Dutch Diagnostics

Human and animal care

Fibrinogen

Reagent for the determination of fibrinogen levels in plasma

SUMMARY

Fibrinogen is a glycoprotein present in plasma and α platelet granules. It is the coagulation factor found in highest concentration of plasma (200-500 mg/dl).

In the presence of a trauma or vascular injury, the thrombin cleaves fibrinogen to form fibrin monomers. These monomers spontaneously form polymers and are stabilized producing the insoluble fibrin.

A decrease in fibrinogen level is found in cases of hereditary disorders such as hypofibrinogenemia, afibrinogenemia, dysfibrinogenemia, and also in other circumstances such as hepatic disease, extended intravascular clotting, fibrinolytic syndromes, etc.

An increase in fibrinogen level is found in cases of diabetes, inflammatory syndromes, etc.

In addition, high fibrinogen levels are now recognized as a risk factor for the development of cardiovascular disease.

PRINCIPLE

This assay is based on the Clauss method, designed as a reference procedure by the NCCLS (National Committee for Clinical Laboratory Standards).

In the presence of high concentrations of thrombin, the time required for clot formation in dilute plasma is inversely proportional to the plasma fibrinogen concentration. The resultant clotting time is compared to a standard fibrinogen preparation.

PROVIDED REAGENTS

Thrombin: vials containing lyophilized thrombin. Once reconstituted the concentration is approximately 100 NIH units of thrombin/ml.

Imidazole Buffer: 0.05 M, pH 7.3 imidazole solution

Reference Plasma: vial containing lyophilized plasma. See assigned Fibrinogen value in label.

NON-PROVIDED REAGENTS

Bidistilled or deionized water.

INSTRUCTIONS FOR USE

Thrombin and Reference Plasma: remove the aluminum seal and open the vial withdrawing the rubber stopper to avoid loss of material. Add the bidistilled or deionized water indicated in the label. Cap, allow the material to stand for 30 minutes and then swirl gently (without agitating) until dilution is complete before use. Date.

It is recommended to maintain the Thrombin reagent in its original vial after reconstitution and during use.

Imidazole Buffer: ready to use. Avoid contamination. Keep in its original vial properly capped.

WARNINGS

The provided reagents are for "in vitro" diagnostic use.

The Reference Plasma has been prepared from human plasma, which has been tested by an FDA approved method and found non-reactive for HBsAg and negative for antibodies to HIV and HCV. However, no known test method can offer complete assurance of the absence of infectious agents. The Reference Plasma, Controls and patient samples should be handled as potentially infectious biological material.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable in refrigerator (2-10°C) until expiration date indicated on the box.

Reconstituted Thrombin: stable 5 days refrigerated (2-10°C) or 30 days frozen (-20°C). Thaw rapidly at 37°C. Do not refreeze. Allow the reagent to stand at room temperature before using again. Avoid extended warming.

Reconstituted Reference Plasma: stable for 8 hours refrigerated (2-10°C).

SAMPLE

Plasma

a) Collection: obtain blood specimens carefully avoiding stasis or foaming, mix gently in a tube with anticoagulant in 9 + 1 proportion (example: 4.5 ml blood + 0.5 ml anticoagulant). Centrifuge and remove plasma before 30 minutes. Perform the extraction with plastic syringes.

b) Additives: sodium citrate 3.8% or 3.2% could be used to obtain plasma. Do not use EDTA or heparin.

c) Known interference substances:

- Icteric, lipemic or hemolyzed specimens may generate erroneous results.
- High levels of fibrinogen or fibrin degradation products may extend the coagulation period, especially when the fibrinogen levels are lower than 150 mg/dl.
- Therapeutic heparin levels do not interfere with the assay, however high levels may cause falsely low fibrinogen results.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: specimen should be stored in plastic tubes until testing to reduce the contact activation effect that can occur with glass tubes. Plasma should be stored in refrigerator (2-10°C) until testing. This period should not be extended more than 4 hours. In case this process could not be performed, plasma should be frozen at -20°C. This pro-

cess should be performed rapidly, alike the thawing (immersing in a 37°C bath) prior to testing.

REQUIRED MATERIAL

1- Provided

- Double logarithm paper sheet

2- Non-provided

- Hemolysis tubes
- Plastic tubes for preparing solutions
- Pipettes and micropipettes to measure indicated volumes
- Water bath at 37°C
- Stopwatch
- Light source for clot observation

PROCEDURE

I- CALIBRATION CURVE

1- Prepare dilutions of fibrinogen Reference Plasma 1:5, 1:10, 1:15, 1:20, 1:30, using 0.1 ml of reconstituted Reference Plasma and 0.4, 0.9, 1.4, 1.9, and 2.9 ml Imidazole Buffer respectively. Plasma diluted 1:10 represent 100% of the assigned value.
2- Prewarm 0.2 ml of each dilution to 37°C for 2 minutes.
3- Set stopwatch with the addition of 0.1 ml reconstituted Thrombin (do not warm thrombin reagent) to the prewarmed dilutions and time clot formation.
4- Calculate the average clotting time for each dilution, in duplicate.
5- Use all of the points to construct a log-log curve that plots fibrinogen concentration vs. clotting time. Draw the straight line of best fit. The final curve must consist of at least 3 consecutive points.

Dilution	Buffer	Ref. Plasma	Fibrinogen concentration(*)	Dilution factor
1:5	0.8	0.2	---- mg/dl	x 2 =
1:10	0.9	0.1	---- mg/dl	x 1 =
1:15	1.4	0.1	---- mg/dl	x 0.67 =
1:20	1.9	0.1	---- mg/dl	x 0.5 =
1:30	2.9	0.1	---- mg/dl	x 0.33 =

(*) fibrinogen concentration indicated on the label of the Reference Plasma

The fibrinogen value of every curve dilution is determined multiplying the fibrinogen concentration in the Reference Plasma by the dilution factor. For example, if there is a fibrinogen level of 260 mg/dl, indicated in the Reference plasma, then the dilutions 1:5, 1:10, 1:15, 1:20 and 1:30 have 520, 260, 173, 130 and 87 mg/dl respectively.

II- PATIENT SAMPLES AND CONTROLS

1- Prepare dilutions 1:10 of the patients' plasmas or control plasmas in Imidazole Buffer.
2- Prewarm 0.2 ml of each dilution to 37°C for 2 minutes.
3- Add 0.1 ml Thrombin rapidly and register clotting time.
4- Repeat testing and calculate the mean result for each specimen.

CALCULATIONS

Given the coagulation time of the patient or control, enter this value to the standard curve and interpolate the fibrinogen value for every case.

INTERPRETATION OF RESULTS

If the specimen's coagulation time is too short (for example, less than 7 seconds) dilute plasma 1:20 with Imidazole Buffer and assay again. Multiply the result by 2.
If the specimen's coagulation time is too much (for example, more than 35 seconds) dilute plasma 1:5 with Imidazole Buffer and assay again. Multiply the result by 0.5.

QUALITY CONTROL METHOD

Control Plasma normal/pathologic

REFERENCE VALUES

The observed range of values for normal patients oscillates between 200-400 mg/dl.
Each laboratory should establish its own Normal Reference Range from individuals representing the population being tested.

PROCEDURE LIMITATIONS

- A new calibration curve should be performed with every change of reagent's lot or any instrument change.
- Failures in the reconstitution of reagents may generate erroneous results.
- Sample collection: specimens and their dilutions should be placed in plastic tubes or siliconized borosilicate glass. It is important to maintain the blood-anticoagulant relation as well as the citrate concentration used.
- It must be checked that the assay be performed at 37°C and that the test tubes be absolutely clean.

PERFORMANCE

Reproducibility: processing replicates from the same samples during the day, the following results were obtained:

Level	S.D.	C.V.
12.1 sec	± 0.3 sec	2.7 %
20.4 sec	± 0.6 sec	3.2 %

PARAMETERS FOR AUTOMATIC ANALYZERS

Fibrinogen reagent is suitable for use with manual, mechanical, and photo-optical methods. For semi-automatic and automatic instrumentation follow the instrument manu-facturer's instructions.

KIT SIZE

Kit for 100 tests (Cat. Nr. 41004) containing:
- Thrombin: 10 x 1 ml
- Reference Plasma: 1 x 1 ml
- Imidazole Buffer: 2 x 60 ml

REFERENCES

- Clauss, A. - Acta Haematol. 17:237, 1957.
- Koepke, J.A. - Am. J. Clin. Pathol. 63:984, 1975.

- Collet, J.P. - Blood 82/8:2462, 1993.
- Ernest, E. - Ann. Intern. Med. 118/12:956, 1993.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

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