# 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 for testing for FVIII inhibitors in patients being treated with the specific antibody emicizumab (Hemlibra).

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This test is for detection of 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:** Plastic vials
**Specimen Volume:** 2 mL in 2 plastic 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. Centrifuge, transfer all plasma into a plastic vial, and centrifuge plasma again.
5. Aliquot plasma (1 mL per aliquot) into 2 separate plastic vials leaving 0.25 mL in the bottom of centrifuged vial.
6. Freeze plasma immediately (no longer than 4 hours after collection) at -20 degrees C or ideally, at or below -40 degrees C.
**Additional Information:**
1. 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

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| 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 Assay
Adults: 55.0-200.0%
Normal, full-term newborn infants or healthy premature infants usually have normal or elevated factor VIII.*
*See Pediatric Hemostasis References section in Coagulation Guidelines for Specimen Handling and Processing.

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

## 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
See Individual Test IDs

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## 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 |