HELENA FIBRINOGEN ASSAY KIT

Cat. No. 5376

The Helena Fibrinogen Assay Kit is intended for use in the quantitative determination of fibrinogen in human plasma.

SUMMARY

Fibrinogen, a high-molecular weight glycoprotein in plasma, plays a key role in hemostasis. Upon generation of thrombin, fibrinogen is converted to the insoluble polymer, fibrin. Sufficient fibrinogen must be present in the circulation to arrest bleeding and repair tissue should vascular trauma or injury occur. Thus, the determination of fibrinogen in plasma is important in an assessment of a thrombolytic disorder.

Fibrinogen is an acute-phase reactant, increasing in the plasma as a result of inflammation, pregnancy and oral contraceptive use.[1] Decreased levels are found in certain pathological states including liver disease and disseminated intravascular coagulation (DIC). Congenital deficiencies include afibrinogenemia (no detectable fibrinogen), hypofibrinogenemia (<1 mg/mL) and dysfibrinogenemia (abnormal fibrinogen).

PRINCIPLES

Clauss[2] developed a simple method for the quantitative determination of fibrinogen by measuring the clotting time of dilute plasma following the addition of thrombin. At relatively high thrombin concentrations (>30 NIH units/mL) and low fibrinogen concentrations (2.0-30.0 mg/dL) the clotting time is dependent on the fibrinogen level. The thrombin clotting time, under these conditions, plotted on a log-log scale versus the fibrinogen concentration, is linear.

REAGENTS

1. Helena Thrombin Reagent

Ingredients: The reagent contains a lyophilized preparation of approximately 100 NIH units/mL of bovine thrombin with added stabilizers.

CAUTION: FOR IN-VITRO DIAGNOSTIC USE

Preparation for Use: Reconstitute a vial of Thrombin Reagent with 2.0 mL or 5.0 mL of distilled or deionized water. Refer to the vial label for the appropriate reconstitution volume. Invert gently to mix and allow to stand until dissolved.

Storage and Stability: The lyophilized product should be stored at 2-8°C. The reconstituted thrombin is stable for 8 hours at room temperature (15-30°C) or 1 week at 2-8°C.

Signs of Deterioration: Discard the vial if it shows signs of precipitation or microbial contamination.

2. Helena Fibrinogen Calibrator

Ingredients: The calibrator consists of a lyophilized citrated normal human plasma assayed for fibrinogen using a functional clotting assay.[2] Refer to the enclosed package insert for the assay value.

CAUTION: FOR IN-VITRO DIAGNOSTIC USE

The Helena Fibrinogen Calibrator has been found negative for Hepatitis B Antigen (HBsAg) and HIV antibody; however, this plasma should be handled with the same precaution as any human plasma sample. Avoid ingestion.

Preparation for Use: Reconstitute each vial with 1.0 mL of distilled or deionized water, restopper vial and allow to stand until dissolved. Invert gently to mix. Do not shake.

Storage and Stability: The unopened vial is stable until the date indicated on the label when stored at 2-8°C. The reconstituted product is stable for 4 hours at 2-8°C.

3. Owren's Veronal Buffer

Ingredients: The buffer contains 28.4 mM barbital, 0.125 M sodium chloride and 0.05% sodium azide as a preservative.

CAUTION: FOR IN-VITRO DIAGNOSTIC USE - DO NOT INGEST.

Preparation for Use: The buffer is ready for use as packaged.

Storage and Stability: The buffer is stable until the date indicated on the label when stored at 2-8°C. Exercise care when pipetting to avoid contamination.

Signs of Deterioration: Discard the buffer if visible signs of microbial contamination occur.

INSTRUMENT

Any high quality electro-mechanical or photooptical coagulation instrument may be used such as the Helena Cascade® M4, Cat. No. 1711.

SPECIMEN COLLECTION AND HANDLING

Specimen: Plasma obtained from whole blood collected with sodium citrate as an anticoagulant is the specimen of choice. The concentration of the sodium citrate should be 3.8% (0.129 M) or 3.2% (0.109 M).

Specimen Preparation: Refer to the NCCLS guideline H-21-AZ on Specimen Collection and Preparation for Coagulation Studies. Whole blood should be collected into a plastic syringe, evacuated blood collection 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:[3]

$$\text{Parts whole blood to one part anticoagulant} = \frac{0.6}{(1 - .\text{HCT})} \times 9.0$$

Particular care should be taken when using evacuated blood collection tubes as they are designed to draw 9 parts blood to 1 part anticoagulant. Therefore, if the hematocrit is abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant. For greater stability of the specimen, an acid citrate anticoagulant solution should be prepared and utilized. The anticoagulant is prepared as follows:[4]

3 parts 0.1 M sodium citrate
2 parts 0.1 M citric acid

Specimen Storage and Stability: The anticoagulated whole blood specimen should be centrifuged at 1600-2000 x G for 10 minutes followed by immediate removal of plasma. Prior to testing, the plasma sample should be stored at 2-8°C in capped plastic test tubes. Studies have shown that there is no significant change in fibrinogen levels on plasma samples stored up to 3 days at 2-8°C[5], nevertheless, it is good laboratory practice to test samples as soon as possible after collection. Plasma may be stored at -20°C, or colder, for at least one month. Thaw quickly at 37°C prior to testing.

