Factor IX

Deficient Plasma

For coagulometric determination of Factor IX with one-stage method

SUMMARY

The IX (FIX) factor is a serine protease, vitamin K dependent, hepatic synthesis.

FIX congenital alteration is associated with a bleeding disorder known as hemophilia B of sex-linked recessive inheritance.

The acquired deficiency may be due to the presence of specific inhibitors or be associated with other factors deficiency, as in the case of treatment with vitamin K antagonists, K hypovitaminosis, liver damage, disseminated intravascular coagulation, etc.

PRINCIPLE

Quantitative determination of FIX consists of measuring the coagulation time of a diluted sample containing factor to be determined, using a deficient plasma provided by the other factors in adequate levels, except FIX, in the presence of phospholipids, negative charge surfaces and calcium (activated partial thromboplastin time: aPTT). The clotting time obtained is inversely proportional to the activity of FIX present in the sample.

This method can be used with any instrument capable of factor assessment testing based on activated partial thromboplastin time.

PROVIDED REAGENTS

A. Reagent A: lyophilized human plasma deficient in factor IX obtained by immunoadsorption with a coagulation activity <1% of FIX.

INSTRUCTIONS FOR USE

Dissolve Reagent A in the distilled water volume stated on the label. Let stand for 30 minutes at room temperature and then homogenize the solution by gentle shaking before use.

NON-PROVIDED REAGENTS

- Distilled water
- Imidazole Buffer from Wiener lab.
- APTT ellagic from Wiener lab.
- Coagulation Control N and Coagulation Control P from Wiener lab.
- Coagulation Calibrator from Wiener lab.

WARNINGS

Reagents are for "in vitro" diagnostic use.

Reagent A has been prepared from non-reactive material for HBsAg, HCV and HIV. However, like blood samples, it should be handled as if they were infectious material.

Use the reagents keeping the usual work precautions in clinical laboratories.

All reagents and samples should be discarded according to local regulations.

STABILITY AND STORAGE INSTRUCTIONS

Factor IX Deficient Plasma is stable in refrigerator (2-10ºC) until the expiration date shown on the box.

Once reconstituted the reagent is stable for 3 hours at room temperature (<25ºC) or for 1 month frozen (-20ºC). Avoid repeated freezing and thawing.

The frozen reagent should be thawed for at least 10 minutes at 37ºC and homogenized before use.

SAMPLE

Citrated plasma

a) Collection: obtain blood carefully (avoiding stasis or trauma) and place in a tube with anticoagulant in 9 + 1 exact proportion (example: 4.5 ml blood + 0.5 ml anticoagulant). Mix gently. Centrifuge for 15 minutes and separate the plasma within 30 minutes. It is advisable to collect with plastic syringes.

b) Additives: to obtain plasma Anticoagulant TP from Wiener lab should be used or 130 mmol/l (3.8%) or 109 mmol/l (3.2%) sodium citrate.

c) Known Interfering Substances:

- Avoid using EDTA or heparin for plasma collection.
- Contamination, visible or not, are the cause of falsely prolonged times.
- Hemolysis and visible lipemias obstruct photo-optical measurement of results.

Refer to the literature of Young for the effects of drugs on the present method.

d) Stability and storage instructions: plasma should be kept at room temperature until testing. This period should not last longer than 4 hours. In case plasma could not be processed at once, it can be frozen up to 2 weeks at -20 ºC. In this case the sample should be frozen immediately and should be thawed quickly at 37ºC, no longer than 10 minutes.

REQUIRED MATERIAL (non-provided)

- Hemolysis tubes.
- Pipettes or micropipettes for measuring stated volumes.
- Stopwatch.
- Water bath at 37ºC.
- Semiautomatic or automatic coagulometer.

PROCEDURE

I- CALIBRATION CURVE

Use Coagulation Calibrator from Wiener lab. and Imidazole Buffer as a diluent.
Perform a 1:10 dilution of the Coagulation Calibrator, mixing 1 part calibrator + 9 parts Imidazole Buffer and from this dilution, the following geometric dilutions in Imidazole Buffer: 1: 2, 1: 4, 1: 8, 1:16 and 1:32. Calibrator diluted 1:20 represents 100% of the value assigned to FIX. The following table shows how to calculate the rest of the activities for each calibration point.
2- Preheat 25 mM calcium chloride (Reagent B of APTT ellagic from Wiener lab) at 37ºC.
3- In a hemolysis tube place:

Reagent A	100 ul
Calibrator dilutions	100 ul
aPTT Reagent (Reagent A)	100 ul

4- Mix and incubate for 3 minutes at 37ºC
5- Start the stopwatch with the addition of 100 uL preheated 25 mM calcium chloride and record the clotting time.
6- Calculate the average clotting time for each dilution, in duplicate.
7- Plot the calibration curve clotting times depending on FIX activity on log-log paper. Join most of the points represented with a straight line. The final line should contain at least 3 consecutive points.

