Factor Deficient Substrate Plasmas (PT Based II, V, VII, X)

Cat. No. 5190, 5191, 5192, 5195

INTENDED USE

The Factor Deficient Substrate Plasmas (II, V, VII, & X) are intended for the quantitative determination of the respective 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]

In 1935, Dr. Armond J. Quick developed the one-stage prothrombin time test.[3] It is on the basis of this test that we are able to perform assays for Factors II, V, VII, and X activities by the incorporation of deficient substrate plasma.

PRINCIPLES

Quantitative measurement of individual coagulation factors by the one stage method depends upon having a substrate plasma lacking the factor being measured. 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]

REAGENTS

Factor II Deficient Substrate Plasma (Cat. No. 5190)
Factor V Deficient Substrate Plasma (Cat. No. 5191)
Factor VII Deficient Substrate Plasma (Cat. No. 5192)
Factor X Deficient Substrate Plasma (Cat. No. 5195)

Ingredients: The plasmas are prepared as follows:

Factor	Plasma Source	Activity
II	Bovine & human	1%
V	Human	2%
VII	Human	2%
X	Human	1%

Precautions: For In-Vitro Diagnostic Use Only. Avoid ingestion.
The Factor Deficient Substrate Plasmas have been found negative when tested for Hepatitis B Antigen (HBsAg) and HIV antibodies. 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 each deficient plasma.

Preparation for Use: Reconstitute each vial of Factor 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.

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

INSTRUMENT

Factor assays using Factor Deficient Substrate Plasmas must be performed using accepted manual methods or by using optical or electromechanical instruments. The Cascade® M or the Cascade® M-4 are recommended.

SPECIMEN COLLECTION AND PREPARATION

Specimen: Plasma obtained from whole blood with 109 mM (3.2%) sodium citrate as an anticoagulant is the specimen of choice.

Specimen Collection:
Accurate coagulation studies depend on whole blood collected. and processed according to CLSI Guideline: Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays, H21-A5.[8] 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 on collection. Collection in 109 mM(3.2%) sodium citrate is recommended by the international Society for Thrombosis and Hemostasis and is used for international reference material International Sensitivity Index (ISI) assignments.[5]

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 capped plastic test tube.

Storage and Stability: The plasma should then be stored 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. Prior to testing, thaw quickly at 37˚C for no more than 5 minutes.

PROCEDURE

Materials Provided:	Cat. No.
Factor II Deficient Substrate Plasma	5190
Factor V Deficient Substrate Plasma	5191
Factor VII Deficient Substrate Plasma	5192
Factor X Deficient Substrate Plasma	5195
Materials required but not provided:	
Helena Thromboplastin Reagent	
10 x 4.0 mL	5380
10 x 10 mL	5381
Owren's Veronal Buffer	5375
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 at 2 to 8˚C 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. 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 Deficient Substrate as described in the REAGENTS section. 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 thromboplastin 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/Specimen 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. Prepare a 1:5 dilution of the patient specimen with Owren's Veronal Buffer.
3. Cover tubes and invert gently but thoroughly. Avoid shaking since excess bubble formation causes prolonged prothrombin times.
4. Perform duplicate prothrombin time tests on each of the standard and unknown dilutions as follows.
   Pipette into the reaction cup in the order specified:
   0.1 mL Factor Deficient Substrate Plasma
   0.1 mL 1:5 dilution of Coagulation S.A.R.P. or test plasma
5. Start a stopwatch immediately and incubate the mixture at 37˚C for 2 minutes.
6. Add 0.2 mL Thromboplastin Reagent to the plasma mixture and record the time required for clot formation.

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.

RESULTS

Using standard 2 x 2 cycle logarithmic paper, plot the percent activity on the X-axis and the corresponding clot time in seconds on the Y-axis. Draw a "line of best fit".

Determine the percent activity for the unknown specimen by finding the point where the clot time obtained for the unknown intersects the standard curve line. This value should be multiplied by the appropriate corrrection factor and by the activity of the reference plasma.

INTERFERENCES

The presence of oxalate, EDTA, or any additive other than sodium citrate may interfere with the test. Hemolysis should not affect the results; however, it is often an indication of poor specimen quality.[8] Moderate lipemia and a hematocrit of 0% to 55% do not normally interfere with the results. Therapeutic levels of heparin may give prolonged results.

INTERPRETATION OF RESULTS

A Factor II, V, VII, or X deficiency may be either congenital or acquired. Congenital conditions affect both sexes, but have been found to be extremely rare. An acquired deficiency as a result of hepatic dysfunction or of non-availability of vitamin K to the liver is much more common.[1] Since other coagulation factors are produced in the liver, more than one may be deficient. Reviewing the patient history and performing other factor assays may be helpful in order to distinguish between congenital and acquired deficiency.

LIMITATIONS

Many commonly administered drugs, diseases, and other factors can affect the results obtained in PT testing. If unexpected results are found, the test should be verified by repeat testing. If the results are confirmed, more in-depth testing may reveal a deficiency of one or more factors. Since normal values vary from laboratory to laboratory, depending on the technique used, each laboratory should establish its own reference interval.

The Factor Deficient Substrate Plasmas are limited to their respective activity determinations based on modified prothrombin 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.

REFERENCE VALUES[7]

Factor Reference 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.[9]

BIBLIOGRAPHY

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3. Quick, A.J., et al., A Study of the Coagulation Defect in Hemophilia and in Jaundice, Am J Med Sci, 190:501-511, 1935.
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9. Clinical and Laboratory Standards Institute. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. Third Edition CLSI Document C28-A3c, Vol. 28, No. 30. 2010.

FACTOR DEFICIENT SUBSTRATE PLASMA

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
Thromboplastin Reagent	
10 x 4 mL	5380
10 x 10 mL	5381
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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