Test Definition: CHF8P

Chromogenic Factor VIII Inhibitor Bethesda Profile, Plasma

Overview

Useful For

Detecting the presence and titer of a specific factor inhibitor directed against coagulation factor VIII for patients on emicizumab (Hemlibra)

Detecting the presence and titer of an inhibitor directed against factor VIII

This test is **not useful** for detecting the presence of inhibitors directed against other clotting factors and will not detect the presence of lupus anticoagulants.

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
CH8BI	Chromogenic FVIII Inhibitor Interp	No	Yes
CHF8	Chromogenic FVIII, P	Yes	Yes
CH8B	Chromogenic FVIII Inhibitor Titer,P	No	Yes

Special Instructions

- Coagulation Guidelines for Specimen Handling and Processing
- Coagulation Patient Information
- Coagulation Profile Comparison

Method Name

Chromogenic

NY State Available

Yes

Specimen

Specimen Type

Plasma Na Cit

Ordering Guidance

This test is indicated if testing for FVIII inhibitors in patients being treated with the specific antibody emicizumab (Hemlibra).

This test detects the presence of specific inhibitors against factor VIII (FVIII). If the presence or type of inhibitor is unknown, APROL / Prolonged Clot Time Profile, Plasma or ALUPP / Lupus Anticoagulant Profile, Plasma should be ordered first.

Multiple coagulation profile tests are available. For testing that is performed with each profile, see Coagulation Profile Comparison.

Shipping Instructions

Send all vials in the same shipping container.

Specimen Required

Specimen Type: Platelet-poor plasma
Collection Container/Tube: Light-blue top (3.2% sodium citrate)
Submission Container/Tube: 13-mm x 75-mm, 5-mL, plastic vial (polypropylene preferred)
Specimen Volume: 2 mL Platelet-poor plasma in two (2) 13-mm x 75-mm, 5-mL, polypropylene vials, each containing 1 mL
Collection Instructions:
1. Specimen **must be** collected prior to factor replacement therapy.
2. If collecting sample through a port/line, be sure to waste the appropriate amount prior to collection.
3. For complete instructions, see Coagulation Guidelines for Specimen Handling and Processing.
4. Within 4 hours of collection, Centrifuge, transfer all plasma into a plastic vial, and centrifuge plasma again.
5. Aliquot plasma (1 mL per aliquot) into 2 separate 13-mm x 75-mm, 5-mL, polypropylene vials, leaving 0.25 mL in the bottom of centrifuged vial.
6. Immediately (within 2 hours of processing) freeze plasma at -20 degrees C or ideally, -40 degrees C or below.
Additional Information:
1. A double-centrifuged specimen is critical for accurate results as platelet contamination may cause spurious results.
2. Each coagulation assay requested should have its own vial.

Forms

1. Coagulation Patient Information (T675)
2. If not ordering electronically, complete, print, and send an Coagulation Test Request (T753) with the specimen.

Specimen Minimum Volume

See Specimen Required

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject
IV heparin contamination	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Plasma Na Cit	Frozen	14 days	

Clinical & Interpretive

Clinical Information

Factor VIII (FVIII) inhibitors are IgG antibodies directed against coagulation FVIII that typically result in development of potentially life-threatening hemorrhage. These antibodies may be alloimmune: developing in patients with congenital FVIII deficiency (hemophilia A) in response to therapeutic infusions of factor VIII concentrate or autoimmune: occurring in patients without hemophilia (not previously factor VIII deficient) either spontaneously, during pregnancy, or in association with autoimmune diseases.

Reference Values

CHROMOGENIC FACTOR VIII ACTIVITY
55.0-200.0%

BETHESDA TITER
< or =0.5 Bethesda Units

Interpretation

An interpretive report will be provided when testing is completed, noting a presence or absence of a chromogenic factor VIII inhibitor.

Cautions

Contamination with excess heparin and hemodilution due to improper specimen collection through an intravenous access device or collection above a running intravenous fluid line may cause spurious results.

Clinical Reference

1. Peyvandi F, Oldenburg J, Friedman KD. A critical appraisal of one-stage and chromogenic assays of factor VIII activity. J Thromb Haemost. 2016;14(2):248-261
2. Verbruggen B, van Heerde WL, Laros-van Gorkom BA. Improvements in factor VIII inhibitor detection: From Bethesda to Nijmegen. Semin Thromb Hemost. 2009;35(8):752-759
3. Miller CH, Platt SJ, Rice AS, Kelly F, Soucie JM. Hemophilia Inhibitor Research Study Investigators. Validation of Nijmegen-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. J Thromb Haemost. 2012;10(6):1055-1061
4. Williams A, Meijer P, Dean E, et al. Performance of factor VIII extended half-life product measurements in external quality assessment programmes. Int J Lab Hematol. 2026;48(2):430-439. doi:10.1111/ijlh.70044

Performance

Method Description

The Chromogenic Factor VIII assay is performed on the Instrumentation Laboratory ACL TOP 700 using the CRYO*check* Chromogenic Factor VIII kit. In this 2-stage assay, patient plasma is diluted and combined with reagents containing bovine factor X, human factors IXa and IIa, calcium chloride, and phospholipids. The factor VIII in the patient's plasma aids in the activation of factor X to factor Xa. After a specified incubation period, chromogenic substrate is added at which time, the factor Xa, present from the previous step, hydrolyzes the substrate into peptide and p-nitroaniline (pNA). The color produced by the release of pNA is measured photometrically at 405 nm and is proportional to the factor VIII in the sample.(Package insert: CRYO*check* Chromogenic Factor VIII. Precision BioLogic Inc; Rev V04, 06/2020)

In the Bethesda procedure, patient plasma is heat inactivated (HI) at 56 degrees C for 30 minutes. Next using the HI patient plasma, serial dilutions are prepared and mixed in equal volumes with normal pooled plasma. The mixture is incubated 2 hours at 37 degrees C. At the end of the incubation, chromogenic factor VIII (CHF8) activity is measured and compared to a control performed at the same time. The difference between the CHF8 activity of the patient's incubation mixture and that of the control is used to calculate the titer. The residual CHF8 activity is converted to Bethesda units: 50% residual CHF8 is equal to 1 Bethesda unit. Assays using the same basic principle as the Bethesda assay are used to quantitate the inhibitors of the other coagulation factors.(Kasper CK, Aldedort LM, Counts RB, et al. A more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh. 1975;34[03]:869-872. doi:10.1055/s-0038-1651378; Miller CH. Laboratory testing for factor VIII and IX inhibitors in haemophilia: A review. Haemophilia. 2018;24[2]:186-197. doi:10.1111/hae.1342)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

3 to 4 days

Specimen Retention Time

7 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification

Test Definition: CHF8P

Chromogenic Factor VIII Inhibitor Bethesda Profile, Plasma

See Individual Test IDs

CPT Code Information

CHF8-85130
CH8B-85335
CH8BI-85390-26

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CHF8P	Chromogenic FVIII Inhibitor Profile	95121-0

Result ID	Test Result Name	Result LOINC® Value
CH8B	Chromogenic FVIII Inhibitor Titer,P	93450-5
606844	Chromogenic FVIII Inhibitor Interp	95122-8
606865	Reviewed by	18771-6
CHF8	Chromogenic FVIII, P	49865-9