Test Definition: CH9

Chromogenic Factor IX Activity Assay, Plasma

Overview

Useful For

Monitoring coagulation factor replacement therapy of selected extended half-life coagulation factor replacements

Aiding in the diagnosis of hemophilia B using a 2-stage assay, especially when a 1-stage assay was normal

Testing Algorithm

This assay is indicated in situations where there is a clinical suspicion of hemophilia B diagnosis, when the 1-stage assay is normal. However, this assay is also recommended for accurate classification of hemophilia B.

For more information see Hemophilia Testing Algorithm.

Special Instructions

- Coagulation Guidelines for Specimen Handling and Processing
- Hemophilia Testing Algorithm

Method Name

Chromogenic

NY State Available

Yes

Specimen

Specimen Type

Plasma Na Cit

Ordering Guidance

Coagulation testing is highly complex, often requiring the performance of multiple assays and correlation with clinical information. For that reason, ALBLD / Bleeding Diathesis Profile, Limited, Plasma is recommended.

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: 1 mL Platelet-poor plasma

Collection Instructions:

1. For complete instructions, see Coagulation Guidelines for Specimen Handling and Processing
2. Centrifuge, transfer all plasma into a plastic vial, and centrifuge plasma again.
3. Aliquot plasma into a separate plastic vial leaving 0.25 mL in the bottom of centrifuged vial.

4. Immediately freeze plasma (no longer than 4 hours after collection) at -20 degrees C or, ideally, at -40 degrees C or below.

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

If not ordering electronically, complete, print, and send a Coagulation Test Request (T753) with the specimen.

Specimen Minimum Volume

Platelet-poor plasma: 0.5 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Factor IX (FIX) is a vitamin K-dependent serine protease synthesized in the liver and participates in the intrinsic coagulation pathway. Its biological half-life is 18 to 24 hours.

Congenital FIX deficiency is inherited as an X-linked recessive bleeding disorder (hemophilia B). Severe deficiency (<1%) characterized by hemarthroses, deep tissue bleeding, excessive bleeding with trauma, and ecchymoses.

Typically, these patients are tested using a 1-stage clotting assay. However, new treatment options using long-acting glycoPEGylated replacement products are being approved for clinical use. Pharmacokinetic studies for these products indicate ideal monitoring of patients should be performed by the 2-stage chromogenic assay.

Reference Values

65-140%

Chromogenic factor IX (FIX) activity generally correlates with the one-stage FIX activity. In full term/premature neonates, infants, children, and adolescents the one-stage FIX activity* is similar to adults. However, no similar data for chromogenic FIX activity are available.(Appel IM, Grimminck B, Geerts J, Stigter R, Cnossen MH, Beishuizen A. Age dependency of coagulation parameters during childhood and puberty. J Thromb Haemost. 2012;10[11]:2254-2263)

*See Pediatric Hemostasis References section in Coagulation Guidelines for Specimen Handling and Processing

Interpretation

Factor IX (FIX} deficiency may be acquired (eg, vitamin K deficiency, warfarin anticoagulation effect, liver disease, or a consumptive coagulopathy) or congenital (hemophilia B).

Optimal laboratory monitoring of selected extended half-life factor IX replacement therapy (eg, glycoPEGylated factor FIX) may be achieved with the chromogenic factor IX assay. Elevated factor IX levels may be associated with acute or chronic inflammation, excess factor IX replacement therapy, or as a result of a rare genetic variant, factor IX Padua.

Cautions

Excess heparin and dilution contamination due to improper specimen collection through an intravenous access device may result in artifactually decreased results.

The 1-stage and chromogenic factor IX (FIX) assay results should correlate in the normal population but may be discordant in the hemophilia population and when measuring factor replacement, especially with PEGylated recombinant FIX products.

Clinical Reference

1. Bowyer AE, Hillarp A, Ezban M, Persson P, Kitchen S. Measuring factor IX activity of noacog beta pegol with commercially available one-stage clotting and chromogenic assay kits: a two-center study. J Thromb Haemost. 2016;14(7 1428-1435. doi:10.1111/jth.13348
2. Kitchen S, Signer-Romero K, Key NS. Current laboratory practices in the diagnosis and management of haemophilia: a global assessment. Haemophilia. 2015;21(4)550-557
3. Sorensen MH, Anderson S, Ezban M. Factor IX-deficient plasma spiked with N9-GP behaves similarly to N9-GP post-administration clinical samples in N9-GP ELISA and FIX activity assays. Haemophilia. 2015;21(6):832-836
4. Dodt J, Hubbard AR, Wicks SJ, et al. Potency determination of factor VIII and factor IX for new product labelling and postinfusion testing: challenges for caregivers and regulators. Haemophilia. 2015;21(4):543-549
5. Wilmot HV, Hogwood J, Gray E. Recombinant factor IX: discrepancies between one-stage clotting and chromogenic assays. Haemophilia. 2014;20(6):891-897

Performance

Method Description

The chromogenic factor IX assay is performed on the Instrumentation Laboratory ACL TOP Family using the Rossix ROX Factor IX kit. In this 2-stage assay, patient plasma is diluted and combined with reagents containing factors VIII, X, V, XIa, and II, fibrin inhibitor, calcium chloride, and phospholipids. The factor IX in the patient's plasma is activated to IXa, which then 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 IX in the sample.(Package insert: ROX FACTOR IX kit. Rossix AB, Molndal, Sweden, Rev 09/2018)

PDF Report

No

Day(s) Performed
Monday through Friday

Report Available
1 to 3 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
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
85130

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CH9	Chromogenic FIX, P	88449-4

Result ID	Test Result Name	Result LOINC® Value
CH9	Chromogenic FIX, P	88449-4