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.

Start a stopwatch immediately and incubate the mixture at 37˚C for 2 minutes.

5. Pipet into the reaction cup in the order specified:

- 1:5 dilution of Coagulation S.A.R.P. or test plasma
- 1:20 dilution of Factor Deficient Substrate Plasma
- 1:40 dilution of Factor Deficient Substrate Plasma
- 1:5 dilution of Owren’s Veronal Buffer

6. 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 correction factor and by the activity of the reference plasma.


8. 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 determining the coagulation factor activities.

BIBLIOGRAPHY


10. Pipette into the reaction cup in the order specified:

- 1:5 dilution of Coagulation S.A.R.P. or test plasma
- 1:20 dilution of Factor Deficient Substrate Plasma
- 1:40 dilution of Factor Deficient Substrate Plasma
- 1:5 dilution of Owren’s Veronal Buffer


15. Factor Deficient Substrate Plasma

<table>
<thead>
<tr>
<th>Item</th>
<th>Cat. No.</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor II Deficient Substrate Plasma (10 x 1.0 mL)</td>
<td>5190</td>
<td>Factor &amp; Human 2%</td>
</tr>
<tr>
<td>Factor VII Deficient Substrate Plasma (10 x 1.0 mL)</td>
<td>5191</td>
<td>V Human 2%</td>
</tr>
<tr>
<td>Factor VIII Deficient Substrate Plasma (10 x 1.0 mL)</td>
<td>5192</td>
<td>VII Human 2%</td>
</tr>
<tr>
<td>Factor IX Deficient Substrate Plasma (10 x 1.0 mL)</td>
<td>5193</td>
<td>IX Human 2%</td>
</tr>
<tr>
<td>Factor X Deficient Substrate Plasma (10 x 1.0 mL)</td>
<td>5194</td>
<td>X Human 2%</td>
</tr>
<tr>
<td>Factor XII Deficient Substrate Plasma (10 x 1.0 mL)</td>
<td>5195</td>
<td>XII Human 2%</td>
</tr>
</tbody>
</table>

PRECAUTIONS

For In-Vitro Diagnostic Use Only. Avoid ingestion. The Factor Deficient Substrate Plasma has 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 plasma. Refer to the container label for requirements of the test.

Factors: Precautions for In-Vitro Diagnostic Use Only. Avoid ingestion. The Factor Deficient Substrate Plasma has 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 plasma. Refer to the container label for requirements of the test.

Precautions for In-Vitro Diagnostic Use Only. Avoid ingestion. The Factor Deficient Substrate Plasma has 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 plasma. Refer to the container label for requirements of the test.

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.

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

INSTRUMENT

Factor assays using Factor Deficient Substrate Plasma must be performed by accepting manual methods or by using optical or electro- mechanical devices. The Cascade® M or the Cascade® M-4 are recommended.

SPECIMEN COLLECTION AND PREPARATION

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

Specimen Collection: Agreement coagulation studies depend on whole blood collected and processed according to CLSI Guidelines: Collection, Transport, Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays, H1-45:1. Add whole blood to

Factor Deficient Substrate Plasma (PT Based II, V, VII, X)
**BIBLIOGRAPHY**

5. Duncan E M, Casey C R, Duncan V M, Loyd J V: Effect of concentra-
tion of trisodium citrate anticoagulant on the calculation of the inter-
7. Tripathi, D.A. and Hurms, C.S. Procedures for the Coagulation Lab-

**INTERFERENCEs**

The presence of oxalate, EDTA, or any additive other than sodium citrate may interfere with the test. Hemolysis should not affect the results; how-
ever, it is often an indication of poor specimen quality.4 Moderate lipemia and a hematocrit of 0% to 55% do not normally interfere with the results.

**LEnNATIONS**

Many commonly administered drugs, diseases, and other factors can affect the results obtained in PT and aPTT testing. Any 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 range.

**REFERENCE VALUES**

The Factor Deficient Substrate Plasma are limited to their respective activity determinations based on modified prothrombin time system. Dilutions of the test reagent 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.

**INTENDED USE**

The Factor Deficient Substrate Plasma (I, VII, IX & X) are intended for the quantitative determination of the relative factor in patients sus-
pected 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 blood clotting. A deficiency of one or more of the fac-
tors may result in a non-notable hemodynamic condition, the severity of which is generally dependent on the degree of deficiency. The deficiencies of the blood factors may be congenital or acquired. The congenital deficiencies are in general single deficiency states while the acquired deficiencies may be due to variations in nature and commonly associated with liver diseases. K deficiency or the ingestion of coumarin type anticoagulant drugs, and defibrination secondary to intravascular clotting.11

In 1935, Dr. Armond J. Quick developed the one-stage prothrombin time test.12 It is on the basis of this test that we are able to perform assays for Factors II, VII, X and V activities by the incorporation of deficient substrate plasma.

**MATERIALS**

Quantitative measurement of individual coagulation factors by the one-stage method depends upon having a substrate plasma lacking the fac-
tor 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 reference plasma with the correction obtained with an acceptable known reference plasma, the percent activity of the coagulation factor may be determined.4

**RECOMMANDATIONS**

1. Factor II Deficient Substrate Plasma (Cat. No. 5190)
2. Factor V Deficient Substrate Plasma (Cat. No. 5191)
3. Factor VII Deficient Substrate Plasma (Cat. No. 5192)
4. Factor X Deficient Substrate Plasma (Cat. No. 5193)

B. Preparation of the test reagent

Plasma with 1.0 mL deionized water. Swirl gently and allow to stand 15 minutes at room temperature to ensure complete dissolution.

Society for Thrombosis and Hemostasis and is used for international ref-

**STANDARD CURVE Preparation**

Factor assays using Factor Deficient Substrate Plasma must be per-
formed using accepted manual methods or by using optical or electro-
mechanical instruments. The Cascade® M or the Cascade® M-4 are rec-
ommended.

**SPECIMEN COLLECTION AND PREPARATION**

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

**SPECIMEN Collection**

Accurate coagulation studies depend on whole blood collected, and processed according to the appropriate Collection, Transport, Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays, H21-A5.8 Add whole blood to

**SPECIFIC REAGENTS**

Factors II, V, VII, and X activities by the incorporation of deficient substrate plasma.