Reactivos GPL

Barcelona, España

CE

- FIBRINOGEN -

FIBRINOGEN
Clauss Method

Store at: +2+8º C.

Presentatión:
Cod. CO030 CONT: *R1 8 x 2 mL.+ R2 1 x 100 mL.*

Procedure

Diagnostic reagent for quantitative determination of fibrinogen.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

Fibrinogen in presence of an excess of thrombin concentration, changes into Fibrin.
The time for clot formation in dilute plasma is inversely proportional to the fibrinogen concentration in the sample.
The thrombin clotting time fibrinogen assay is based on the method originally described by Clauss.1 In the presence of high concentrations of thrombin, the time required for clot formation in dilute plasma is inversely proportional to the fibrinogen concentration.

REAGENTS COMPOSITION

R 1	Bovine thrombin ≈ 100 NIH u /mL
R 2	Imidazole Buffer
R 3	Caolin Solution (Not included)
Optional	COAGULATION CAL REF: CO060 CONTROL N REF: CO040 CONTROL P REF: CO050

PRECAUTIONS

R2: H290- May be corrosive to metals. H302-Harmful if swallowed. H360-May damage fertility or the unborn child. H412-Harmful to aquatic life with long lasting effects
Follow the precautionary statements given in MSDS and label of the product.

REAGENT PREPARATION AND STABILITY

R1: Dissolve (→) the contents with 2.0 mL. of distilled water. Cap vial and mix gently to dissolve contents. Stability: 7 days at 2-8º C or 1 month at –20º C, if immediately frozen and stored in the original container. Do not re-freeze.
R2: Mix before use.
R3: Ready for use reagent.
COAGULATION CAL (Calibrator): Dissolve (→) the contents with 1.0 mL of distilled water. Cap vial and mix gently to dissolve contents. Stability: 8 h at 2-8º C.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Quality control values outside established ranges.
- Product colour variations.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8º C. Do not use reagents over the expiration date.

SPECIMEN

Plasma from venous puncture diluted 1/10 in trisodium citrate solution 3.8% (105 mmol/L).
Mixing, immediately, the blood with anticoagulant. Avoid foaming the specimen.
Centrifuge the sample at 3000 x g for 10 min and transfer the plasma to siliconized glass or plastic containers.
Turbid, icteric, lipemic or hemolyzed samples may generate erroneous results.
The sample is stable for 4 hours at room temperature (15-25º C) or 28 days if immediately frozen at below 20º C.

MATERIAL REQUIRED BUT NOT PROVIDED

- Coagulometer or stopwatch and bath at 37º C ± 0.5º C.

General laboratory equipment.

TEST PROCEDURE

The reagent can be used by manual procedure, mechanical, photo-optical or other means of end clot detection (Note 2).

1. Dilute the citrated plasma and Control 1/10 with Imidazole buffer:
   50 µL plasma + 450 µL Imidazole buffer.
   The diluted sample must be processed in 1 hour.
2. Prepare the following dilutions of the Calibrator in Imidazole buffer.

Calibrator Dilution	1/40	1/30	1/20	1/10	1/5
IMIDAZOLE BUFFER (mL)	3.9	2.9	1.9	0.9	0.4
COAGULATION CAL (mL)	0.1	0.1	0.1	0.1	0.1
Factor	10/40*	10/30*	10/20*	10/10*	10/5*
Concentration (mg/dL)	0.25* x c	0.33* x c	0.5* x c	1* x c	2* x c

(c = Calibrator value)

3. Add 20 µL of R3 to 0.2 mL of each dilution, and allow to reach 37ºC for 4-6 minutes.
4. Add 0.1 mL of R1 and time clot formation. Do not pre-warm thrombin R1.

CALCULATIONS

1. Calculate the mean of duplicate clotting times immediately after reaction. Use all five of the calibrator points to construct a log-log curve that plots fibrinogen concentration (mg/dL) vs. clotting time (s).
2. Draw the straight line of best fit. Examine the curve and, if necessary, omit non-linear points. The final curve must consist of at least three consecutives points. Constructing the curve with only the most linear points will produce the best recovery on control and patient samples.
3. The following curve is **only orientative**. It will change with lot and concentration of the calibrator, a well as, with the instrument used.

Time (s)	Concentratation (mg/dL)	Concentratation (g/L)
18.1	608	6.08
26.4	304	3.04
49.6	152	1.52
84.7	76	0.76
153	38	0.38

4. Find the clotting time of quality control and patient samples on the curve and read the corresponding fibrinogen value.
5. If clotting times for the 1/10 dilution fall outside the linear curve, prepare 1/5 or 1/20 dilutions as needed. If the sample is diluted 1/5, divide the result from the standard curve by 2; if the sample was diluted 1/20, multiply the curve result by 2 to get the final result.

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES

200 – 400 mg/dL[1]. (2.0 – 4.0 g/L)
(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

Fibrinogen (Factor I), protein synthesized by the liver, is the substance used in the blood to form a clot. Its determination is used to evaluate abnormal blood clotting.
Elevated Fibrinogen levels are observed in acute inflammations and in pregnancy; low values are observed in trombolitic therapy, in hepatic disease, in the congenital non fibrinogen, in DIC (Disseminated Intravascular Coagulation) and in pancreatitis (low values)[1].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE

- *Precision:*

	Inter-assay (n=30)		
Mean (U/L)	144	294	488
CV (%)	5.9	3.4	2.9

- *Accuracy:* Results obtained using CHEMELEX reagents did not show systematic differences when compared with other commercial reagents.

INTERFERING SUBSTANCES

Has been observed interferences in samples with fibrinogen degradation. Acute inflammatory reactions can elevate circulating fibrinogen. Hemolysis can cause clotting factor activation and end point detection interference. High paraprotein levels,and drugs that activate the fibrinolytic system can interfere with fibrinogen assays. A list of drugs and other interfering substances with the determination has been reported[2,3].

NOTES

1. All labware must be clean and free of trace amounts of detergents.
2. Always follow instrument manufacturer's instructions; the results must be validated by the test laboratory.

BIBLIOGRAPHY

1. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

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