SPECTRUM

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FIBRINOGEN-LS

Clauss Clotting Time Method

REF: 631 001
Thrombin reagent (2 x 1 ml) – Buffer (5 ml) 20 test

REF: 631 002
Thrombin reagent (6 x 1 ml) – Buffer (15 ml) 60 test

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in-vitro diagnostic use		Use by/Expiration Date
LOT	Batch Code/Lot number		CAUTION. Consult instructions for use
REF	Catalogue Number		
	Consult instructions for use		Manufactured by

Intended Use

Reagent for quantitative determination of plasma Fibrinogen using Clauss Clotting Time method on both manual and automated coagulation analysers.

Background

The enzyme ,Thrombin, is the penultimate protein in the clotting sequence, acting upon soluble Fibrinogen and converting it to insoluble fibrin. Normal plasma Fibrinogen levels range from 200-400 mg/dl, however levels as low as 10-20 mg/dl may occur in acquired or congenital hypo-fibrinogenemia. Determination of plasma Fibrinogen levels has proven to be a useful test in the diagnosis of hemorrhagic disorders relating to plasma Fibrinogen content. These include hyper-fibrinogenomia , hypo-fibrinogenomia , dys-fibrinogenomia and afibrinogenomia.

Principle

Bovine Thrombin utilizes the Clauss clotting time method for the determination of plasma fibrinogen levels, wherein excess Bovine Thrombin is used to clot diluted plasma. First, a standard curve is prepared using reference plasma of known fibrinogen content at dilutions of 1/5, 1/10 and 1/20. When Thrombin is added, the clotting time obtained is inversely proportional to the Fibrinogen content. Next, patient plasma, at a dilution of 1/10, is clotted with Thrombin and the resultant clotting time is used to interpolate Fibrinogen level from the standard curve.

Reagent

***Thrombin reagent*:** Bovine Thrombin 100 NIH units/ml liquid ready to use.

***Buffer* :** Owren's buffer**,** ready to use (pH 7.35).

Fibrinogen calibrator : lyophilized preparation of human plasma , concentration stated on the vial label .

Storage and Stability

Bovine Thrombin : Open vial is stable up to 25 days when stored at 2-8°C.

Don't freeze. Warm to room temperature prior to use. Avoid prolonged heating.

Buffer: Open vial is stable up to 30 days when stored at 2-8°C .

Calibrator : reconstitute individual vials with the volume of distilled water stated on the vial label and mix for 2 minutes.

Reconstituted calibrator is stable for 5 hours at room temperature **,** 2 days at 2-8°C and 1 week at -20°C.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only.
2. Reagents contain 0.1% sodium azide as preservative.
3. FIBRINOGEN thrombin reagent is not from a human source hence contamination due to HBsAg, HIV and HCV is practically excluded.
4. Fibrinogen calibrator provided is from a human source, which was tested and found to be non-reactive for HBsAg, HCV and HIV. However no known test methods can assure that infectious agents are absent. Handle all human products as potentially infectious.
5. Avoid exposure of the reagent to elevated temperatures, direct light and contamination. Immediately replace the cap after use and store at recommended temperature.

Sample Collection and Preparation

No special preparation of the patient is required prior to sample collection by approved techniques. Withdraw blood without undue venous stasis and without frothing

Mix nine parts of freshly collected blood with one part of sodium citrate (0.109 mol/l, 3.2%). Centrifuge immediately for 15 minutes at 3000 rpm (approximately 2000 g) and transfer the plasma into a clean test tube. **Plasma must be tested within 3 hours of collection.**

Additional Material Required

10 x 75 mm glass test tubes, 0.2 ml and 0.1 ml precision pipettes, water bath at 37°C , automated / semi-automated instrument .

Test Procedure

1- Pre-warm the instrument to stable state.

2- Directly use the reagent.

3- Prepare the ref. plasma and use according to the insert.

4- Prepare the FIB calibration curve:

a) Dilute the plasma as the table below (e.g. FIB ref. concentration is 300 mg/dl)

Proportion	Standard plasma	Buffer	FIB conc. (mg/dl)
1:5	50 µl	200 µl	60 mg/dl
1:10	25 µl	225 µl	30 mg/dl
1:20	25 µl	475 µl	15 mg/dl

b) Every plasma should be tested in duplicates to get the mean value.

c) Enter the calibration data to the instrument and construct the curve.

5- Determination of Fibrinogen concentration:

a) Dilute patient's plasma at 1/10 dilution (e.g. 180 µl buffer + 20µl plasma).

b) Add 100 µl of thrombin reagent to the diluted plasma and record clotting time in seconds .

c) Get the Fibrinogen concentration by the mean value of the clot time from calibration curve.

Quality Control

A known normal control should be run in parallel with each batch of tests. This control may be plasma coagulation control (PLASMATROL) or freshly drawn normal plasma.

Performance Characteristics

Users should establish product performance characteristics for the specific instrument used.

The intra-batch precision CV% ≤ 10%

The duplicate precision CV% ≤ 5%

Expected Values

150 mg/dl - 400 mg/dl

Remarks

1. Significant levels of heparin and elevated levels of fibrinogen degradation products (FDP) in the patient plasma can cause falsely low fibrinogen results.

2. Insufficient pre-warming of plasma and reagent or contaminated glassware may cause erroneous results.

3. EDTA should not be used as an anticoagulant.

4. Use reagents of the same lot for performing the test.

5. Do not interchange reagents from different lots.

Bibliography

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