NoFACT VIII

Immunodepleted Factor VIII Deficient Substrate Plasma

INTENDED USE

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

SUMMARY AND PRINCIPLE

Factor VIII is a glycoprotein zymogen of approximately 330,000 Daltons that circulates at a concentration of 300 pM (1). When converted to its active form, Factor VIIIa, it complexes with activated FIX (FIXa) and accelerates the conversion of FX to FXa.

Factor VIII has decreased activity in a congenital condition known as Hemophilia A. An acquired Factor VIII deficiency state may occur in disseminated intravascular coagulation (DIC) and in patients who develop specific Factor VIII inhibitors.

The quantitative clot-based assay for Factor VIII uses a modification of the activated partial thromboplastin time (APTT) test and Factor VIII deficient plasma (2, 3). In this system a dilution of the test plasma is mixed with a FVIII 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 FVIII 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 VIII Deficient Substrate Plasma

Package Contents: 10 vials x 1 mL, lyophilized.

Ingredients: The reagent is human plasma, which has been immunodepleted to contain less than 1% Factor VIII activity. The plasma has been buffered and lyophilized to maximize stability.

WARNING: Potential Biohazard: The NoFACT VIII 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 VIII 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 (room temperature; 18-25°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
Imidizole 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 FVIII 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 FVIII 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 controls.

RESULTS

Results of a factor assay may be expressed in % activity or IU/mL. The analytical measurement range (linearity) is 1% - 160% FVIII 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 % FVIII recoveries using NoFACT VIII Deficient Plasma with Stago PTT-A on the Stago Compact. Unfractionated heparin, Low Molecular Weight Heparin, and direct thrombin inhibitors interfere with FVIII determinations. Lupus anticoagulants may also interfere (6).

The performance characteristics of NoFACT VIII Deficient Plasma were not evaluated for other coagulation analyzers and APTT reagent combinations or coagulation systems.

PERFORMANCE CHARACTERISTICS

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

Precision:

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

Plasma	Mean FVIII activity, %	% CV, Within-run (S-r)	% CV, Lot-to-Lot (S-lot)	% CV, Within-Device (S-device)
System N (Normal Control Plasma) n = 240	91.6%	4.2%	0.63%	6.8%
System P (Abnormal Control Plasma) n = 240	33.3%	4.9%	3.8%	8.0%
Low FVIII pooled patient plasma n = 120	11.8%	5.7%	0.0%	8.5%

Correlation:

A total of two hundred and thirty-three frozen plasma samples from patients and donors were assessed in three laboratories in parallel with the Stago PTT-A FVIII assay using Stago VIII Deficient plasma and with the Stago PTT-A FVIII assay using NoFACT VIII Deficient Plasma. The regression statistics were:

	All Labs n = 233	Site 1 n = 90	Site 2 n = 90	Site 3 n = 53
Slope	0.845	0.861	0.914	0.831
Intercept	4.2	2.8	2.5	5.9
r^2	0.968	0.991	0.986	0.953
r	0.984	0.995	0.993	0.976

EXPECTED VALUES

Factor VIII activity reference range: 50% - 150% (7). The normal range can be affected by pre-analytical as well as analytical variables. Each laboratory should therefore determine the normal range for FVIII activity for its particular population, instrument / reagent system, and laboratory practice.

LITERATURE REFERENCES

1) Kaufman K, Antonarakis S, and Fay P, "Factor VIII and Hemophilia A", 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) Brandt JT, et al, Effect of lupus anticoagulants on the activated partial thromboplastin time, Arch Pathol Lab Med 115: 109, 1991.
7) Kitchen, Olson, and Preston (ed), Quality in Laboratory Hemostasis and Thrombosis, 1st edition, Blackwell Publishing, 2009, page 90. ISBN 978-1-4051-6803-8.