

D-Dimer Turbidimetric



REF 3145005

1 x 54 mL

CONTENTS

R1. Reagent 1 x 48 mL

R2. Reagent 1 x 6 mL

CAL. 1 x 1 mL

For *in vitro* diagnostic use only

D-Dimer Turbidimetric

Latex Turbidimetry

PRINCIPLE

The latex particles coated with murine monoclonal anti-D-Dimer antibody are agglutinated when they react with human plasma samples that contains D-Dimer. This antibody is highly specific for the cross-linked region of human D-Dimer. The latex particles agglutination is proportional to the concentration of the D-Dimer in the sample and can be measured by turbidimetry.

REAGENTS COMPOSITION

R1 **Diluent.** Borate buffer, 0.1 mol/L, pH 8.2

R2 **Latex.** Latex particles coated with murine monoclonal antibody anti-D-Dimer, pH 8.2.

CAL **Calibrator** Ref: 3945005 (2 x 1 mL). Lyophilized solution of highly purified human D-Dimer. Contains bovine albumin, stabilizers and preservative.

Precautions: The reagents contain sodium azide <0.1%. Avoid any contact with skin or mucous.

REAGENT PREPARATION

R1 Ready to use.

R2 Ready to use. Mix gently the vial before use avoiding the formation of foam.

CAL Lyophilized. Dissolve the contents with exactly **1.0 mL** of distilled water. Replace the stopper and swirl carefully. Let stand the vial at 15-25°C for 20-30 minutes before use. Do not shake.

Calibration curve (6 points): Prepare two-fold serial dilutions of the Calibrator with NaCl 9 g/L. Multiply the concentration of the Calibrator by the corresponding factor indicated in the table below to obtain the D-Dimer concentration of each point of the curve.

Dilution	1:1	1:2	1:4	1:8	1:16
NaCl 9 g/L (µL)	--	200	200	200	200
D-Dimer CAL (µL)	400	200	200	200	200
	Mix and transfer				
Factor	1.0	0.5	0.25	0.125	0.0625

Calibrator 0 µg/L: Prepare a tube with NaCl 9 g/L.

STORAGE AND STABILITY

- The reagents will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use the reagents after the expiration date.
- Reagent deterioration: Presence of particles and turbidity.
- Calibrator: Stability after reconstitution: 2 days at 15-25°C, 15 days at 2-8°C and 2 months at -20°C.

SAMPLE

Mix 9 vol. of freshly venous blood in 1 vol. of trisodium citrate. Sample collection must be in conformity with the recommendations for haemostasis tests. For further information refer to the CLSI document H21-A5.

Citrated plasma is stable 4 days at 2-8°C, 1 month at -20°C. **Do not freeze more than once!** Thaw frozen samples at 37°C and then allow at room temperature before use. Thawed samples must be assayed within 2 hours. (Note 1)

MATERIAL REQUIRED

- Thermostatic bath at 37°C.
- Spectrophotometer or photometer thermostatable at 37°C with a 630 ± 20 nm filter.

PROCEDURE

Preliminary Procedure

Prewarm the reagent R1 and the photometer (cuvette holder) to 37°C.

Analytic Procedure

- Using distilled water zero the instrument at 630 nm.
- Pipette into a cuvette:

Diluent (R1)	800 µL
Sample/Calibrator/Water (Blank)	25 µL
Latex (R2)	100 µL

- Mix well and record the absorbances immediately (A_1) and after 2 minutes (A_2) of the first reading.

Calculation

Calculate the absorbance difference ($A_2 - A_1$) of each point of the calibration curve and plot the values obtained against the D-Dimer concentration of each calibrator dilution. D-Dimer in the sample is calculated by interpolation of its ($A_2 - A_1$) in the calibration curve. (Note 2)

QUALITY CONTROLS

D-Dimer controls are recommended to monitor the performance of manual and automated assay procedures. It is recommended to use D-Dimer Turbidimetric Control Set (ref: 3945010).

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUES

Samples with a D-Dimer concentration ≤250 ng/mL are considered normal. Use of a 250 ng/mL cut-off value is recommended for the VTE exclusion. (Note 3)

Each laboratory should establish its own reference range. D-Dimer levels increases in pregnancy and also rises with age.



CLINICAL SIGNIFICANCE

D-Dimer is a degradation product of fibrin. After the initial formation of the fibrin clot generated by the influence of thrombin on fibrinogen, Factor XIII links two D-domains and generates a solid fibrin clot. Plasmin degrades the cross-linked fibrin forming a variety of soluble derivatives with several molecular weights. These degradation products contain the D-Dimer domain.

The D-Dimer is a measure of fibrinolytic activity of plasmin in the bloodstream. Its determination is becoming a widespread tool for diagnosing thrombosis and monitoring thrombolytic therapy for the Disseminated Intravascular Coagulation (DIC).

Increased levels of D-Dimer are found in clinical conditions of Venous Thromboembolism (VTE) such as Pulmonary Embolism (PE) and Deep Vein Thrombosis (DVT) and also in DIC. Other conditions could increase D-Dimer such as pregnancy, malignancy, recent surgery, active or recent bleeding, trauma, hematomas, inflammation and liver disease.

When D-Dimer values below the cut-off level are obtained and in conjunction with a low clinical pretest probability, DVT of the lower limb and PE can be excluded with a negative predictive value of approximately 95%.

