# Fluoro-Check™ D-Dimer Test

***For in vitro Diagnostic Use***

*One–step quantitative time resolved fluorescence immuno-chromatographic assay for the detection of D-Dimer in human Whole blood or plasma*

## 1. INTENDED USE

The Fluoro-Check™ D-Dimer Test is a time resolved fluorescence immunoassay for the quantitative determination of D-Dimer in human whole blood and plasma specimen with Fluoro-Checker™ TRF reader. The test is used as an aid in the diagnosis of Disseminated Intravascular Coagulation (DIC), or Venous Thromboembolism (VTE), which includes Deep Venous Thrombosis (DVT) and Pulmonary Embolism (PE).

## 2. SUMMARY AND EXPLANATION OF THE TEST

D-Dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. Fibrinolysis is a process that prevents blood clots from growing, in which a fibrin clot, the product of coagulation, is broken down. Fibrin consists of D- and E- units, and the cleavage of fibrin leads to so called D-Dimers, which contains two D fragments of the fibrin protein joined by a cross-link.

D-Dimers are not normally present in human blood plasma. As D-Dimer is released into the circulation during the fibrinolytic process, the measurement of D-Dimer and higher molecular weight oligomers containing D-Dimer epitopes is considered to reflect the overall activity of clot formation and lysis. Elevation of D-Dimer in the blood stream of patients with pulmonary embolism and deep venous thrombosis have been reported by Goldhaber.

## 3. PRINCIPLE

The Fluoro-Check™ D-Dimer Test is lateral flow immunochromatographic assays for quantitative determination of D-Dimer in whole blood or plasma, using time resolved fluorescence particle as detection method.

Two D-Dimer antibodies in the dye pad of the membrane, one labeled with capturing molecule, the other with fluorescence particle as detection molecule, bind with D-Dimer protein in the sample. These antigen-antibody complexes move along the nitrocellulose membrane and bind to molecule immobilized on the test line to produce specific test signal. Signal intensity of fluorescence, which reflects the amount of D-Dimer protein in the sample, is analyzed by Fluoro-Checker™ TRF Reader, and presented as D-Dimer concentration. Fluorescence labeled antibody, unbound with D-Dimer protein, passes through the test line, and then captured by IgY antibody on the control line, to produce control signal which indicates assay validity.

## 4. REAGENT

The Fluoro-Check™ D-Dimer Test contains all the reagents necessary for the detection of D-Dimer in human whole blood or plasma. The device contains a membrane strip coated with streptavidin on the test line and dye pad infused with biotinylated monoclonal anti-D-Dimer antibody and fluorescence particles coupled with D-Dimer specific antibody. A stabilizer containing 0.05% sodium azide, BSA protein and other chemicals are deposited on the dye pad in dried form.

## 5. MATERIALS

*Provided*

- 20 Fluoro-Check™ D-Dimer Test devices containing membrane strip in a sealed aluminum foil pouch with desiccant
- 20 Disposable transfer pipette (10 µl)
- 1 Bottle of developer
- Instructions for Use
- QR card

*Required but not provided*

- Whole blood or Plasma Collection Container
- Positive and negative quality control materials
- Timer
- Fluoro-Checker™ TRF reader

## 6. STORAGE AND STABILITY

The test kit should be stored at 2°C - 30°C in the original sealed pouch for the duration of shelf life.

## 7. PRECAUTIONS

- For *in-vitro* diagnostic and professional use only.
- Do not use hemolyzed specimens as hemolysis may affect test results
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- To avoid cross contamination, use a fresh transfer device for each clinical sample tested.
- Do not use test kit if the pouch is damaged or improperly sealed.
- Do not use test kit beyond expiration date.

## 8. SPECIMEN COLLECTION AND PREPARATION

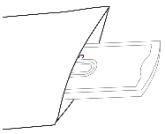
- This test can be used for finger-pricking or venous blood. For venous drawn samples, whole blood or plasma specimen using heparin or EDTA as the anticoagulant can be used with this product. Other blood anticoagulants have not been evaluated. Each laboratory should determine the acceptability of its own blood collection tubes and anticoagulant products. Variation in these products may exist between manufacturers and, at times, from lot-to-lot.
- The samples should be collected under standard laboratory conditions.
- Optimal results were obtained when patient samples were tested immediately after collection. Plasma samples may be refrigerated for 24 hours at 2-8ºC. If testing cannot be performed within 24 hours, or for shipment of samples, freeze the samples at -20ºC or below. Do not repeat freezing and thawing cycles, as freezing and thawing may damage D-Dimer molecule in the sample.
- Sodium azide can be added as a preservative up to 0.1% without affecting the test results.
- Refrigerated or frozen plasma specimen should reach room temperature and be homogeneous prior to testing

## 9. TEST PROCEDURE AND PROTOCOL

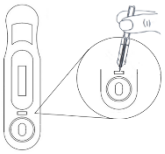
a) Collect specimen according to instructions in “Specimen Collection”.

b) Test device and sample should be brought to room temperature (20°C-30°C) prior to testing.

