FIBRINOGEN
Immunoturbidimetric

INTENDED USE
Plasma fibrinogen assay kit is an in-vitro immunoturbidimetric test for quantifying the amount of native, intact, immunoreactive fibrinogen present in human blood plasma.

Assessment of plasma fibrinogen will help in the evaluation and treatment of patients with thrombotic disease. The Immunoturbidimetric Assay Kit can be performed manually, with spectrophotometers, available in clinical laboratories.

Cat. No. FIB300
250 Tests

R1: PEG in guanidine 1 x 50 mL
R2: Antihuman fibrinogen 1 x 36 mL
Calibrator: human fibrinogen 1 x 1 mL
Control Level 1: human fibrinogen 1 x 1 mL
Control Level 2: human fibrinogen 1 x 1 mL

SUMMARY AND EXPLANATION OF THE TEST
Fibrinogen, also known as clotting factor I is a soluble plasma glycoprotein that participates in the final phase of the blood coagulation cascade. The molecule consists of two sets of alpha, beta, and gamma polypeptide chains with molecular weights of 66,000, 52,000, and 46,500, respectively. The six chains, (alpha, beta, gamma)2 are linked by 29 disulfide bonds to form a trinodular structure as seen in the electron microscope. During the coagulation process, the procoagulant enzyme thrombin removes the aminoterminal fibrinopeptides A and B by minor proteolysis, converting fibrinogen to fibrin monomers. The fibrin monomers then polymerize to form an insoluble fibrin clot. It is noteworthy that fibrinogen is stable in EDTA-plasma, because EDTA inhibits the thrombin-catalyzed conversion of fibrinogen to fibrin.

cDNA cloning has shown that the alpha, beta, and gamma chains of fibrinogen are encoded by three independent genes and are synthesized in liver hepatic parenchymal cells. The individual chains are processed, glycosylated, assembled, and eventually secreted into the circulating blood as mature fibrinogen molecules. The "acute phase" alterations in the composition of blood have been known for many years. Today, we understand that the increase in the erythrocyte sedimentation rate during acute illness reflects, in large part, a change in plasma protein composition, particularly an increase in the fibrinogen level. Fibrinogen is an acute-phase liver protein whose synthesis is positively regulated during inflammation. The main mediators of this phenomenon are glucocorticoids and interleukin-6 (IL-6), a pleiotropic cytokine that also controls hematopoiesis.

PRINCIPLE OF PROCEDURE
The Immunoturbidimetric method for fibrinogen is based on the standard well known concept of light scattering properties of antigen-antibody aggregates. In turbidimetry, the transmitted light is measured at the same wavelength and direction as the incident light while in nephelometry the scattered light leaving the solution is measured at some angle other than the incident beam. Thus, the light remaining after the scatter is measured in turbidimetry. This concept has been used commonly to quantify plasma/serum proteins with the aid of enhancers such as polyethylene glycol (PEG). Improvements in optical instrumentation and detection systems have now made homogeneous immunoturbidimetric methods of analysis as sensitive as their immunonephelometric counterparts.

The plasma specimen (calibrator, control or patient) is first allowed to incubate at 37°C with Reagent I (a buffered solution of PEG and other immunocomplex enhancers) to obtain a reference absorbance blank at 340 nm - 700 nm with a bichromatic analyzer, which minimizes the interference(s) due to bilirubin, hemoglobin, lipoproteins, or chylomicrons. Addition of Reagent II (anti-human fibrinogen in buffer) results in the formation of an insoluble fibrinogen immunocomplex whose absorbance is measured at 340 nm after three minutes. The blank absorbance reading is subtracted from the test absorbance and compared to a calibrator to obtain the fibrinogen concentration in the plasma specimen.

