Factor Inhibitor (Bethesda Titer) Assays

Physical Characteristics

Factor inhibitors (also called circulating anticoagulants or inactivators) are endogenously produced antibodies, commonly specific for their respective coagulation factors, that interfere with coagulation *in vivo* or *in vitro*.

Physiology, Incidence, and Etiology

Specific factor inhibitors can be classified as neutralizing or non-neutralizing. Neutralizing inhibitors interact with the functional component of the coagulant protein and may cause clinical bleeding. Non-neutralizing antibodies react with the protein somewhere other than the functional epitopes and may be clinically silent.

Factor VIII inhibitors may arise as IgG_4 (non-complement fixing) alloantibodies or autoantibodies. Factor VIII alloimmunization occurs in 15-20% of hemophilia A patients in response to treatment with factor VIII concentrates. Alloimmunization occurs primarily in severe hemophilia A when the patient's plasma factor VIII concentration is less than 1%. Alloimmunization is idiosyncratic, affecting some, but not all severe hemophilia A patients. Autoimmune factor VIII inhibitors occur in 1 of one million individuals, primarily in pregnancy and the post partum period, in patients over 55 years old, and in people with primary autoimmune disorders. Porcine factor VIII inhibitors may arise in hemophilia A patients treated with porcine factor VIII (Hyate C®) therapy.

Factor IX inhibitors arise as alloantibodies in 3-5% of hemophilia B (Christmas disease) patients in response to factor IX concentrate, prothrombin complex concentrate, or fresh frozen plasma therapy. Inhibitors of other coagulation factors are extremely rare as deficiencies of these factors are rare. Autoimmune anti-factor V inhibitors have been described.

Clinical Consequences of Factor Inhibitors

Alloimmune factor inhibitors are suspected when hemophilia patients' symptoms increase in severity, or bleeding continues despite infusion of ever increasing dosages of factor concentrates. For example, the normal plasma half-life of factor VIII, is 12 hours; however, Hemophilia A patients with inhibitors may have undetectable factor VIII levels a few minutes after factor VIII concentrate infusion and may require specialized therapy to halt acute bleeding.

Factor inhibitors form covalent complexes with their target coagulant factor molecules, rendering the factors non-functional. For alloimmune factor VIII inhibitor, this is a first order reaction with complete *in vitro* inactivation of available factor VIII. Autoimmune factor VIII inhibitors exhibit *in vitro* second order kinetics, achieving equilibrium with factor VIII leaving some residual factor VIII unbound in the plasma. *In vivo*, however, autoimmune factor VIII inhibitors cause "acquired hemophilia" with symptoms as severe as the more common congenital severe hemophilia. The mortality rate for acquired hemophilia is up to 22%.

Factor Inhibitor (Bethesda Titer) Assay Principle

Factors VIII, IX, XI, and XII Inhibitors

(The factor VIII inhibitor assay is used as an example of testing principle.)
Serial dilutions are made of patient plasma with veronal buffered saline, then mixed with normal plasma containing close to 100% factor VIII activity and are then incubated for 2 hours. An activated partial thromboplastin time (APTT)-based factor VIII assay using factor VIII-depleted plasma substrate is then performed on these incubated mixtures. Results are compared to those of incubated normal plasma. One Bethesda unit is defined as the amount of factor VIII inhibitor that neutralizes 0.5 IU of factor VIII in this system.

The number of serial dilutions tested is based on the anticipated level of the inhibitor.

Factor IX, XI, and XII inhibitor assays are performed in the same manner as the factor VIII inhibitor assay, substituting the respective factor IX, XI, and XII depleted substrate plasmas in place of the factor VIII depleted substrate plasma.

Porcine Factor VIII Inhibitors

The porcine factor VIII inhibitor assay is performed using the factor VIII inhibitor test protocol above, except that porcine factor VIII (Hyate: C®) concentrate is used in place of normal plasma. The porcine factor VIII (Hyate: C®) concentrate is mixed with human factor VIII free substrate prior to mixing with the patient's serial dilutions.

Factors II, V, VII, and X Inhibitors

Assays for these factor inhibitors are performed in a manner similar to the factor VIII inhibitor assay, except that the testing method used is a prothrombin timer-based system, using thromboplastin reagent, rather than an APTT testing system.

Factor Inhibitor (Bethesda Titer) Assays cont'd

Factor Inhibitor (Bethesda Titer) Assay Performance Characteristics

Autoimmune inhibitors cannot be accurately measured in the Bethesda titer system because of their second order kinetics. If there is residual coagulation factor, it could falsely lower the result. Lupus anticoagulant activity must be ruled out prior to assaying for factor inhibitors. Some inhibitors may fully neutralize factors in less than two hours.