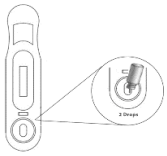
c) Remove the test device from the sealed pouch immediately before use. Label the device with patient or control identification.



d) Using sample transfer pipette, deliver 10 µl of whole blood, or 5 µl of plasma sample into the sample well.



e) Deliver two drops of develop buffer (60 ~ 80 µl) to the developer well by squeezing diluents bottle.



f) Read the results at 15 minutes. The tested device should be analyzed by the Fluoro-Checker™ TRF reader following by the instruction manual.

## 10. INTERPRETATION OF RESULTS

The signal intensity of test line and control can be analyzed by Fluoro-Checker™ TRF Reader and reading results are expressed as a concentration of analytes using

predetermined calibration curves specific for Fluoro-Check™ D-Dimer Test kit. The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture.

## 11. LIMITATIONS

- The test is for professional and *in-vitro* diagnostic use only.
- Test result obtained by current device may only be used as an indicator of thrombosis and requires further confirmation.
- As with all diagnostic tests, a definitive clinical diagnosis should not be made based on the results of a single test. The test result should be used in conjunction with other clinical information such as clinical signs and symptoms and other test results to diagnose thrombosis. Confirmation of test results should only be made by a physician along with clinical symptoms and laboratory findings.
- Samples containing unusually high titers of certain antibodies such as human anti-mouse or human anti-rabbit antibodies have been known to affect the performance of this device. However, these studies using the Fluoro-Check™ D-Dimer Test have not been performed.
- Patients taking more than 30 µg/day of biotin may have falsely negative results and should not use this test, unless it is conformed that the patient is not taking more than 30 µg/day of biotin.

## 12. QUALITY CONTROL

The presence of fluorescence band in the Control area of the window acts as an internal control to ensure that an adequate volume of sample has been added. In the absence of this Control band, the test is invalid and must be repeated. Good laboratory practice recommends the use of control materials to ensure proper kit performance. Quality control specimens are available from commercial sources, and should be assayed using the same procedures followed when running patient samples. Controls should minimally be run before using each new lot of Fluoro-Check™ D-Dimer Test, at regular intervals afterwards and any time the validity of the test results are questioned.

For the calibration of the assay performance, QR card is supplied with the assay kit. Refer to the Fluoro-Checker™ TRF reader for QR card.

## 13. REFERENCE RANGE

The cutoff values of the Fluoro-Check™ D-Dimer Test were estimated by comparison to the VIDAS® D-dimer Exclusion II Assay (bioMérieux, Inc). The cutoff level of D-Dimer is 500 ng/mL for patients. However, each laboratory should establish its own reference range. It has been noted that the cutoff levels are different if a quantitative assay system other than VIDAS® D-dimer Exclusion II Assay (bioMérieux, Inc) is used.

## 14. PERFORMANCE CHARACTERISTICS

### *1. Detection limits*

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of quantification (LoQ) of Fluoro-Check™ D-Dimer Test were determined as follows.

| LoB | LoD | LoQ |
|---|---|---|
| 68 ng/mL | 120 ng/mL | 120 ng/mL |

### *2. Linearity / Reportable range*

Linearity range of Fluoro-Check™ D-Dimer Test is between 120 ng/mL and 4400 ng/mL, with 11.2% repeatability within this interval.

### *3. Interference & Specificity test*

The following endogenous substances do not interfere with the performance of the Fluoro-Check™ D-Dimer Test at the levels below (less than 10% bias).

| Substances | Concentration |
|---|---|
| Hemoglobin | 50 mg/mL |
| Triglyceride | 10 mg/mL |
| Cholesterol | 2 mg/mL |
| Bilirubin | 0.5 mg/mL |
| Fibrinogen | 1 mg/mL |
| Biotin, Vitamin B7 | 600 ng/mL |

The following substances do not show any significant interference with this assay at the level tested, 10 µg/mL.