MATERIALS PROVIDED:
1. 50mL of Reagent I: Contains polyethylene glycol in 7mM guanidine buffer with stabilizers and preservatives.
2. 36 mL of Reagent II: Contains antihuman fibrinogen in 20mM guanidine buffer with dye, stabilizers and preservatives.
3. 1 mL of Calibrator: Contains human fibrinogen
4. 1 mL each controls Level 1 and 2: Contains human fibrinogen

MATERIALS REQUIRED BUT NOT PROVIDED:
1. Pipettes; variable volume, 2-20 ul, 100 - 1000 ul.
2. Manual spectrophotometers, capable of bichromatic measurements in the region of 340 nm at 37° C. or automated chemistry analyzer.
3. Timer.
4. Deionized Water

PRECAUTIONS
1. For In-Vitro Diagnostic Use.
2. If either Reagent contains visible particulates, do not use the Reagents.
3. Do not mix reagents from different lots.
4. The reconstituted calibrators and controls should not have undissoved or precipitated material. A slightly cloudy solution is normal.
5. Because no test method can offer complete assurance that HIV, HBsAg, or other infectious agents...
are absent, it is recommended that human blood based products be handled with the same precautions used for any potentially biohazardous patient specimens. Each donor unit used in preparation of Fibrinogen controls level 1 & 2 has been tested and found to be non-reactive for antibodies to Human Immunodeficiency Virus (HIV), Hepatitis B Surface Antigen (HBsAg) and Hepatitis C Virus (HCV).

6. Fibrinogen calibrator is intended solely for in vitro diagnostic use for the purpose described on the labeling. MEDTEST DX shall not be liable for any claimed damages arising from other usage.

REAGENT STORAGE AND STABILITY
Unopened kit components are stable through the expiration date on the label when stored at the suggested temperature on the label. Opened bottles of Reagents I and II are stable for four weeks at 2 - 8° C.

Upon receipt, store at 2 - 8° C. Unopened, this product is stable until the expiration on the vial label. Once the calibrator and controls are open and reconstituted, they are stable for at least 48 hours at 2 - 8° C and 8 hours at room temperature. Vials should be kept tightly closed after use to avoid evaporation. DO NOT FREEZE. DO NOT EXPOSE TO EXCESSIVE HEAT.

SPECIMEN COLLECTION AND STABILITY
Non hemolyzed EDTA plasma is the specimen of choice for the fibrinogen assay. Sufficient blood should be drawn to yield 100 ul of non-hemolyzed plasma. Blood should be collected as recommended by the Diagnostic guidelines (National Committee for Clinical Laboratory Standards). Procedures for the collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard, 2nd. ed. Villanova, Pa. NCCLS; 1984. NCCLS Publication H3-A2C)

INTERFERENCES
The following showed no detectable interferences.
- Bilirubin up to 3 mg/dl
- Hemoglobin up to 300 mg/dl
- Triglyceride up to 1500 mg/dl

PROCEDURE (Manual):
- Read the entire procedure before beginning the test.
- Allow all reagents, controls, calibrators and specimens to come to room temperature before beginning the test. The reagents should be used as supplied.
- Preparation of specimen: Use plasma specimen without any diluent.

Manual Procedure:
Set the wavelength of the spectrophotometer to 340 nm, the dichromatic correction wavelength to 700 nm and the temperature of the cuvette holder to 37° C. With DI water both in the reference cell and test cuvette, set the instrument reading to zero (0). Pipet 555 ul of reagent 1 into the test cuvette followed by 21 ul of calibrator or control or patient sample. Cover the cuvette opening (e.g. parafilm), mix gently and place the cuvette in the holder at 37° C. Immediately start the timer set for 2 minutes. At the end of 2 minutes, read and record the absorbance (Blank) at 340 nm.

To the same test cuvette, add 390 ul of Reagent II, mix by gentle inversion as above and replace the cuvette in the holder at 37° C. Start the timer set for 3 minutes. At the end of 3 minutes, read and record the absorbance at 340 nm (Test).

CALIBRATION
Use a fibrinogen calibrator provided with the kit. The fibrinogen value of each lot is provided with the flyer enclosed. Reconstitute the contents in 1 mL of distilled or de-ionized water at room temperature by swirling motion until the material is completely dissolved. Any undissolved or precipitated material should not be used. A slightly cloudy solution is normal.

