BIOGENIX
INC. PVT. LTD.

D-DIMER ASSAY
(Turbidimetry Method)

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INTRODUCTION

BIPL D Dimer Turbidimetry is intended for In-vitro quantitative determination of D-Dimer in human plasma. D-dimer is a fibrin degradation product, a small protein fragment present in blood after fibrinolysis degrades a blood clot. D-dimer is normally undetectable in the blood and is synthesized only after a clot has formed and is in the process of being broken down. D-dimer levels rise when a significant formation and breakdown of blood clots occurs. D-dimer is useful in diagnosis of deep vein thrombosis (DVT), pulmonary embolism (PE), and disseminated intravascular coagulation (DIC).

PRINCIPLE

The Kit utilizes latex-enhanced immunoturbidimetry to measure the D-Dimer level in human plasma. During the test, D-Dimer in the sample binds with the specific anti-d-dimer antibody that is coated on latex particles to cause agglutination. The turbidity caused by agglutination is detected optically by chemistry analyzer. The change in absorbance is proportional to the level of d-dimer in the sample. The actual concentration is obtained by comparing with a calibration curve with known concentrations.

KIT CONTENTS

R1 - D-Dimer Buffer	1 x 30 ml
R2 - D-Dimer antibody	1 x 10 ml
D-Dimer Calibrator	1 vial
D-Dimer Calibrator Diluent	1x 1ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 10 days on board the analyser at 2-10°C. Protect from light and avoid contamination.

WORKING REAGENT PREPARATION AND STABILITY

Assay can be performed with use of separate R1-D-D and R2-D-D reagents of 3 parts of R1-D-D with 1 part of R2-D-D.
Avoid foaming.

CONCENTRATIONS IN THE TEST

R1 - Tris buffer solution. Sodium azide < 0.1%

R2 - Latex suspension, anti-d-dimer antibodies, buffer solution, sodium azide < 0.1%

WARNINGS AND NOTES

1. The Kit is for in vitro diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed

ADDITIONAL EQUIPMENT

- Automatic/semi automatic analyzer or photometer able to read at 700/578 nm
- Thermostat at 37ºC
- General laboratory equipment

SPECIMEN

Follow standard laboratory procedures to collected in sodium citrated plasma samples.

It is recommended to perform test immediately after sample collection. If the test cannot be done immediately, store sample at 2- 8° C for up to 1 day or at -80° C for up to 6 months. Avoid repeated freezing and thawing.

PLOTTING OF MULTIPOINT CURVE (FOR SEMI AND FULLY AUTOMATIC)

The D-Dimer Turbidimetry Assay is based on Non-Linear Reactions, hence it is strongly recommended to run Multi-standard mode to plot the Multi-point curve to have better accuracy and precise result.

SERIAL DILUTION STEP FOR FULLY AUTOMATIC ANALYSER

Dissolve the lyophilized D-D calibrator with 1ml distilled water, and dilute to 6 calibrators as follows,

Dilute	1	2	3	4	5	6
CAL (ul)	200	100	50	50	25	0
Normal Saline (ul)	0	100	150	350	375	200
ratio	1	1/2	1/4	1/8	1/16	0

PROCEDURE FOR FULLY AUTOMATIC ANALYSER

Wavelength **700nm**
Temperature **37°C**
Cuvette **1 cm**

	Blank Tube	Calibration Tube	Sample Tube
Sample	-	-	18ul
Calibrator	-	18ul	-
DI water	18ul	-	-
R1	225ul	225ul	225ul
Mix, Incubate at 37 C for 5 minutes.			
R2	75ul	75ul	75ul
Mix, incubate at 37 C for 5 minutes, zero setting for blank tube, read the absorbance $\Delta A_{calibrator}$ and ΔA_{sample}			

CALCULATION

The analyzer automatically calculate the analyte concentration of each sample.

EXPECTED VALUES

<0.5 µg/mL

The reference range should be determined by each hospital to confirm with the characteristics of the region being tested.

PERFORMANCE CHARACTERISTICS

The following performance data was obtained using analyzer at 37 C. Results obtained in individual laboratories may differ.

MEASURING RANGE

0.2µg/mL - 10.0µg/mL

Determine the samples with higher concentrations via the rerun function. In the event of a rerun the upper limit of the assay ranges is increased to approximately 20µg/mL.These values are dependent on the lot specific values of the calibrators in use.

REAGENT BLANK
≤1.600

ANALYTICAL SENSITIVITY
≥0.03 at 1µg/mL

The lower detection limit represents the lowest measurable total uric acid concentration that can be distinguished from zero. It is calculated as three standard deviations of 20 replicates of the lowest standard.

ACCURACY
≤±15%

PRECISION
≤10%

BATCH ERROR
R≤15%

SERIAL DILUTION STEP FOR SEMI-AUTOMATIC ANALYSER

	1ST	2ND	3RD	4TH	5TH
Calibrator	100 µl	50 µl from 1st Tube	50 µl from 2nd Tube	50 µl from 3rd Tube	50 µl from 4th Tube
Normal saline	0	50 µl	50 µl	50 µl	50 µl
Ratio of dilution	Neat	1/2	1/4	1/8	1/16

PROCEDURE FOR SEMI-AUTOMATIC ANALYSER

These reagents may be used both for manual assay Sample Start and in several semi-automatic analyzers. Applications for them are available on request.

Wavelength **578 nm**
Temperature **37°C**
Cuvette **1 cm**

REAGENT	CALIBRATOR (C)	TEST (T)
R1 D-DIMER BUFFER	750 µl	750 µl
CALIBRATOR	10 µl	
Sample	-	10 µl
Bring up to the temperature of determination. Then add		
R2 - D-Dimer Antibody	250 µl	250 µl

Mix well, after about 60 sec. (37°C) read the absorbance A1 of the test (T) and calibrator (C) against air or water.After exactly 180 secs. (for all temperature) read the absorbance A2 of the test (T) and calibrator (C). Calculate ΔA/min. (A2 - A1) for the test and calibrator.

CALCULATION
D-Dimer concentration = ΔA (T) / Δ A(C) x calibrator concentration

REFERENCE VALUES
upto 0.5 µg/mL

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL
To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls

PERFORMANCE CHARACTERISTICS

- **Linearity :** 0 – 20 mg/L (R≥0.990)
- **Precision :** Within Run: CV≤8%; Run-to-Run: Cv≤10%
- **Interference:** No interference detected for: ascorbic acid (50 mg/dL), Bilirubin (19.6 mg/dL), free bilirubin (18.4 mg/dL) Rhuematoid factor (500 IU/ml) and hemoglobin (460 mg/dL).

SYSTEM PARAMETERS FOR SEMI- AUTOMATIC ANALYSER

Method	Fixed Time (2-Point)
Wavelength	578 nm
Zero Setting	Distilled Water
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	----
Delay Time	60 secs
Read Time	180 secs
No. of Reading	2
Interval Time	----
Sample Volume	0.01 ml (10 ul)
Reagent Volume (3R1 + 1R2)	1.0 ml (1000 ul)
Standard Concentration	Refer Calibrator Vial
Units	µg/mL
Factor	----
Reaction Slope	Increasing
Linearity	20 µg/mL

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

1. Adam S.S., Key N.S.,Greenbery C.S. D-dimer antigen: current concepts and future prospects. Blood 113 (13): 2878-87.
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