INTENDED USE
The Immunospec Fibrinogen reagent is an in vitro diagnostic assay intended for quantitative determination of fibrinogen in plasma.

SUMMARY
Thrombin converts soluble fibrinogen into insoluble fibrin, which when cross-linked becomes the fibrin clot as the last step in the coagulation cascade. Fibrinogen is an acute-phase reactant protein in that the concentration rises sharply in response to many different physiological stimuli such as tissue inflammation or injury. High fibrinogen levels are associated with atherosclerotic cardiovascular disease and with the occurrence of myocardial infarction and stroke. Other conditions in which fibrinogen is elevated are cancers of the stomach, breast, or kidney, and inflammatory disorders like rheumatoid arthritis. Reduced fibrinogen levels are prevalent in liver disease, prostate cancer, lung disease, bone marrow lesions, malnourishment, and disseminated intravascular coagulation. Other conditions of deficient fibrinogen are congenital afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia.

PRINCIPLE
Immunospec Fibrinogen test kit is based on the Clauss method of quantifying plasma fibrinogen. The Clauss method measures the rate of fibrinogen fibrin conversion in the presence of excess thrombin and has been shown to be rapid, sensitive and precise. When diluted plasma is clotted with excess thrombin, the fibrinogen level is inversely proportional to the clotting time. A calibration curve is prepared from a fibrinogen reference and plotted on log-log paper. This calibration curve is used to determine the fibrinogen concentration in the test sample.

REAGENTS
1. Fibrinogen reagent is a lyophilized preparation of bovine thrombin, approximately 100 NIH U/mL, buffer, stabilizers and preservative. Reconstitute individual vials with 2 mL of high purity water. Allow to stand at room temperature for 30 minutes before use. Ensure all particulate matter is well dissolved before use. After reconstitution, stable for 5 days at 2-8°C, or frozen for up to 30 days. Warm to room temperature prior to re-use.

2. Fibrinogen Normal Control is a lyophilized citrated human plasma containing buffer and preservative. Assigned fibrinogen value is on the vial label. Reconstitute individual vials with 1 mL of high purity water. Allow to stand at room temperature with occasional swirling for 30 minutes before use. Ensure all particulate matter is well dissolved. After reconstitution stable for 8 hours at 2-8°C.

3. Imidazole Buffer is ready for use. Store at 2-8°C. Use until expiration date printed on bottle. Avoid contamination.

PRECAUTIONS
Do not ingest. Avoid contact with skin, eyes or clothing. Fibrinogen Normal control is a potentially biohazardous material. Source materials from which this product was manufactured were found negative for HBsAg and for antibodies against HCV, HIV-1 and HIV-2 using approved methods; however, no test method can offer complete assurance that infectious agents are absent. As with all materials of human origin, this product should be handled as a potentially infectious material.

SPECIMEN COLLECTION AND PREPARATION
Test plasma should be prepared from citrated whole blood without heparin, EDTA or oxalate.

1. Blood Collection using Syringe Method: Draw venous blood into a plastic or siliconized syringe. Immediately transfer 9.0 mL of blood into a tube containing 1.0 mL of 3.2% or 3.8% sodium citrate solution.

2. Blood Collection using an Evacuated Blood Collection Tube: Draw venous blood into a commercial vacuum tube containing 3.2% or 3.8% sodium citrate solution. Insure that a full draw has been obtained since the ratio of 9 parts blood to 1 part citrate is critical. A heparinized lock or transfer line should not be used. It is generally recommended that the second or third tube draw be used for coagulation tests.

3. Plasma Preparation: Mix well by inversion and centrifuge at 2,500 x g for 15 minutes soon after blood collection. Unless samples are to be processed immediately, transfer the plasma into a plastic tube. Plasma that is clearly hemolyzed or contains > 10,000 platelets per cubic milliliter or red cells is not suitable for coagulation testing.

4. Plasma Storage: Plasma samples may be stored at room temperature (18 to 26°C) for up to 2 hours; refrigerated (2 to 8°C) for up to 4 hours; frozen at –20°C for up to 6 months. Plasma may be re-centrifuged prior to freezing to assure that all cells are removed. Quick thaw frozen samples and test immediately. The samples must not have any contact with glass.

PROCEDURE
Materials Provided
1. Fibrinogen, 5 X 2 mL
2. Fibrinogen Normal Control, 3 X 1 mL
3. Imidazole Buffer, 6 X 15 mL

This procedure pertains to manual or semi-automated coagulation systems. Refer to your instrument manual for more detailed instrument specific instructions.

1. Prepare 1/5, 1/10, 1/20, 1/40 dilutions of normal plasma using the following table:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Normal Plasma µL</th>
<th>Imidazole Buffer µL</th>
<th>Total Volume µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/5</td>
<td>100</td>
<td>400</td>
<td>500</td>
</tr>
<tr>
<td>1/10</td>
<td>100</td>
<td>900</td>
<td>1000</td>
</tr>
<tr>
<td>1/20</td>
<td>100</td>
<td>1900</td>
<td>2000</td>
</tr>
<tr>
<td>1/40</td>
<td>100</td>
<td>3900</td>
<td>4000</td>
</tr>
</tbody>
</table>

2. Prepare a standard curve, plot the concentration (mg/dl) on the x-axis and clotting time (sec) on the y-axis. Use the assigned fibrinogen value on the normal control to determine fibrinogen values for the dilutions. If the calibrated normal control has 286 mg/dl of fibrinogen undiluted, then multiply the 286 by the dilution factor to determine the fibrinogen content. Use the following table as an example, assuming the normal control was assigned a fibrinogen concentration of 286 mg/dl.

3. Ensure the reconstituted Fibrinogen is at room temperature prior to use.

4. Pipette 200 µL of each plasma dilution into a test cuvette.

5. Incubate at 37°C for 2 minutes. (Not longer than 5 minutes)

6. Rapidly add 100 µL of each plasma dilution into a test cuvette.

7. Record the clotting time in seconds. All samples should be done in duplicate.

8. Create a standard curve, plotting the average clotting time against fibrinogen concentration on a log-log graph. Plot the concentration (mg/dl) on x-axis and clotting time (sec) on the y-axis. Use the assigned fibrinogen value on the normal control to determine fibrinogen values for the dilutions. If the calibrated normal control has 286 mg/dl of fibrinogen undiluted, then multiply the 286 by the dilution factor to determine the fibrinogen content. Use the following table as an example, assuming the normal control was assigned a fibrinogen concentration of 286 mg/dl.
Precision: Within-run precision was assessed using Immunospec Fibrinogen Normal and Low Control plasmas on both optical and a mechanical instruments. The results are shown in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Coatron IV (Optical)</th>
<th>Amelung (Mechanical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.9 %</td>
<td>2.5 %</td>
</tr>
<tr>
<td>Abnormal</td>
<td>2.7 %</td>
<td>1.8 %</td>
</tr>
</tbody>
</table>

Correlation: Correlation studies were performed against the Fibrinogen reagent of a competitor on the Coatron IV coagulometer. The results are shown in the following table.

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.920</td>
<td>0.99</td>
<td>0.06</td>
</tr>
</tbody>
</table>

REFERENCES