Factor IX Deficient Substrate Plasma

Cat. No. 5194

Helena Laboratories

INTENDED USE
The Factor IX Deficient Substrate Plasma is intended for the quantitative determination of Factor IX (Christmas factor) in patients suspected of having a congenital or acquired deficiency of this coagulation protein.

SUMMARY
Numerous coagulation factors have been identified in the blood and are required for normal blood clotting. A deficiency of one or more of the factors may result in a notable hemorrhagic condition, the severity of which is governed by the degree of the deficiency. Deficiencies of the blood clotting factors may be congenital or acquired. The congenital deficiencies are, in general, single deficiency states while the acquired deficiencies may be multiple in nature and commonly associated with liver disease, vitamin K deficiency or the ingestion of coumarin type anticoagulant drugs, and defibrination secondary to intravascular clotting.[1, 2]

Factor IX, known as Christmas Factor or plasma thromboplastin component (PTC), is decreased in a congenital disease known as Hemophilia B or Christmas Disease.[1, 2] Hemophilia B is clinically indistinguishable from hemophilia A, and it has a sex-linked recessive mode of inheritance. There are about 7 cases of hemophilia B to every case of hemophilia B.

An acquired Factor IX deficiency may occur in conjunction with a vitamin K deficiency and/or hepatocellular dysfunction.[1]

In an effort to devise a quantitative assay for Factor IX, several methods based on the thromboplastin test were used and were found to be time consuming and complicated. Langdell, Wagner and Brinkhous (1953) developed a one-stage "partial thromboplastin time" which was simple to perform but not reproducible. Helena's procedure determines Factor IX activity by using a modification of the activated partial thromboplastin time (APTT) test and a Factor IX deficient substrate plasma.[3]

PRINCIPLES
Quantitative measurement of individual coagulation factors by the one stage method depends upon having a substrate plasma lacking the factor being measured. A severely deficient plasma (less than 1% activity) has a prolonged activated partial thromboplastin time (APTT). A dilution of the test plasma is mixed with an equal volume of factor deficient plasma, and the clotting time of the mixture is determined. By comparing the degree of correction provided by the test plasma with the correction obtained with an acceptable known reference plasma, the percent activity of the coagulation factor may be determined.[4]

REAGENT
Factor IX Deficient Substrate Plasma (Cat. No. 5194)

Ingredients: The reagent is human plasma which contains less than 1% Factor IX activity.

Precautions: For In-Vitro Diagnostic Use Only. Avoid ingestion. The Factor IX Deficient Substrate Plasma has been found negative when tested for Hepatitis B Antigen (HBsAg) and HIV antibody; however, the deficient plasma should be handled with the same precautions as those observed when handling patient plasmas. Refer to the container label for results of HCV testing.

Preparation for Use: Reconstitute each vial of Factor IX Deficient Substrate Plasma with 1.0 mL deionized water. Swirl gently and allow to stand 15 minutes at room temperature to ensure complete dissolution.

Storage and Stability: The lyophilized product is stable until the expiration date printed on the vial and box labels when stored at 2 to 6°C. The reconstituted product is stable for 8 hours at 2-6°C. After the initial reconstitution period, the product should be kept on ice for the duration of testing.

Signs of Deterioration: The lyophilized product may appear as a dry, straw colored plug or pieces.

INSTRUMENT
Factor IX assays using Factor IX Deficient Substrate Plasma must be performed using accepted manual methods or by using optical or electro-mechanical instruments. The Cascade® 480, the Cascade® M or the Cascade M-4 are recommended.

SPECIMEN COLLECTION AND PREPARATION
Specimen: Plasma obtained from whole blood with 3.8% sodium citrate as an anticoagulant is the specimen of choice.

Specimen Collection: Blood may be collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique. Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. For blood specimens with hematocrits (HCT) of 40-50% (normal), 9 parts of freshly collected whole blood should be immediately added to one part anticoagulant. For blood specimens with hematocrits outside the normal range, adjust the amount of whole blood added to the anticoagulant according to the following formula.[5]

$$\text{Parts whole blood to one part anticoagulant} = \frac{0.6}{(1 - .\text{HCT}) \times 9}$$

Particular care should be taken when using evacuated test tubes. These tubes are designed to draw 9 parts blood to 1 part anticoagulant. If the hematocrit is determined abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant.

