Lab Dept: Coagulation

Test Name: CHROMOGENIC FVIII INHIBITOR BETHESDA PROFILE

General Information

Lab Order Codes:	CHF8I
Synonyms:	Chromogenic FVIII Inhibitor Profile
CPT Codes:	85390 – Chromogenic FVIII Inhibitor Interp 85130 – Chromogenic FVIII, P 85335 – Chromogenic FVIII Inhibitor Titer, P
Test Includes:	Activity of factor VIII in percentage Chromogenic Factor VIII titer in Bethesda Units

Logistics

Test indications:	Useful for detecting the presence and titer of a specific factor inhibitor directed against coagulation factor VIII for patients on emicizumab (Hemlibra).
Lab Testing Sections:	Coagulation – Sendouts
Referred to:	Mayo Medical Laboratories (MML Test: CHF8P)
Phone Numbers:	MIN Lab: 612-813-6280 STP Lab: 651-220-6550
Test Availability:	Daily, 24
Turnaround Time:	Results in 2 to 3 days.
Special Instructions:	N/A

Specimen

Specimen Type:	Blood
Container:	Light-Blue top (3.2% sodium citrate)
Draw Volume:	2.7ml in 2 blue top tubes.
Processed Volume:	2ml Plasma in 2 plastic vials, each containing 1ml

Collection:

● A clean venipuncture is essential, avoid foaming
● Entire sample must be collected with single collection, pooling of sample is unacceptable.
● Capillary collection is unacceptable.
● Patient's with a hematocrit level >55% must have a special tube made to adjust for the hematocrit; contact lab for a special tube.
● Mix thoroughly by gentle inversion. Deliver immediately to the laboratory at room temperature via courier or pneumatic tube.

Off campus collections:
● Must be tested within 4 hours.
● Do not refrigerate.
● If not received in our lab within 4 hours of collection, sample must be centrifuged and "platelet-poor plasma removed from cells and transferred to an aliquot tube being careful not to distribute to disturb the cell layer. Centrifuge the plasma a second time and transfer into a clean aliquot tube being careful not to include any residual platelets on the bottom of the tube. Freeze at -20 degrees C and deliver to the lab on dry ice within 2 weeks. ***Validation of your lab's centrifuge for platelet poor plasma is required.**

Special Processing:

Lab Staff: Spin sample collected in blue top tube(s) for 5 minutes on the Stat Spin centrifuge, remove plasma and transfer to a 4 mL BCS sample cup(s), spin remaining plasma again for 5 minutes in the Stat Spin centrifuge. Transfer plasma to 2 mayo sendout tubes for analysis leaving approximately 200 mL in the bottom of the original cup to discard.

Patient Preparation:

Specimen must be collected prior to factor replacement therapy.

Sample Rejection:

Improper tube; clotted samples; under filled tube; mislabeled or unlabeled specimens; gross hemolysis; gross lipemia; gross icterus; IV contamination

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Interpretive

Reference Range:

Pediatrics:	Normal, Full-term newborn infants or health premature infants usually have normal or elevated factor VIII
Adults:	55.0-200.0%
Bethesda Titer:	< or =0.5 Bethesda Units

Critical Values:

N/A

Limitations:

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.

Methodology:

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.

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.

References:

Mayo Clinical Laboratories August 2022

Updates: