**INTENDED USE**
The Factor VIII Deficient Substrate Plasma is intended for the quantitative determination of Factor VIII (antihemophilic factor) in patients suspected of having congenital or acquired deficiencies 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 result from vitamin K deficiency or the ingestion of coumarin type anticoagulants, and depletion secondary to conditions such as bleeding disorders.

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. In an effort to deviate a quantitative assay for Factor VIII, several methods based on the thromboplastin test were used and were found to be time consuming and complicated. Lendell, Wagner and Brinhus (1953) developed a one-stage "partial thromboplastin time" which is 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 plasma sample.

**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 partial thromboplastin time (APTT). A dilution of the test plasma is mixed with an equal volume of a factor deficient plasma to arrive at the clotting time of the mixture is determined. By comparing the degree of correction provided by the substrate plasma with the correction obtained with an acceptable known reference plasma, the percent activity of the factor being measured in the test plasma can be determined.

**REAGENT**
Factor VIII Deficient Substrate Plasma (Cat. No. 5193)

**INSTRUMENT**
Factor VIII assays using Factor VIII Deficient Substrate Plasma must be performed using accepted manual methods or by using opalescent or centrifugal devices. The Cascade® M or the Cascade M-4 are recommended.

**SPECIMEN COLLECTION AND PREPARATION**
Specimens should be obtained from whole blood collected in 1.8% sodium citrate as an anticoagulant. For the specimen of choice.

**SPECIMEN STORAGE**
Blood may be collected with evacuated test tubes containing an anticoagulant according to the following formula.

<table>
<thead>
<tr>
<th>Parts whole blood</th>
<th>mL</th>
<th>1 part anticoagulant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**PROCEDURE**
Materials Provided: Cat. No. 5193

**MATERIALS AVAILABLE FROM HELENA**
Helena APTT Reagent Kits
10 x 5.0 mL - 250 tests 5383
10 x 10 mL - 500 tests 5384
5190
10 x 10 mL - Calcium Chloride 5386
Helena APTT-SA Reagent Kits
10 x 10 mL - 500 tests 5387
5190
10 x 10 mL - APTT-ES Reagent 5388
Kits
10 x 10 mL - APTT-ES SA Reagent 5389
Kits
10 x 5 mL - 500 tests 5390
Owners' Veronal Buffer 5375

**MATERIALS REQUIRED BUT NOT PROVIDED:**
12 x 75 mm plastic test tubes
Sterile disposable syringes
Plastic or siliconized glass serological pipettes and syringes

**GENERAL COMMENTS**
1. All plates and tubes must be clean and dry. Plates and tubes must be free of dust and debris.

**LIMITATIONS**
The Factor VIII 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. The amount of clotting factor being investigated is so small. When less than 1% of the factor is added to Factor VIII deficient plasma the clotting times become less reproducible and the standard curve will begin to plateau.

**EXPECTED VALUES**
Factor VIII Standard: 50-150% of the normal plasma

**BIBLIOGRAPHY**


**FACTOR VIII DEFICIENT SUBSTRATE PLASMA**

**INGREDIENTS:**
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 90 days when stored at 2 to 6°C.

**STORAGE AND SHIPMENT:**
The lyophilized product is shipped cold and must be stored at 2 to 6°C.

**SIGNS OF DETERIORATION:**
If the reconstituted product appears to be cloudy or if there is any turbidity in the vial, the product should be discarded and a new vial used.

**LIMITATIONS**
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**SUMMARY**

Numerous coagulation factors have been identified in the blood and are required for normal blood clotting. A deficiency of one or more of these factors may result in a notable hemostatic 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 can be associated with liver disease, vitamin K deficiency or the ingestion of coumarin type anticoagulant drugs, and deficiency secondary to anticoagulant clotting drugs.

Factor VIII, known as antihemophilic factor (AHF) or antihemophilic globulin (AHL), 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. In an effort to deivate a quantitative assay for Factor VIII, several methods based on the thromboplastin test were used and were found to be time consuming and complicated. Langdoll, Wagner and Brinholz (1953) developed a one-stage "partial thromboplastin time" (aPTT) test that is 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.1

**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 partial thromboplastin time (aPTT). A dilution of the test plasma is mixed with an equal volume of aPTT reagent. The clotting time of the mixture is determined by comparing the degree of coagulation of the test plasma with the correction obtained with an acceptable known reference plasma, the percent activity of which is determined by the procedure. A coagulation factor may be determined.2

**REAGENT**

Factor VIII Deficient Substrate Plasma (Cat. No. 5193)

**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® 480, the Cascade M or the Cascade M-4 are recommended.

**SPECIMEN COLLECTION AND PREPARATION**

Specimens obtained from whole blood centrifuged at 400-500 x g for 10 minutes. The clotting times of the mixture is determined. By comparing the degree of coagulation of the test plasma with the correction obtained with an acceptable known reference plasma, the percent activity of which is determined by the procedure. A coagulation factor may be determined.2

**MATERIALS**

**Tube**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>mL</th>
<th>Standard Buffer</th>
<th>Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1</td>
<td>0.4</td>
<td>0.1</td>
<td>2.5</td>
</tr>
<tr>
<td>1:1</td>
<td>0.4</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>1:2</td>
<td>0.8</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>1:4</td>
<td>1.6</td>
<td>0.1</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**QUICK QUALITY CONTROL**

- Perform all quality control procedures using the Helena APPT-SA Reagents.
- Perform all quality control procedures using the Helena APTT-ES Reagents.
- Use the Helena APTT-ES Reagents for all dilutions and calculations.

**INTERPRETATION OF RESULTS**

A Factor VIII deficiency indicates the possible presence of Hemophilia A, a congenital coagulation disorder. 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 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 levels.

**LIMITATIONS**

- The Factor VIII 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.
- Any amount of clotting factor assay is an unacceptable result. The 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**

Factor VIII Standard:
- 50-150% of the normal plasma

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

**BIBLIOGRAPHY**


**FACTOR DEFICIENT SUBSTANCES**

**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 XII Deficient Substrate Plasma (10 x 1.0 mL) | 5196 |

**Equipment and Supplies**

Cascade® 480 1430
Cascade M 1710
Cascade N 1711
Coagulation S.A.R.P. 5185
S.A.C.-1 5301
S.A.C.-2 5302

For Sales, Technical and Order Information, and Service Assistance, call 800-231-5663 toll free.