CALCULATIONS
The results are calculated as follows for the manual procedure:

- The calibrator factor is calculated as follows:
  - Factor = Conc of Fibrinogen in the calibrator / (Abs of Calib. Test - Abs of Calib. Blank)
  - Example: Fibrinogen level in the calibrator = 245 mg/dl
    - Abs of calibrator blank = 0.033
    - Abs of calibrator test = 0.285
    - Abs of Test - Abs of Blank = 0.285 - 0.033 = 0.252
    - Factor = 245 mg/dl ÷ 0.252 = 972.22
  - Fibrinogen conc in unknown sample = (Abs of sample test - Abs of sample blank) x Factor.
  - Example 1. Abs of sample test - Abs of sample blank = 0.658
    - Fibrinogen concentration of sample #1 = 0.658 x 972.22 = 639.7 mg/dl
  - Example 2. Abs of sample test - Abs of sample blank = 0.315
    - Fibrinogen concentration of sample #2 = 0.315 x 972.22 = 306.2 mg/dl

QUALITY CONTROL
It is recommended that two levels of Fibrinogen Controls be included in duplicate as per the practice followed in the laboratory. Reconstitute the contents in 1 mL of distilled or de-ionized water at room temperature by swirling motion until the material is completely dissolved. Any undissolved or precipitated material should not be used. A slightly cloudy solution is normal. For exact fibrinogen target value and range, see flyer for respective Controls. It is recommended that a daily record of the Control results be plotted to observe any trends or results outside the expected range.

LIMITATIONS
- Extraneous light scatter must be minimized for optimal performance.
- Reagents I and II must be clear. If any visible particulate matter is seen, do not use the reagents.
- Samples that are grossly hemolyzed (plasma
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hemoglobin >300 mg/dl) and very lipemic (triglyceride >1500 mg/dl) should not be used. Do not use serum samples.

- Plasma, calibrator and controls must be clear. Filter through 0.2 micron membrane, if necessary.
- If the fibrinogen concentration exceeds the measuring range as stated in the application sheet, dilute the specimen and reassay. For dilution, use a plasma with a known low fibrinogen value.

EXPECTED VALUES

It is recommended that each laboratory establish its own reference range and use the expected range given in this package insert only as a guideline.

Based on 317 known normal healthy male and female patient samples, the fibrinogen values ranged from 195 to 370 mg/dl with a mean of 298 mg/dl. Immunoturbidimetric fibrinogen is a simple and useful test to evaluate coagulation disorders.

PERFORMANCE CHARACTERISTICS

All data was obtained using the manual assay method on a Hitachi model U-2000 spectrophotometer.

Sensitivity:
The sensitivity of the fibrinogen assay was determined by the lower limit of the reference curve which was -61 mg/dl. If no fibrinogen is detected, it is recommended that a fresh plasma sample be obtained and tested. If no fibrinogen is again detected, result is "no fibrinogen detected".

Precision:
The precision of the immunoturbidimetric test was determined following the "Tentative Guideline for User Evaluation of Precision Performance of Clinical Chemistry Devices," National Committee for Laboratory Standards, Vol. 4, No. 8, June 1984 (order code EP5-T). Briefly, the recommended precision determination involves the assay in duplicate of two specimens (controls) once a day for twenty days. The statistical treatment described yields a measure of: 1. Within run precision, and 2. Total precision. (Two normal human plasma pools with target values of 249mg/dl and 268mg/dl respectively were used to obtain the precision results.)

Within run precision:
Mean = 247 mg/dl 1 ± SD = 3.347
Mean = 269.8 mg/dl 1 ± SD = 4.153

Total Precision:
Mean = 247 mg/dl 1 ± SD = 5.824
Mean = 269.8 mg/dl 1 ± SD = 8.299

Linearity
This method is linear from 50 to 1000 mg/dL.

Method Comparison
Comparison was made on 167 specimens between immunoturbidimetric fibrinogen test and the commercially available Behring Diagnostic Fibrinogen kit. The overall correlation (r) between the two methods was 0.96, n=167, Intercept = 25.4.

REFERENCES


Manufactured for

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Rev. 02/16 M803-FIB300-01