CE

REF 5196

Factor XI Deficient Substrate Plasma

INTENDED USE

The Factor XI Deficient Substrate Plasma is intended for the quantitative determination of Factor XI (plasma thromboplastin antecedent) 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 XI, also referred to as plasma thromboplastin antecedent (PTA), activity is decreased in Rosenthal's disease or hemophilia C.[1, 2] It is an autosomal recessive disease that occurs mainly in individuals of Jewish ancestry. The disease occurs with equal frequency in males and females.

In an effort to devise a quantitative assay for Factor XI, 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 XI activity by using a modification of the activated partial thromboplastin time (APTT) test and a Factor XI 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 XI Deficient Substrate Plasma (Cat. No. 5196)

Ingredients: The reagent is human plasma which contains less than 1% Factor XI activity. The plasma has been buffered and lyophilized to assure stability.

Precautions: For In-Vitro Diagnostic Use Only. Avoid ingestion. The Factor XI 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 on the deficient plasma.

Preparation for Use: Reconstitute each vial of Factor XI 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 8°C. The reconstituted product is stable for 8 hours at 2 to 8°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 XI assays using Factor XI Deficient Substrate Plasma must be performed using accepted manual methods or by using optical or electro-mechanical instruments. The Cascade® M or M-4 are recommended.

SPECIMEN COLLECTION AND PREPARATION

The specimen of choice is citrated whole blood or plasma collected and processed according to the CLSI Guideline: Collection, Transport and Processing of Blood Specimens for Testing and Plasma-Based Coagulation Assays and Molecular Hemostasis Assays, H21-A5.[5] Add whole blood to 109 mM (3.2%) of the dihydrate form of sodium citrate, in a proportion of nine parts whole blood to one part anticoagulant. Mix the blood by gentle inversion with the anticoagulant immediately after collection.

Helena recommends testing whole blood specimens within 30 minutes of collection, especially if the patient is being monitored for heparin therapy.

Specimen Preparation: Centrifuge the whole blood specimen at 1600-2000 X G for 10 minutes. 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 8°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 XI Deficient Substrate Plasma	5196
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
10 x 25 mL Owren's Veronal Buffer	5375
10 x 10 mL - Calcium Chloride	5386

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 may be needed. To eliminate vial-to-vial variation, multiple vials should be reconstituted, allowed to dissolve and 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 XI 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 XI 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 XI is decreased in Rosenthal's disease or hemophilia C. The disorder tends to be mild, with bruising, epistaxis and menorrhagia as the most common clinical manifestations. Intramuscular hemorrhages are unusual. Affected individuals may have persistent bleeding following tooth extraction, tonsillectomy, or some other surgical procedure.

LIMITATIONS

The Factor XI Deficient Substrate Plasma is limited to Factor XI 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 2% of the factor is added to the deficient substrate, the clotting times become less reproducible and the standard curve may begin to plateau.

EXPECTED VALUES[7]

Factor XI 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.
2. Williams, W.J. et al., Hematology, 2nd Ed., McGraw-Hill, Inc., New York 1404-1413, 1434-1438, 1977.
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. Clinical and Laboratory Standards Institute: Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition. CLSI Document H21-A5, Vol. 28, No. 5, 2008.
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, 41, 1981.

FACTOR DEFICIENT SUBSTRATES

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-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
10 x 25 mL Owren's Veronal Buffer	5375
10 x 10 mL - Calcium Chloride	5386
Equipment and Supplies	
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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