PROCEDURE

Materials Provided:	**Cat. No.**
Helena Fibrinogen Assay Kit	5376
Contains:	
Helena Thrombin Reagent (5 x 2.0 mL)	
Helena Fibrinogen Calibrator (2 x 1.0 mL)	
Owren's Veronal Buffer (2 x 25 mL)	
Fibrinogen Graph Paper	
Components sold separetely:	
Helena Thrombin Reagent (10 x 2.0 mL)	5374
Helena Thrombin Reagent (10 x 5.0 mL)	5378
Helena Fibrinogen Calibrator (10 x 1.0 mL)	5379
Owren's Veronal Buffer (10 x 25 mL)	5375
Additional Equipment and Supplies	
Coagulation S.A.R.P.® (10 x 1 mL)	5185
Cascade M4	1711
S.A.C. -1	5301
S.A.C. -2	5302
Materials Required but not Provided:	
Reaction cups	
Stopwatch	

Plastic test tubes, 12 x 75 mm
37°C heat block or water bath
Centrifuge
Pipetting Devices – 0.05 mL, 0.1 mL, 0.2 mL

STEP BY STEP METHOD

I. Calibration

1. Allow all reagents to equilibrate to room temperature (15-30°C).
2. Prepare 1:5, 1:10, 1:20, 1:30 and 1:40 dilutions of Fibrinogen Calibrator using Owren's Veronal Buffer as follows:

	1:5	1:10	1:20	1:30	1:40
Buffer	0.8 mL	0.9 mL	1.9 mL	2.9 mL	3.9 mL
Fibrinogen Calibrator	0.2 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL

3. Run duplicate determinations on each dilution of the fibrinogen calibrator as follows:
   a. Incubate 0.2 mL of calibrator dilution for 2 minutes at 37°C. Do not exceed 5 minutes at 37°C.
   b. Add 0.1 mL of Thrombin Reagent (room temperature) to immediately initiate the timed reaction.
   c. Determine the clotting time. Average the duplicate times for each calibrator.
   d. Plot the average clotting times obtained versus the respective fibrinogen concentration. The Helena Fibrinogen Calibration Graph Paper is printed to accomodate the 1:10 dilution factor. Refer to the instructions for plotting the standard curve given on the Fibrinogen Graph Paper. The concentration value for each lot of calibrator is given on the assay card.

II. Testing

1. Dilute patient plasma and controls 1:10 with Owren's Veronal Buffer (1 part specimen and 9 parts buffer).
2. Test patient and control dilutions in duplicate in the following manner.
   a. Pipette 0.2 mL of diluted specimens into a specimen cup and incubate it for 2 minutes at 37°C. Do not exceed 5 minutes at 37°C.
   b. Add 0.1 mL of Thrombin Reagent (room temperature) to initiate the timed reaction.
   c. Determine the clotting time by averaging the readings of the duplicate determinations.

Quality Control

A control should be run with each standard curve or set of assays. Helena S.A.C.-1 and/or S.A.C.-2 and S.A.R.P.® have been assayed for fibrinogen and are highly recommended. When the control is used, the fibrinogen values must fall within the stated variation (usually $\bar{x} \pm 2$ S.D.) given on the package insert or the data should be repeated or considered suspect.

Calculation of Unknown

Read the results of the patient and control dilutions from the standard curve by drawing a line from the test clotting time on the curve, down through the X-axis, to give the concentration of fibrinogen in mg/dL. The Cascade® M4 will automatically perform calculations and print results.

RESULTS

The fibrinogen assay on the test specimen is generally performed using a 1:10 dilution of plasma. The concentration can then be read in mg/dL directly from the standard curve. For plasma dilutions other than 1:10, the concentration from the curve should be multiplied by the dilution factor (i.e. multiply the concentration value by 2 when assaying a 1:20 dilution, or by 0.5 when assaying a 1:5 dilution). If no detectable clotting occurs using a 1:3 dilution of patient plasma, this is indicative of either a fibrinogen concentration of less than 20 mg/dL, or the presence of some interfering substance (see Limitations of Procedure).

EXPECTED VALUES

Plasma of healthy adults contains about 150 to 350 mg/dL of fibrinogen.[6,7] Helena tested thirty-two (32) presumed healthy donors for fibrinogen levels and obtained the following data.

$\bar{x}$ = 275.0 mg/dL
Range (2SD) = 162.4 - 387.7 mg/dL

Each laboratory should establish its own normal range on a representative sample population since normal values vary from laboratory to laboratory.

LIMITATIONS

Significant levels of heparin or fibrinolytic degradation may cause the test to indicate a falsely low fibrinogen level. However, because of the high thrombin concentrations used and the dilution of the plasma, heparin concentrations below 0.6 USP units/mL and levels of fibrinolytic degradation products below 100 µg/mL do not significantly affect fibrinogen values.

PERFORMANCE CHARACTERISTICS

Precision Studies

Within Run: Twenty normal and twenty abnormal control samples were tested and the following data was noted:

	Normal mg/dL	Abnormal mg/dL
$\bar{x}$	302.9	101.4
SD	8.98	5.05
CV%	2.96	4.98

Run-to-Run: The same forty samples were tested for successive days and gave the following data:

	Normal mg/dL	Abnormal mg/dL
$\bar{x}$	309.0	101.2
SD	10.76	7.44
CV%	3.43	7.35

Comparison Studies

Comparison studies were done on 36 (normals and abnormals) specimens using a reference method and the Helena procedure. The linear regression equation derived from the study was Y = 0.857X + 23.8 with a correlation coefficient of r = 0.942.

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