Specimen Preparation: Centrifuge the whole blood specimen at 1600-2000 X G for 10 minutes. A refrigerated centrifuge set at 2 to 6°C is preferred. Immediately separate the plasma from the red blood cells, and place it in a plastic test tube with cap.

Storage and Stability: Prior to testing, the plasma should be stored in the capped plastic tubes at 2 to 6°C. If testing is delayed for more than 2 hours, the plasma may be stored at -20°C or colder for up to one month. Thaw quickly at 37°C prior to testing, but do not allow to stand at 37°C for more than 5 minutes.

PROCEDURE
Materials Provided:

	Cat. No.
Factor IX Deficient Substrate Plasma	5194

Other Supplies Available from Helena

Helena APTT Reagent Kits	
10 x 5.0 mL - 250 tests	5383
10 x 10 mL - 500 tests	5384
10 x 10 mL - APTT Reagent	5385
10 x 10 mL - Calcium Chloride	5386
Helena APTT-SA Reagent Kits	
10 x 10 mL - 500 tests	5389
10 x 10 mL - APTT-SA Reagent	5387
10 x 5 mL - 250 tests	5388
Helena APTT-ES Reagent Kits	
10 x 10 mL - APTT-ES Reagent	5396
10 x 10 mL - 500 tests	5397
Owren's Veronal Buffer	5375

Materials required but not provided:
- 12 x 75 mm plastic test tubes
- Stopwatch
- Plastic or siliconized glass serological pipettes and syringes

General Comments
1. Assay patient samples as soon after collection as possible.
2. Sample dilutions must be assayed within 30 minutes after preparation and maintained on ice until tested.
3. Sample dilutions exceeding 1:40 and serial dilutions are not recommended.
4. Run all four of the recommended dilutions on plasma samples to avoid erroneous results due to possible dilution errors.
5. When performing factor assays, more than one vial of reagent maybe needed. To eliminate vial-to-vial variation multiple vials

should be reconstituted, allowed to dissolve, and then pooled.

6. Prepare a new standard curve each time assays are performed. Even though the same lot of reagents may be used, vial-to-vial variation, technique differences and instrument variability require this procedure. Helena's Coagulation S.A.R.P. (Cat. No. 5185) is recommended for use as the standard.

STEP-BY-STEP METHOD

A. Specimen and Reagent Preparation

NOTE: Throughout the procedure, all test tubes, syringes, and pipettes, must be plastic or siliconized glass.[6]

1. Reconstitute the appropriate number of vials of Factor IX Deficient Substrate with 1.0 mL deionized water. Swirl gently and allow to stand approximately 15 minutes at room temperature to ensure complete dissolution. Approximately 0.8 mL is required for each specimen assayed.
2. Reconstitute one vial of Coagulation S.A.R.P. with 1.0 mL deionized water. Swirl gently and allow to stand for 10 minutes to ensure complete dissolution. This will be used as the standard.
3. Prepare APTT reagent according to the package insert. Prewarm the reagent to 37°C.
4. Number a set of four 12 x 75 mm test tubes for the standard curve and each test specimen.

B. Standard Curve Preparation

1. Prepare the following dilutions of Coagulation S.A.R.P. with Owren's Veronal Buffer.

Tube	Dilution Ratio	mL Standard	mL Buffer	Actual % Activity
1	1:5	0.1	0.4	20
2	1:10	0.1	0.9	10
3	1:20	0.1	1.9	5
4	1:40	0.1	3.9	2.5

2. Cover tubes and invert gently but thoroughly. Avoid shaking since excess bubble formation causes prolonged prothrombin times.
3. Perform duplicate APTT tests on each of the standard and unknown dilutions as follows.
   Pipette into the reaction cup in the order specified:
   0.1 mL Factor IX Deficient Substrate Plasma
   0.1 mL 1:5 dilution of Coagulation S.A.R.P. or test plasma
4. Start a stopwatch immediately and incubate the mixture at 37°C for 2 minutes. Use this mixture to perform APTT assays according to the APTT reagent package insert.

