

BE DDimer Turbidimetric Immunoassay

Reagent for quantitative determination of D-Dimer (DD) in human plasma

INTENDED USE

This reagent is designated for professional use in laboratory (automated method). This Latex immunoassay is a quantitative test is to determine D-Dimer in citrated human plasma. It can be used to exclude the presence of thrombosis in patients with suspected thrombotic disorders and as an aid in the management of patients with Covid-19 disease. This test should be used with other clinical and diagnostic information to diagnose and manage patients.

PRINCIPLE

BE DDimer reagent consists in sub-micron sized polystyrene particles coupled to monoclonal antibodies specific for D-Dimer. When plasma specimen containing D-Dimer is exposed to the reagent, the particles will agglutinate, giving rise to increased light-scattering. This phenomenon leads to an increase of absorbance measured at 400-800 nm which is proportional to the concentration of D-Dimer in the specimen.

GENERALITIES (1)-(4) (10) (11)

Fibrin fragments containing D-Dimer antigen is always present in plasma as a result of plasmin degradation. After an injury or in case of conditions associated with increased haemostatic activity, the D-Dimer concentration increases in plasma. The determination of D-Dimer concentration helps in the diagnosis of thrombosis. Deep vein thrombosis (DVT), pulmonary embolism (PE) and disseminated intravascular coagulation (DIC) are associated with elevated level of D-Dimer. A negative D-Dimer test result has a high negative predictive value for patient with a suspected thrombotic disorder.

In patients with Covid-19 disease, increasing plasma D-dimer is seen with worsening disease. Markedly elevated D-dimer is a prognostic marker for mortality and can be used as an aid in managing anticoagulant treatment of hospitalized Covid-19 patients.

REAGENTS

BU	DD BU	Reaction Buffer
		Buffer, Preservatives < 0.1%
AC	DD AC	Latex Reagent
		Polystyrene particles coated with monoclonal antibodies
		Buffer, Preservatives < 0.1%
CAL	DD CAL	DDimer Calibrator
		Lyophilised plasma enriched with D-Dimer



DIL **DD DIL** Dilution Buffer
For dilution of D-Dimer Calibrator and to dilute plasma of patients.
According to 1272/2008 regulation, these reagents are not classified as dangerous.

SAFETY CAUTIONS

Behnk reagent kits are designated for professional in vitro diagnostic use. Good Laboratory Practices must be applied during use of reagents, reference or control plasmas, and human samples should be handled as potentially infectious. For further information, Material Safety datasheet is available upon request. Dispose of waste in accordance with the local regulations. I Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REPARATION OF REAGENTS

CAL: Add 1ml of demineralised water (15-25°C). Close the vial and let stand for 15min at room temperature. Mix gently by swirling before use. Other reagents are ready to use.

STABILITY AND STORAGE

Unopened vials stored at 2-8 °C are stable until the expiry date stated on the label.
• Once opened, when free from contamination, reagents BU, AC and DIL are stable for 2 weeks at 20-25 °C and 8 weeks at 2-8 °C.
• CAL: transfer the necessary quantity, well recapped and stored in the original vial. Once reconstituted and without contamination, standard (CAL) is stable for 24 hours at 20-25 °C, 1 week at 2-8 °C

SAMPLES COLLECTION AND HANDLING (5)

Plasma from careful venipuncture with anticoagulant ratio of 1/10 (trisodium citrate solution 0.109 M). The ratio is essential. Mix immediately the blood with anticoagulant. Avoid drawing with a syringe that could result in the formation of micro-clots. Centrifuge 10 minutes at 2500 g.

LIMITS (6)

I Turbid or opalescent plasma may cause erratic results and should be interpreted with caution: dilute the sample and re-assay. Patients who have received mouse monoclonal antibodies for diagnosis or therapy may have plasmas containing anti-mouse antibodies (HAMA). Such antibodies may lead to false enhance D-Dimer concentration. The same may occur with Rheumatoid Factor. For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S

MATERIAL REQUIRED BUT NOT PROVIDED

- Basic medical analysis laboratory equipment
- Coagulation analyzer useful with turbidimetric detection (400-800 nm wavelength)
- Demineralised water

REFERENCE RANGE (6) (7) (12) (13)

- Plasma: < 200 ng/mL (DDU)
 - D-Dimer concentrations increase during pregnancy and with age.
- Each laboratory should establish its own normal ranges for the population that it serves.

REF 771500:

BU (3 x7 mL) AC (3 x 4 mL), CAL (2 x 1 mL) DIL (2x 7 mL)

CALIBRATION (9)

REF 771500: DD CAL DDimer Calibrator

This Calibrator is traceable to an In-House Reference Preparation which underwent a one-time value assignment to align with another commercially available assay which reports results in ng/mL (DDU).

Concentration of this Calibrator is batch-specific. (See **Assigned value** on the label of the vial) Follow the D-Dimer calibration procedure of the analyzer.

QUALITY CONTROL

REF 773200 BE DDTrol 1 and REF 773201 BE DDTrol 2

Controls are required for checking the accuracy and reproducibility of the results.

The control intervals should be adapted to each laboratory's individual requirements.

Values obtained should fall within the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

PROCEDURE

Automated method on Behnk Thrombolyzer series

Refer to the full detailed application specific to the automated system

- Performances and stability data have been validated on Thrombolyzer Compact X (available on request).
- Procedure on other automated coagulation analyzer, performances and stability data must be validated by user.

CALCULATION (6)

Results are expressed in DDU directly given by the analyser.

To convert results in Fibrinogen eq. units (FEU), multiply the result (DDU) by 2.5.

PERFORMANCES

The within run and between run studies were performed with two levels (middle and high) of plasma on Thrombolyzer Compact X:

Within Run N=40	Level 1	Level 2
Mean (ng/mL)	595	1203
S.D.:	11,2	33,3
C.V. % :	1,9	2,8

Run to run N=40	Level 1	Level 2
Mean (ng/mL)	595	1203
S.D.:	6,4	25,9
C.V. % :	1,1	2,2

Detection limit: approx. 98 ng/mL

Prozone effect: tested up to 97468 ng/mL, no effect found within the measuring range (approx. up to 6363 ng/mL)

Measuring range: between 100 (QL) and 3200 ng/mL

I Cut off: 200 ng/mL

Comparison with a commercially available reagent (same method) on Thrombolyzer Compact X and Sysmex CA-1500; using 50 specimens between 114 and 3095 ng/mL:

$$y = 0.95x \quad r = 0.9742$$

INTERFERENCES:

Lipids	No interference up to 200 mg/dL
Bilirubin	No interference up to 855 µmol/L
Low molecular weight Heparin	No interference up to 100 U/mL
Non-fractionated Heparin	No interference up to 100 U/mL

Other substances may interfere with the results (see § Limits)

Calibration Stability:

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

REFERENCES

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| = Significant modifications

Manufacturer	Use by	In Vitro Diagnostic	Temperature limitation	Catalogue number	See insert	Batch number	Store away from light	Sufficient for	Dilute with	Demineralized water	Biological hazard