D-Dimer assay could provide significant utility for monitoring patients with suspected DVT.

ANALYTICAL PERFORMANCE

– **Linearity limit:** Up to 3300 ng/mL, under the described assay conditions. Samples with higher concentrations should be diluted 1:5 with NaCl 9 g/L and retested again. (Note 4)

– **Detection limit:** Values ≤ 92 ng/mL give non-reproducible results.

– **Analytical sensitivity:** $0.3 \text{ mAb} \cdot \text{min}^{-1} / \text{ng/mL}$.

– **Prozone effect:** Concentration of D-dimer >45.000 ng/mL still gives positive result. (Note 5)

– **Precision:**

	Intra-assay (n=10)			Inter-assay (n=10)		
Mean (ng/mL)	284	432	1338	284	432	1338
CV (%)	3.7	2.4	1.4	3.8	3.3	2.6

– **Specificity:** The monoclonal antibody has specificity for the D-Dimer domain of cross-linked region of Fibrin degradation products. No cross reaction with fibrinogen and E fragments is observed. Cross-reactivity is observed with D fragment at concentration above $10 \mu\text{g/mL}$ in plasmas spiked with purified D fragment. (Note 8)

– **Interferences:** Bilirubin (40 mg/dL), hemoglobin (1600 mg/dL), lipemia (2000 mg/dL) and rheumatoid factor (90 IU/mL), do not interfere. Other substances may interfere. (Note 9)

– **Method comparison:** A correlation study with 50 plasmas whose D-Dimer levels was in a range from $40 - 2000 \text{ ng/mL}$ was performed with Linear D-Dimer test (y) to the HemosIL[®] D-Dimer test (x). Obtained results are the following: $r: 0.995$, slope: 0.96 , intercept: 40.3 .

NOTES

1. Due to lack of stability of D-Dimer analyte in the sample it is recommended to keep plasma samples at $2-8^{\circ}\text{C}$.
2. This method may be used with different instruments. It is recommended to use fixed time method although it should be validated to demonstrate that results meet the performance characteristics. Contact to the distributor for any question in the application method.
3. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
4. The linearity limit depends on the sample/reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
5. In automatic analyzers with auto rerun capability perform a 1:10 dilution in order to increase the test range to $33,000 \text{ ng/mL}$.
6. For automatic instruments, avoid the presence of bubbles in the reagents that may interfere with the assay results.
7. D-Dimer levels could be also expressed in ng FEU/mL . FEU means Fibrinogen Equivalent Unit. The equivalence between these two measurements is approximately $1 \text{ ng FEU/mL} \sim 0.5 \text{ ng D-Dimer/mL}$.
8. In physiological conditions the presence of α_2 -antiplasmin prevents the formation of Fragment D from Fibrinogen.
9. Although it has not been observed, the presence of Human Anti-Mouse Antibodies (HAMA) in certain samples may cause over-estimation of D-Dimer levels.
10. Since a D-Dimer International Standard is not currently available, an internal Standard has been prepared according to the criteria and recommendations proposed by Nieuwenhuizen W. and Meijer Piet for the harmonisation of D-Dimer test results.

BIBLIOGRAPHY

1. Nieuwenhuizen W. A reference for harmonisation of D-Dimer assays, Thrombosis and Haemostasis. 1997; 77 (5): 1031-1033.
2. Mojca Stegnar. et al. Determination of D-Dimer by different quantitative assays. Biochemia Medica. 2008; 18 (2): 216-23.
3. Meijer Piet, et al. A model for the harmonization of test results of different quantitative D-Dimer methods. Thrombosis and Haemostasis. 2006; 95: 567-72.
4. Wilson David B. Evaluation of and automated Latex enhanced turbidimetric D-Dimer test. Am J Clinical Pathology 2003; 120:930-937.
5. Legnani C. et al. Different cut-off values of quantitative D-Dimer methods. Haematologica. 2008; 96 (6).
6. Kelly J. et al. Plasma D-Dimers in the diagnosis of venous thromboembolism. Arch intern Med. 2002;162:747-756.
7. Righini M. et al. Clinical usefulness of D-Dimer depending on clinical probability and cutoff value. Arch intern Med. 2004;164:2483-2487.
8. Wells Philip S. Evaluation of D-Dimer in the diagnosis of suspected Deep-Vein thrombosis. New England J of Med. 2003;349:1227-1235.
9. Soheir S. Adam, et al. D-Dimer antigen: current concepts and future prospects. Blood J. 2009;113 (13).
10. Greenberg CS, et al. Measurement of plasma fibrin D-Dimer levels with the use of a monoclonal antibody coupled to latex beads. Am J Clin. path. 1987; 87:94-100.
11. Brown Michael D. et al. Turbidimetric D-Dimer test in the diagnosis of pulmonary embolism: a Metaanalysis. Clinical chemistry 2003;49:1846-1853.
12. Gaffney PJ, et al. Monoclonal antibodies to crosslinked fibrin degradation products. British J. of Haematology. 1988;68:83-90.
13. CLSI Document H21-A5: Collection, Transport, and processing of Blood Specimens for Testing Plasma-based Coagulation assays; Approved Guideline Flth Edition.
14. Francalanci I. et al. D-dimer concentrations during normal pregnancy, as measured by ELISA. Thromb. Res. 1995; 78(5): 399-405.

