D-DIMER TURBILATEX

Latex Turbidimetry

PRODUCT CODE
TL007

INTENDED USE
D-Dimer turbilatex Test is a quantitative turbidimetric test highly specific for circulating derivatives of cross- linked fibrin degradation products (XL-FDP) in human plasma.

CLINICAL SIGNIFICANCE
During blood coagulation, fibrinogen is converted to fibrin by the activation of thrombin. The resulting fibrin monomers polymerize to form a soluble gel of non-cross-linked fibrin. This fibrin gel is then converted to cross-linked fibrin by thrombin activated Factor XIII to form an insoluble fibrin clot. Production of plasmin, the major clot-lysing enzyme, is triggered when a fibrin clot is formed. Fibrinogen and fibrin are both cleaved by the fibrinolytic enzyme plasmin to yield degradation products, but only degradation products from cross-linked fibrin contain D-Dimer. Therefore, cross- linked fibrin degradation products (XL-FDP) are a specific marker of fibrinolysis.

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
REAGENT 1 (DILUENT) Borate buffer, 0.1 mol/L, pH 8.2
REAGENT 2 (LATEX) Latex particles coated with murine monoclonal antibody anti D-Dimer, pH 8.2.
CALIBRATOR: 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.

PREPARATION
Reagent 1 & 2 are ready to use.
Calibrator: Reconstitute with 1.0 mL of distilled water. Mix gently and incubate at room temperature for 20-30 minutes before use. DO NOT SHACKE.

CALIBRATION CURVE
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
All the kit components are ready to use, and 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 freeze: frozen reagents could change the functionality of the test.
Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.

Reagent's deterioration: Presence of particles and turbidity.

PRECAUTIONS
Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However, handle cautiously as potentially infectious.

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, 1 week at 2-8ºC and 2 months at -20ºC.

IVD CE

SPECIMEN AND SAMPLE PREPARATION
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.
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)
Fresh serum or plasma, Stable 7 days at 2-8°C or 3 months at –20°C. Samples with presence of fibrin should be centrifuged.
Do not use highly hemolyzed or lipemic samples.

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 (A1) and after 2 minutes (A2) of the first reading.

Calculation

Calculate the absorbance difference (A2-A1) 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 (A2-A1) in the calibration curve. (Note 2)

QUALITY CONTROLS

D-Dimer controls are recommended to monitor the performance of manual and automated assay procedures.
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.

Bio Research For Medical Diagnostics
Muslim Al Attar Street,P.O.Box:1235,
Amman-11953,Jordan
Tel:+962 64892525, Fax: +962 64892526,
www.bioresearch.com.jo

EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

D-Dimer levels increases in pregnancy and also rises with age.

PERFORMANCE CHARACTERISTICS

- ***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:***

Description	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 forthe D-Dimer domain of cross-linked region of Fibrindegradation 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.
- **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.

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.

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

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