FIBRINOGEN

Method based on Thrombin clotting time

CE

REF. 1004 5x2 ml (without Calibrator and Controls) IVD
REF. 1005 10x2 ml (without Calibrator and Controls)

DNV CERTIFIED COMPANY
UNI EN ISO 9001:2008
UN EN ISO 13485:2012

GIESSE DIAGNOSTICS

PRINCIPLE

The thrombin clotting time fibrinogen assay is based on the method originally described by Clauss. In the presence of high concentrations of thrombin, the time required for clot formation in dilute plasma is inversely proportional to the fibrinogen concentration.

SAMPLE

Plasma in trisodium citrate 3.2 % (0.109 M).
Avoid hemolysis and contamination by tissue fluids. Centrifuge blood for 15 minutes at 1500 x g. Test within 2 hours if samples are held at 22-24°C.

KIT COMPONENTS

BovineThrombin 200 (5/10 vials)	Lyophilized buffered Bovine Thrombin
Imidazole Buffer (1x100 ml)	Imidazole buffer in saline Sodium azide 0.2 g/l

Optional:

Fibrinogen Calibrator REF. 1006 (1x1 ml)	Human plasma collected with sodium citrate anticoagulant
Control Plasma N REF. 1007 (1x1 ml)	Human plasma collected with sodium citrate anticoagulant < 0.4 %
Control Plasma P REF. 1008 (1x1 ml)	Human plasma collected with sodium citrate anticoagulant < 0.4 %

The reagents are stable until the expiration date indicated on the label if stored tightly closed at 2-8°C .
Erratic values, product color variations, or lack of vacuum in the viala of Bovine Thrombin, Calibrator or Controls could indicate product deterioration. However, poor control performance could also be due to other factors within the test system.

REAGENT PREPARATION

Reconstitute the contents of one vial of **Bovine Thrombin** with 2.0 ml of distilled water. Agitate gently until solution is complete.
The reconstituted material is stable 7 days at 2-8°C, 8 hours at 15-30°C or may be frozen within 4 hours for use within 30 days. Thaw rapidly at 37°C. Do not refreeze.

Using photo-optical or mechanical instruments it is advisable to reconstitute the Bovine Thrombin, with distilled water instead, with 2.0 ml of Kaolin (available separately – REF. 1010).

Reconstitute the **Fibrinogen Calibrator,** the **Control Plasma N** and the **Control Plasma P** with 1.0 ml of distilled water. Swirl gently and let stand undisturbed for 15 minutes at room temperature. Do not invert vial or mix vigorously. Afer proper reconstitution, calibrator and controls are stable for 2 respectively and for 8 hours at 2-8°C .

All reagents must been at room temperature before use.

PRECAUTIONS AND WARNINGS

Each unit of source material used in the preparation of this product has been tested by an FDA licensed method and found non-reactive for HbsAg and negative for antibodies to HIV and HCV. However, no known test method can offer complete assurance that products derived from human blood will not transmit hepatitis, AIDS or other infectious diseases. This product, like all materials of human origin, should be handled as potentially infectious biological material.
Dispose of waste according to local laws.

TEST PROCEDURE

Diluite samples and controls 1:10 with Imidazole buffer (50 µl + 450 µl).
Pipette into plastic or siliconized glass tubes:

Prediluted samples	200 µl

Incubate 4 – 6 minutes at 37°C. Add:

Bovine Thrombin	100 µl

Time clot formation. Do not prewarm thrombin.

The frequency of curve preparation is partially determined by the method of clot detection used. Always prepare a new curve with each change in reagent lots, instrumentation, or when controls no longer fall within established ranges

RESULTS INTERPRETATION

Prepare several dilutions of Fibrinogen Calibrator in Imidazole Buffer and test as described in the procedure.
Calculate the mean of duplicate clotting times and construct a log-log curve that plots fibrinogen concentration vs. clotting time.

Plasma diluted 1:10 represents 100 % of the assigned value. The dilution factor indicates the relatioship between the 1:10 dilution and other dilutions.

Example: Fibrinogen Calibrator = **304 mg/dl** (each laboratory must prepare curves with their reagents and instrumentation).

Dilution	Dilution factor	Fibrinogen (mg/dl)	Mean CT (sec)
1:3.5	10/3.5 = **2.9**	304x2.9 = **882**	5.8
1:5	10/5 = **2**	304x2= **608**	7.3
1:10	10/10 = **1**	304x1 = **304**	13.4
1:15	10/15 = **0.67**	304x0.67 = **204**	20.8
1:35	10/3.5 = **0.29**	304x0.29 = **88**	49.2

Find the clotting time of controls and patient samples on the curve and read the corresponding fibrinogen value.

LIMITATIONS

1. Blood must be immediately added to trisodium citrate anticoagulant and gently mixed. **EDTA ed Heparin are unsuitable anticoagulants**.
2. Hemolysis can cause clotting factor activation and end point detection interference. Icteric and lipemic specimens may also be inappropriate for end point detection methods.
3. The sample should only contact nonwettable surfaces.
4. The ratio of blood to anticoagulant is usually 9:1 and results in a citrate concentration of 10.9 to 12.9 mmol/l. This concentration must be adjusted for patients with hematocrits above 55 % .
5. Freezing and thawing of plasma that contains residual cells will generate damaged cell membranes that can affect results.
6. Acute inflammatory reactions can elevate circulating Factor I (Fibrinogen).
7. High Fibrinogen Degradation Products (FDP) may prolong clotting times, especially when the fibrinogen level is below 150 mg/dl.
8. In patients with qualitative fibrinogen abnormalities, the thrombin clotting time assay may indicate decreased fibrinogen. The quantitative fibrinogen results may be normal on these same samples if tested by other methods.

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9. Heparin does not interfere at therapeutic levels. However, very high heparin levels may cause low fibrinogen results. Batroxobin enzyme can be substitued for thrombin in this assay if heparin interference is suspected.
10. High paraprotein levels, thrombin antibodies, and drugss that activate the fibrinolytic system can interfere with fibrinogen assays.
11. The kit Fibrinogen and individual components are designed to work at 37°C. Ensure that all heating elements are functioning properly.

EXPECTED VALUES

Generally the normal reference interval is:

130 – 350 mg/dl (1.5 – 3.5 g/l)

These values should only be used as a guideline. Each laboratory should estabilish own Reference Range.

PERFORMANCE

Precision: a low, a normal, and a high fibrinogen plasma were tested on multiple days using reagents on a photo-optical instrument. Ten standard curves were determined on each test day, for a total of 30 standard curves. The percent CV was determined to be 5.9 % (low), 3.4 % (normal) and 2.9 % (high).

Accuratezza: A low, a normal, and a high fibrinogen plasma were tested on multiple days using our reagents. These results were compared to results obtained using other manufacturer's reagents in multiple laboratories.

Sample	Giesse Reagent	n =	All reagents	n =
Low	144 mg/dl	10	163 mg/dl	195
Normal	294 mg/dl	10	297 mg/dl	195
High	488 mg/dl	16	474 mg/dl	300

REFERENCES

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