Quality Control

Quality Control for factor assays involves multiple components. Instrumentation should be evaluated on a routine basis as outlined by the manufacturer. A normal control plasma such as Helena's S.A.C.-1 (Cat. No. 5301) and an abnormal control, such as S.A.C.-2 (Cat. No. 5302), can be used to verify instrument and reagent performance. Careful attention should be given to other reagents and instruments used in the assay. These include pipettes, deionized water, timing devices and diluting fluids.

INTERPRETATION OF RESULTS

Factor IX has a decreased activity in a congenital condition known as hemophilia B or Christmas Disease, which is sex-linked recessive. Hemophilia B is clinically indistinguishable from hemophilia A. The severity of bleeding is directly related to the amount of Factor IX in the blood. Severely affected patients experience prolonged bleeding after injury and spontaneous hemorrhages into deep muscle tissues and joints. The mildly affected patients characteristically bleed after trauma, but generally are spared the painful and crippling hemorrhages into joints and muscles.[1] The general management of patients and the principles of replacement therapy are similar to those in hemophilia.

The most common cause of an acquired deficiency of blood clotting factors is hepatic dysfunction due to liver cell damage or non-availability of vitamin K to the liver. Fibrinogen, factors II, VII, IX, X and possibly factor V are produced in the liver and all except fibrinogen and factor V require vitamin K for normal synthesis.[1]

LIMITATIONS

The Factor IX Deficient Substrate Plasma is limited to Factor IX activity determinations based on a modified APTT test system. Dilutions of the test specimen exceeding 1:40 are not recommended since the amount of clotting factor under investigation is so small. When less than 1% of the factor is added to the deficient substrate, the clotting times become less reproducible and the standard curve will begin to plateau.

EXPECTED VALUES[7]

Factor IX Expected Values:

50-150% of the normal plasma

Each laboratory should determine an expected range for its particular population and instrument-reagent system.

BIBLIOGRAPHY

1. Biggs, R., ed. Human Blood Coagulation, Hemostasis Thrombosis, 2nd Ed., Blackwell Scientific Publications, London, 231-248, 1976.
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3. Hardisty, R.M., et al., A One-Stage Factor VIII Assay and Its Use on Venous and Capillary Plasma, Throm. et Diath. Haemorr., 7:215-229, 1972.
4. Penner, J.A., The University of Michigan Medical School Blood Coagulation Laboratory Manual, 14th Ed. University Publications, Ann Arbor, 72-78, 1979.
5. Triplett, D.A., ed., Standardization of Coagulation Assays: An Overview, College of Am Path, Skokie, IL., 4-5, 1982.
6. Jaques, L.B. et al., Silicones and Blood Coagulation, Canadian Med Assoc Journal, 55:26-31, 1946.
7. Triplett, D.A. and Harms, C.S. Procedures for the Coagulation Laboratory. Am Society for Clin Path, Chicago, 36, 1981.

FACTOR DEFICIENT SUBSTRATES PLASMAS

Item	Cat. No.
Factor II Deficient Substrate Plasma (10 x 1.0 mL)	5190
Factor V Deficient Substrate Plasma (10 x 1.0 mL)	5191
Factor VII Deficient Substrate Plasma (10 x 1.0 mL)	5192
Factor VIII Deficient Substrate Plasma (10 x 1.0 mL)	5193
Factor IX Deficient Substrate Plasma (10 x 1.0 mL)	5194
Factor X Deficient Substrate Plasma (10 x 1.0 mL)	5195
Factor XI Deficient Substrate Plasma (10 x 1.0 mL)	5196
Factor XII Deficient Substrate Plasma (10 x 1.0 mL)	5197
Helena APTT Reagent Kits	
10 x 5.0 mL - 250 tests	5383
10 x 10 mL - 500 tests	5384
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Helena APTT-ES Reagent Kits	
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10 x 10 mL - 500 tests	5397
Owren's Veronal Buffer	5375
Equipment and Supplies	
Cascade® 480	1430
Cascade® M	1710
Cascade® M-4	1711
Coagulation S.A.R.P.	5185
S.A.C.-1	5301
S.A.C.-2	5302

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