D-Dimer Turbidimetric $\quad$ CE

REF 3145005 1 x 54 mL **CONTENTS** R1.Reagent 1 x 48 mL R2. Reagent 1 x 6 mL CAL. 1 x 1 mL	# D-Dimer Turbidimetric *Latex Turbidimetry*
For *in vitro* diagnostic use only	

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 15-25ºC for 20-30 minutes before use. Do not shake.

Calibration curve (5 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
NaCl 9 g/L (µL)	--	200	200	200
D-Dimer CAL (µL)	400	200	200	200
		Mix and transfer		
Factor	1.0	0.5	0.25	0.125

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

STORAGE AND STABILITY

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

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

MATERIAL REQUIRED

- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 405 nm filter.

PROCEDURE

Preliminary Procedure
Prewarm the reagent R1 and the photometer (cuvette holder) to 37ºC.

Analytic Procedure
1. Using distilled water zero the instrument at 405 nm.
2. Pipette into a cuvette:

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

3. 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 cutt-off level are obtained and in conjuction 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 2600 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 ≤41 ng/mL give non-reproducible results.

– **Prozone effect:** Concentration of D-dimer >44,000 ng/mL still gives positive result (Note 5).

– **Precision:**

	Intra-assay (n=10)			Inter-assay (n=10)		
Mean (ng/mL)	297	421	976	297	421	976
CV (%)	3.1	2.4	1.3	2.3	2.4	1.9

– **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 µg/mL in plasmas spiked with purified D fragment. (Note 8)

– **Interferences:** Bilirubin (40 mg/dL), hemoglobin (1700 mg/dL), lipemia (500 mg/dL) and rheumatoid factor (120 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 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 2-8ºC.
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 26,000 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 ng FEU/mL ~ 0.5 ng D-Dimer/mL.
8. In physiological conditions, the presence of α2-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.

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