NoFACT IX

Immunodepleted Factor IX Deficient Substrate Plasma

INTENDED USE

NoFACT IX Deficient Plasma is a human plasma immunodepleted of Factor IX and intended for the quantitative determination of Factor IX activity in citrated plasma from patients suspected of FIX deficiency. FIX activity is based on the activated partial thromboplastin time. For in vitro diagnostic use.

SUMMARY AND PRINCIPLE

Factor IX is a glycoprotein zymogen of approximately 56,000 Daltons that circulates at a concentration of 89 nM (1). When converted to its active form, Factor IXa, and in complex its cofactor FVIIIa, FIXa accelerates the conversion of FX to FXa.

Factor IX has decreased activity in a congenital condition known as Hemophilia B. An acquired Factor IX deficiency state may occur in conjunction with vitamin K deficiency, oral anticoagulant therapy, and liver disease.

The quantitative clot-based assay for Factor IX uses a modification of the activated partial thromboplastin time (APTT) test and Factor IX deficient plasma (2, 3). In this system a dilution of the test plasma is mixed with a FIX deficient plasma and the clot time of an APTT determined for the mixture. Under these conditions the clot time is inversely proportional to the concentration of FIX in the test plasma (3).

SPECIMEN COLLECTION AND PREPARATION

Perform sample collection, handling, and storage according to the CLSI document H21-A5 "Transport and Processing of Blood Samples for Testing Plasma-based Coagulation Assays and Molecular Hemostasis Assays" (4). Nine parts of freshly drawn whole blood should be collected into one part 3.2% trisodium citrate anticoagulant. Fresh plasma samples up to 4 hours post-collection and frozen samples stored up to two weeks at -20C and up to six months at -70C are acceptable. Thaw frozen samples rapidly in a 37C water bath and mix gently and thoroughly before testing.

REAGENTS

For *In-Vitro* Diagnostic Use Only.

Factor IX Deficient Substrate Plasma

Package contents: 10 x 1 mL vials, lyophilized.
Ingredients: The reagent is human plasma, which has been immunodepleted to contain less than 1% Factor IX activity. The plasma has been buffered and lyophilized to maximize stability.
WARNING: Potential Biohazard: The NoFACT IX Deficient Plasma has been found negative when tested for Hepatitis B Antigen (HBsAg) and antibodies to HCV and HIV by FDA licensed tests; however, the deficient plasma should be handled with the same precautions as those observed when handling patient plasmas.
Preparation for Use: Reconstitute each vial of NoFACT IX Deficient Plasma with 1.0 mL distilled water. Swirl gently; do not shake. Allow to stand for 20 minutes at room temperature to insure complete dissolution before use.

Storage and Stability: The lyophilized product is stable until the expiration date printed on the vial when stored at 2 to 8°C. The reconstituted product is stable for 8 hours when stored at 2 to 8°C and 4 hours when stored at RT (18-26°C).

MATERIALS REQUIRED BUT NOT PROVIDED

Supplies available from r2 Diagnostic (or equivalent products from other manufacturers):
Phospholin ES, an APTT reagent
0.025 M Calcium Chloride
Imidazole Buffered Saline
PlasmaRef ARN calibration plasma

Supplies not provided by r2 Diagnostics:
Semi-automated or automated coagulation analyzer
Normal and abnormal quality control plasmas approved for FIX activity
Common clinical laboratory equipment and materials such as centrifuges, test tubes, pipettes, and distilled water.

TEST PROCEDURE

Contact r2 Diagnostics for instrument applications using APTT reagent to test for FIX concentration.

Quality Control

Quality control of coagulation tests involves multiple components. Each laboratory should establish a quality control program that includes both normal and abnormal control plasmas.

RESULTS

Results of a factor assay may be expressed in % activity or IU/mL. The analytical measurement range (linearity) is 1% - 188% FIX activity.

LIMITATIONS

Hemolysis to 500 mg/dL hemoglobin, icterus to 20 mg/dL unconjugated bilirubin, and lipemia to 2000 mg/dL triglycerides cause less than a 10% shift in % FIX recoveries using NoFACT IX Deficient Plasma with Stago PTT-A on the Stago Compact. Unfractionated heparin, Low Molecular Weight Heparin, and direct thrombin inhibitors interfere with FIX determinations. Lupus anticoagulants may also interfere (7).

The performance characteristics of NoFACT IX Deficient Plasma were not evaluated for other coagulation analyzers and APTT reagents or coagulation systems.

PERFORMANCE CHARACTERISTICS

The method comparison and analytical studies of NoFACT IX Deficient Plasma were assessed using Diagnostica Stago STA Compact coagulation analyzers, Stago PTT Automate 5 (PTT-A), and Stago Unicalibrator and controls.

Precision:

CLSI EP5-A2 (5) precision estimates of the Stago PTT-A Factor IX assay using NoFACT IX Deficient Plasma, as % CV of the recovered FIX values, were:

Plasma	Mean FIX activity, %	% CV, Within-run (S-r)	% CV, Lot-to-Lot (S-lot)	% CV, Within-Device (S-device)
System N (Normal Control Plasma) N=240	109%	5.1%	1.1%	7.2%
System P (Abnormal Control Plasma) N=240	43%	4.6%	2.9%	7.7%
Low FIX pooled patient plasma N=120	14%	5.7%	2.2%	7.8%

Correlation:

Two hundred and thirty-three plasma samples from patients and donors were assessed in three laboratories in parallel with the Stago PTT-A FIX assay using Stago IX Deficient plasma and with the Stago PTT-A FIX assay using NoFACT IX Deficient Plasma. The regression statistics were:

	All Labs N=233	Site 1 N=100	Site 2 N=80	Site 3 N=53
Slope	0.858	0.785	0.923	0.817
Intercept	5.729	6.168	5.328	11.206
r^2	0.915	0.977	0.907	0.830
r	0.956	0.988	0.988	0.911

REFERENCE VALUES

A typical reference range is 78%-184% (6), however each laboratory should determine a reference range for FIX activity for its particular population and instrument / reagent system.

LITERATURE REFERENCES

1) Bajaj S and Thompson A, "Molecular and Structural Biology of Factor IX", in Hemostasis and Thrombosis: Basic Principles and Clinical Practice, 5th edition, editors Coleman R et al, Lippicott Williams and Wilkins, 2006.
2) Marques MB and Fritsma GA, Quick Guide to Coagulation Testing, AACC Press2006.
3) Laposata M, et. al., The Clinical Hemostasis Handbook, Year Book Medical Publishers, 1989.
4) H21-A5, Collection, Transport and Processing of Blood Samples for Testing Plasma-based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition, Clinical Laboratory Standards Institute, 2008.
5) EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-2nd Edition, Clinical Laboratory Standards Institute, 2004.
6) Bennett ST, Lehman CM, and Rodgers GM, Laboratory Hemostasis: A Practical Guide for Pathologists, Springer, 2007.
7) Brandt JT, et al, Effect of lupus anticoagulants on the activated partial thromboplastin time, Arch Pathol Lab Med 115: 109, 1991