™ Reader Operation Manual for the complete instructions on use of the Test.
REF Catalog No. FR-203

RESULT
The i-CHROMA™ Reader calculates D-Dimer test results automatically and displays concentration of D-Dimer in blood sample on the LCD as form of ng/mL. For further information, refer to the Operation Manual for the i-CHROMA™ Reader.

Quality Control
• A quality control test using commercially available controls should be performed as a part of good testing practice, to confirm the expected QC results, to confirm the validity of the assay, and to assure the accuracy of patient results. If you want to perform QC of Test Kit, we recommend using Bio-Rad D-Dimer Control.

• A quality control test should be performed at regular intervals, and before using a new kit with patient specimens, controls should be tested to confirm the test procedure, and to verify the tests produce the expected QC results. QC specimens should also be run whenever there is any question concerning the validity of results obtained. Upon confirmation of the expected results, the test device is ready to use with patient specimens. Control standards are not provided with this test kit. For information about obtaining the controls, contact Boditech Med Incorporated’s Technical Services for assistance.

Procedure Control
• Each i-CHROMA™ D-Dimer test device contains internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by i-CHROMA™ Reader. An invalid result from the
internal control causes an error message on i-CHROMA™ Reader indicating that the test should be repeated.

LIMITATIONS OF THE PROCEDURE

- The results of i-CHROMA™ D-Dimer should be evaluated with all clinical and laboratory data available. If D-Dimer Test results should not agree with the clinical evaluation, additional tests should be performed.

- The false positive results include cross-reactions with some components of serum from individual to antibodies; and non-specific adhesion of some components in human blood that have similar epitopes to capture and detection antibodies. In the case of false negative results, the most common factors are: non-responsiveness of antigen to the antibodies by that certain unknown components are masking its epitope, such that antigen cannot be seen by the antibodies; instability of D-Dimer antigen, resulting in degradation with time and, or temperature, such that they become no longer recognizable by antibodies; and degraded other test components. The effectiveness of the test is highly dependent on storage of kits and sample specimens at optimal conditions.

- Plasma using anticoagulants (e.g. heparin or EDTA) other than Sodium Citrate has not been evaluated with the i-CHROMA™ D-Dimer Test and thus should not be used.

- Other factors may interfere with i-CHROMA™ D-Dimer Test and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.

Performance Characteristics

1. Analytical Sensitivity
   Analytical sensitivity is the lowest concentration of D-Dimer that the test system can detect with CV<10%. Analytical sensitivity of i-CHROMA™ D-Dimer Test was determined by testing 10 times each using 3 lots of reagents and i-CHROMA™ D-Dimer test was 50 ng/ml.

2. Specificity
   Other bio-molecules, such as Hb, CEA, AFP, ALP, CRP, Troponin I, CK-MB, Myoglobin, Albumin and specially hyperlipid were added to test specimen with much higher level than their physiological level in normal blood. There was no significant interference with the D-Dimer measurement, nor was their any significant assay cross-reactivity with those bio-molecules tested.

3. Precision

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<thead>
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<th>Intra- and inter-assay precision</th>
<th>Intra-assay</th>
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<tr>
<td></td>
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4. Linearity: The high concentration was diluted with the low concentration to the following final percentages; 100%, 75%, 50%, 25%, 10%, 5% and 0%. Sample was assayed in triplicate in one analytical run at each D-Dimer level. The coefficient of linear regression was R=0.998. Linearity of i-CHROMA™ D-Dimer test device was 50 ~ 10,000ng/mL.

5. Comparability
   Total D-Dimer concentrations of 150 clinical specimens were quantified independently with i-CHROMA™ D-Dimer test device and Biomerieux VIDAS automatic analyzer according to established standard test procedure. Test result was compared and their compatibility was investigated with linear regression and correlation of coefficient (R). Linear regression and correlation of coefficient were Y=0.6161X + 46.34 and R=0.9334, respectively.

REFERENCES

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10. VIDAS#{174}D-dimer: fast quantitative ELISA for measuring D-dimer in plasma JEAN-LOUIS PITTET,1* PHILIPPE DE MOERLOOSE,5 GuIDo REBER,5 CATHERINE DURAND,1 CECILE VILLARD,2 NADIA PIGA,2 DOMINIQUE ROLLAND,3 SERGE COMBY,4 and GEORGES Dupuy1 Clinical Chemistry 42, No. 3, 1996

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For Technical Assistance call Boditech Med’s Technical Services at
Tel: +82 (33) 243-1400
E-mail: sales@boditech.co.kr

Boditech Med Incorporated

1144-2 Geodu-ri, Dongnae-myeon,
Chuncheon-si, Gangwon-do 200-883
Republic of Korea
Tel: +82 (33) 243-1400
Fax: +82 (33) 243-9373 www.boditech.co.kr

EU Representative: Jai Jun Choung, Ph.D.
Eu Biotech Development Ltd.

81 Oxford Street, LONDON, W1D 2EU
United Kingdom
Tel: +44 207 903 5441
Fax: +44 207 903 5333
E-Mail: jjchoung@eubio.co.uk

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