Factor VIII Deficient Substrate Plasma

Cat. No. 5193

INTENDED USE

The Factor VIII Deficient Substrate Plasma is intended for the quantitative determination of Factor VIII (antihemophilic 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 hemorrahagic 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 VIII, known as antihemophilic factor (AHF) or antihemophilic globulin (AHG), is decreased in two congenital diseases, Hemophilia A or "classical hemophilia" which is a sex-linked recessive trait, and von Willebrand's disease, which is an autosomal dominant trait.[2] In an effort to devise a quantitative assay for Factor VIII, 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 VIII activity by using a modification of the activated partial thromboplastin time (APTT) test and a Factor VIII 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

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

Precautions: For In Vitro Diagnostic Use Only. Avoid ingestion. The Factor VIII Deficient Substrate Plasma has been found negative when tested for Hepatitis B Antigen (HBsAg), and HIV antibody. Testing the HCV anitbody on this product has ben found positive. The deficient plasma should be handled with the same precautions as those observed when handling patient plasmas.

Preparation for Use: Reconstitute each vial of Factor VIII 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. 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 VIII assays using Factor VIII Deficient Substrate Plasma must be performed using accepted manual methods or by using optical or electro-mechanical instruments. The Cascade M-4 is 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 and 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 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:

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Other Supplies Available from Helena

Helena APTT Reagent Kits	
10 x 10 mL Calcium Chloride (0.025M)	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
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 two preferred user 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 VIII Deficient Substrate Plasma 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 when running standard curve or two when running 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 VIII 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

A Factor VIII deficiency indicates the possible presence of Hemophilia A or von Willebrand's disease. Hemophilia A is a sex-linked recessive trait. Hemophilia patients are classified by the amount of Factor VIII activity measured in their plasma, severe (0-5%), moderate (5-10%) and mild (10-15%). Von Willebrand's disease is an autosomal dominant trait exhibiting decreased levels of Factor VIII coagulant activity, affecting both sexes equally. A differential diagnosis is made based on the results of other specialized coagulation tests, in conjunction with Factor VIII coagulant activity level.

LIMITATIONS

The Factor Deficient Substrate Plasma is limited to Factor VIII 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 being to plateau.

EXPECTED VALUES[7]

Factor VIII Expected Values:

50-150% of the normal plasma

For best results, 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, 1992.
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. Triplet, D.A. and Harms, C.S. Procedures for the Coagulation Laboratory. Am Society for Clin Path, Chicago, 36, 1981.

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