FIX activity at each dilution curve is determined by multiplying the Coagulation Calibrator FIX% by the dilution factor indicated in the table. Example for an assigned value of 110% to Coagulation Calibrator:

Coagulation Calibrator Dilution	Final Dilution	FIX Calculation (%)	FIX activity (%)
2:1	2.0	110 x 2.0	220
1:1	1.0	110 x 1.0	110
1:2	0.5	110 x 0.5	55
1:4	0.25	110 x 0.25	27.5
1:8	0.125	110 x 0.125	13.8
1:16	0.063	110 x 0.063	6.9

II- PATIENT SAMPLES

1- Prepare 1:20 dilutions from patients plasmas in Imidazole Buffer (1 part sample + 19 parts Imidazole Buffer).
2- Preheat 25 mM calcium chloride (Reagent B of APTT ellagic from Wiener lab) at 37ºC.
3- In a hemolysis tube place:

Reagent A	100 ul
Diluted sample	100 ul
aPTT Reagent (Reagent A)	100 ul

4- Mix and incubate for 3 minutes at 37ºC
5- Start the stopwatch with the addition of 100 uL preheated 25 mM calcium chloride and record the clotting time.
6- Repeat the determination and average the results for each sample.

CALCULATIONS

The values of the 1:20 diluted plasma samples are interpolated on the calibration curve.

INTERPRETATION OF RESULTS

If FIX activity obtained by direct interpolation of the calibration curve is below the lowest point of the curve, repeat the determination of the sample with a lower dilution (1:10), multiplying the result by 0.5.
If the activity of FIX obtained by direct interpolation of the calibration curve is higher than the highest point of the curve, repeat the determination of the sample with a higher dilution (1:40), multiplying the result by 2.

QUALITY CONTROL METHOD

Coagulation Control N and Coagulation Control P from Wiener lab.
The control shall be processed in the same manner as the samples.

REFERENCE VALUES

50-150%

Each laboratory should establish its own reference values from the techniques and instruments used.

PROCEDURE LIMITATIONS

See Known Interfering Substances and Stability and Storage Instructions under SAMPLE.
Keep plasma samples at room temperature to avoid low temperature activation.
It is recommended to respect the incubation time of samples with Reagent A.
Improper handling of samples may result in partial activation of coagulation factors which would cause erroneous results.
Lupus anticoagulant may affect factor activity determination.
If FIX inhibitors are suspected several sample dilutions must be assayed. A new calibration is required for each reagent batch and for each instrument used.

PERFORMANCE

a) Reproducibility: was determined using different samples (in series and day-to-day). The following results were obtained:

Intra-assay precision:

Level	S.D.	C.V.
52.5 sec	0.69 sec	1.31%
60.3 sec	0.42 sec	0.70%

Inter-assay precision:

Level	S.D.	C.V.
52.5 sec	1.04 sec	1.98%
60.3 sec	1.14 sec	1.88%

b) Measuring range: 5-180%

PARAMETERS FOR AUTOMATIC ANALYZERS
Refer to the specific applications for each instrument.

WIENER LAB PROVIDES
- 5 x 1 ml (Code 1705018).

REFERENCES

- White G - Definitions in Hemophilia, Thromb Haemost 85:560, (2001).
- Kasper C - Hemophilia B: Characterization of Genetic Variants and detection of carriers, Blood, 50 (3): 351:366, (1977).
- Barrowcliffe T - Standardization of FIX & FIX assays, Haemophilia, 9: 397–402, (2003).
- Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Haemostasis Assays: Approved Guideline, 5th ed. CLSI: H21-A5.
- Triplett DA - New Methods in Coagulation, Crit Rev Clin Lab Sci. 1981;15 (1):25-84, (1981).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed. 2001.

SYMBOLS
The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
Σ	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
⟶	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
	Harmful
	Corrosive / Caustic
	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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