| | | |
|---|---|---|
| Acetaminophen | Dopamine | PCP |
| Acetylsalicylic Acid | Erythromycin | Phenobarbital |
| Allopurinol | Fluoxetine | Phenytoin |
| Ampicillin | Furosemide | Probenecid |
| Ascorbic Acid | Hydrocodone | Procainamide |
| Caffeine | Ibuprofen | Propranolol |
| Captopril | Indomethacin | Quinidine |
| Chloramphenicol | Metoprolol | Sulfamethoxazole |
| Cocaine | Morphine | Theophylline |
| Digoxin | Nicotine | Trinitroglycerin |
| Diltiazem | Nitrofurantoin | Verapamil |
| Dipyridamole | Oxytetracycline | Warfarin |

### *4. Precision Test*

Precision study was performed at four levels of D-Dimer protein in plasma sample with 200 replicates per each level of sample for 20 days, based on CLSI guideline EP5-A. The within-run and total precision data are summarized in the table below.

| Mean (ng/mL) | Within Run | | Total Run | |
|---|---|---|---|---|
| | SD (ng/mL) | CV (%) | SD (ng/mL) | CV (%) |
| 375.1 | 26.8 | 7.1 | 30.8 | 8.2 |
| 510.9 | 34.2 | 6.7 | 39.1 | 7.7 |
| 1206.2 | 76.6 | 6.4 | 91.2 | 7.6 |
| 2008.6 | 151.7 | 7.6 | 178.1 | 8.9 |

Precision data of whole blood sample is comparable to that of plasma sample. Whole blood specimen from 3 donors were spiked with D-Dimer protein to prepared three levels of samples, and tested in 15 replicates.

| Mean (ng/mL) | Within Run | | Total Run | |
|---|---|---|---|---|
| | SD (ng/mL) | CV (%) | SD (ng/mL) | CV (%) |
| < 120 | N/A | N/A | N/A | N/A |
| 491.4 | 50.8 | 10.3 | 50.5 | 10.3 |
| 1972.4 | 270.9 | 13.7 | 264.2 | 13.4 |

### *5. Matrix Comparison Study*

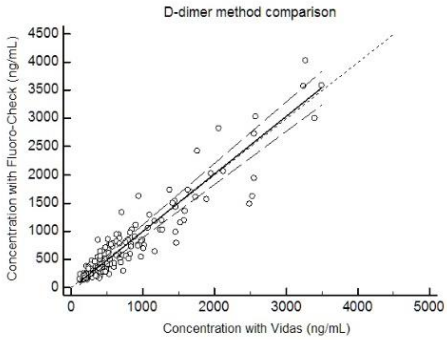
Performance of the assay using samples of five matrices - whole blood and plasma, in heparin or EDTA as anticoagulant, and "finger prick-mimicking" whole blood sample - were compared at D-Dimer ranges between 200 ng/mL – 2800 ng/mL. "Finger prick-mimicking" whole blood is venous whole blood specimen in no anticoagulant. The results were evaluated by regression and correlation method. Data presented by heparinized plasma as reference in table below, demonstrate that the assay performs equivalently in those matrices.

| | EDTA Plasma | Heparinized Whole Blood | EDTA Whole Blood | finger prick-mimicking whole blood |
|---|---|---|---|---|
| correlation coefficient | 1.000 | 0.988 | 0.988 | 0.988 |

### *6. Method Comparison Study*

Method comparison study was performed for Fluoro-Check™ D-Dimer in conjunction with Fluoro-Checker™ TRF reader versus
VIDAS® D-dimer Exclusion II Assay (bioMérieux, Inc). Plasma of clinical samples were collected from 179 peoples, D-Dimer level between 120 ng/mL and 4400 ng/mL. The results were analyzed for correlation regression and summarized in below.

| n | Ranges (ng/mL) | Intercept (ng/mL) | Slope | Correlation Coefficient |
|---|---|---|---|---|
| 179 | 121.21 ~ 4034.72 | -19.710 | 1.020 | 0.901 |




Comparison data were further analyzed for concordance at the D-Dimer Cut-off level of 500 ng/mL which was determined by a ROC analysis with the acceptance criteria: Area under curve (AUC) ≥ 0.85, and Sensitivity (Se) ≅ or > Specificity (Sp). The Fluoro-Check™ D-Dimer Test showed 85.90% (95% CI: 76.49-91.94%) PPA and 92.08% (95% CI: 85.14-95.93%) NPA. The OPA was 89.39%.

| Fluoro-Check™ D-Dimer Test | VIDAS® D-Dimer Exclusion™ II | | Total | Performance (95% CI) |
|---|---|---|---|---|
| | < 500 ng/mL | ≥ 500 ng/mL | | |
| < 500 ng/mL | 93 | 11 | 104 | NPA: 92.08% (85.14-95.93%) |
| ≥ 500 ng/mL | 8 | 67 | 75 | PPA: 85.90% (76.49-91.94%) |
| Total | 101 | 78 | 179 | OPA: 89.39% |