Reference Ranges

Assay	Reference Range
Factor II inhibitor	0.0 – 0.8 Bethesda units
Factor V inhibitor	0.0 – 0.8 Bethesda units
Factor VII inhibitor	0.0 – 0.8 Bethesda units
Factor VIII inhibitor	0.0 – 0.8 Bethesda units
Factor VIII inhibitor (porcine)	0.0 – 0.8 Bethesda units
Factor VIII inhibitor (porcine screen)	Negative
Factor IX inhibitor	0.0 – 0.8 Bethesda units
Factor X inhibitor	0.0 – 0.8 Bethesda units
Factor XI inhibitor	0.0 – 0.8 Bethesda units
Factor XII inhibitor	0.0 – 0.8 Bethesda units

Factor inhibitors are not normally present in plasma. When present, factor inhibitors are measured by Bethesda titer units (BU). Titers of less than 5 BUs are classified as low responders, titers greater than 10 BUs as high responders. Responder status influences the approach for clinical treatment.

Specimen Requirements

Two citrated plasma specimens: Collect blood into two blue-stopper collection tubes containing 3.2% (0.109M) buffered sodium citrate. Ensure the blood reaches the fill line. Invert gently six times immediately after filling. Centrifuge the capped tubes at 2000g for ten minutes. Transfer the plasma to two plastic centrifuge tubes using a plastic pipette, then recentrifuge for ten additional minutes. The plasma must be platelet free (platelet count $<10 \times 10^9$/L). Transfer the plasma to two additional plastic tubes, seal, label, and freeze immediately at -20°C or lower. Ship the frozen specimens overnight on dry ice.

Test Request Information

All factor inhibitor assays are ordered individually as required and can be ordered separately from the Esoterix Service Directory.

Assays	Catalog #	CPT Code
Factor II inhibitor	300724	85210, 85730, 85610, 85611 x 3, 85335
Factor V inhibitor	300706	85220, 85730, 85610, 85611 x 3, 85335
Factor VII inhibitor	300721	85230, 85730, 85610, 85611 x 3, 85335
Factor X inhibitor	300725	85260, 85730, 85610, 85611 x 3, 85335
Factor VIII inhibitor	300701	85240, 85730, 85732 x 3, 85335
Factor VIII inhibitor porcine	300702	85240, 85730, 85732 x 3, 85335
Factor VIII inhibitor porcine screen	300703	85730, 85732 x 2, 85335

Factor Inhibitor (Bethesda Titer) Assays cont'd

Assays	Catalog #	CPT Code
Factor IX inhibitor	300704	85250, 85730, 85732 x 3, 85335
Factor XI inhibitor	300707	85270, 85730, 85732 x 3, 85335
Factor XII inhibitor	300708	85280, 85730, 85732 x 3, 85335
Factor VIII Bethesda Titer (includes factor VIII activity and factor VIII Bethesda Titer only)	300188	85240, 85335
Factor IX Bethesda Titer (includes factor IX activity and factor IX Bethesda Titer only)	300189	85250, 85335

Reflex Recommendations

Factor inhibitor assays are ordered in response to clinical impression, and are performed periodically on individuals who have inhibitors and are receiving therapy. During therapy, periodic assays are essential, both to manage the dosage and to establish whether a patient is a low or high responder. Low responders maintain titers consistently under 5 BUs while high responders may generate extremely high titers and rapid anamnestic responses to therapy. Assays must also be performed repeatedly during inhibitor suppression therapy.

Therapy for Factor Inhibitors

Acute Bleeding Episodes

To resolve acute or life-threatening hemorrhage, low responders may be given increased factor concentrate (VIII or IX) dosages to neutralize the inhibitor and achieve therapeutic factor levels. Fibrinolysis inhibitors such as Amicar or tranexamic acid may be given simultaneously. High responders, or patients demonstrated to be unresponsive to therapy, are given porcine factor VIII concentrate (Hyate C®) or recombinant Factor VIIa (Novoseven®). Patients who develop porcine factor VIII inhibitors may then be given factor VIII bypassing preparations such as prothrombin complex concentrate (PCC, Proplex®), activated PCC (FEIBA®), or recombinant factor VIIa. Low dose heparin infusions may be given with activated preparations such as Proplex® and FEIBA® but not Novoseven® to reduce the risk of thrombosis or DIC.

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