## 15. REFERENCES


1. Oger E., *et al*., Evaluation of a New, Rapid, and Quantitative D-Dimer Test in Patients with Suspected Pulmonary Embolism. *Am J Respir Crit Care Med*. 1998; 158: 65-70.
2. Fancher TL, White RH, Kravitz RL. Combined Use of Rapid D-dimer Testing and estimation of clinical probability in the diagnosis of deep vein thrombosis: systematic review. *BMJ*. 2004; 329: 821–824.
3. Fumeaux T, Cornuz J. The use of the rapid D-dimer test for the exclusion of acute venous thromboembolism in a regional hospital. *Swiss Med. Wkly*. 2003; 133: 178-183.
4. Wells PS *et al*. Evaluation of D-Dimer in the Diagnosis of Suspected Deep-Vein Thrombosis. *N. Eng. J. Med.*, 2003; 349: 1227-1235.
5. S.Z. Goldhaber. Pulmonary embolism, *New England Journal of Medicine* 339: 93-104, 1998.
6. Wells, P.S., Anderson, D.R., et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. *New England Journal of Medicine* 349: 1227-1235,2003.
7. Kahler ZP, and Kline JA. Standardizing the d-dimer Assay: Proposing the d-dimer International Managed Ratio. *Clin Chem* 61:5, 2015.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# *Quick Reference Instruction for Fluoro-Check ™ D-dimer with Fluoro-Checker ™ TRF analyzer*

Read the complete test procedure, including recommended QC before performing the test. Refer to the IFU for complete information about the test. Ensure ALL components are at room temperature (20℃-30℃) when running the test.

## *QR card registration*

① On test page, Insert the QR card for automatic registration of lot information

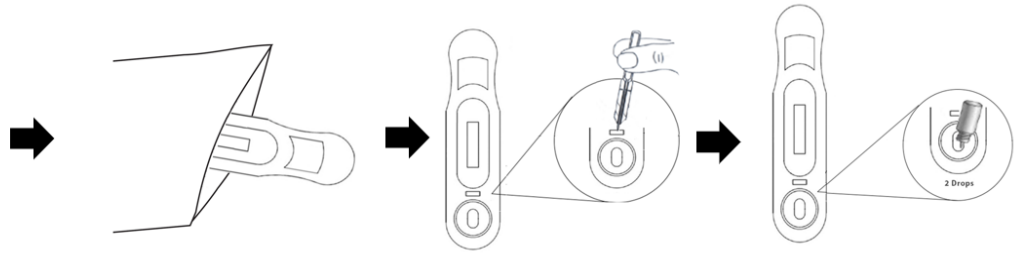

## *Sample preparation*

② Collect whole blood, plasma, or serum specimen. Both the test cassette and sample should be brought to room temperature (20℃~30℃) prior to testing.

③ Remove the test cassette from the sealed pouch immediately before use.

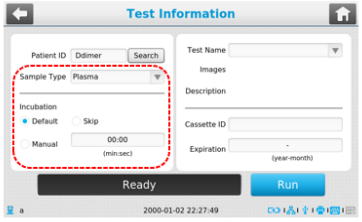
④ Deliver 10 µl of whole blood or 5 µl of plasma or serum sample into the sample well.

⑤ Deliver two drops of develop buffer (60 – 80 µl) to the developer well by squeezing diluents bottle.

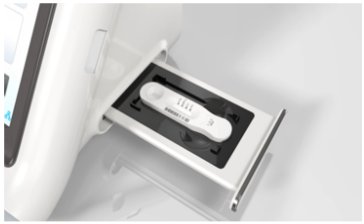



## *Using Fluoro-Checker ™ TRF analyzer to read the cassette*

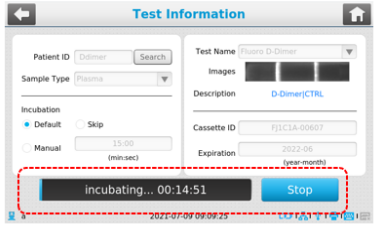
Select "Sample Type"
Set "Default" Mode for Incubation



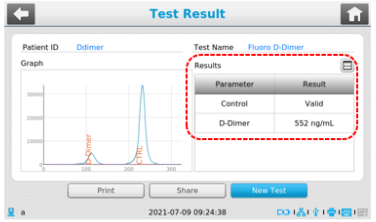

⑥ Insert the cassette immediately and press Run.



⑦ 15-minute Incubation will automatically start.




⑧ Result will appear on screen in 15 min.

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