SIEMENS Healthineers

Dade® Fibrinogen Determination Reagents

FIBRINOGEN DETERMINATION

Revision bar indicates update to previous version.

Intended Use

For the quantitative determination of fibrinogen in human plasma.

Summary and Explanation

Fibrinogen is a plasma protein which is converted from a soluble protein to an insoluble polymer by the action of thrombin resulting in the formation of a fibrin clot. The thrombin clotting time of dilute plasma is inversely proportional to the fibrinogen concentration of the plasma[1-3]. Using this principle, Clauss[1] developed a simple quantitative assay for fibrinogen by measuring the clotting time of dilute plasma when excess thrombin is added. The clotting time obtained is then compared with that of a standardized fibrinogen preparation.

Reagents

For *in-vitro* diagnostic use

Note

Dade® Fibrinogen Determination Reagents can be used manually or on automated coagulation analyzers. Siemens Healthineers provides Reference Guides (Application Sheets) for several coagulation analyzers. The Reference Guides (Application Sheets) contain analyzer/assay-specific handling and performance information which may differ from that provided in these Instructions for Use. In such a case, the information contained in the Reference Guides (Application Sheets) supersedes the information in these Instructions for Use. In addition, please also consult the instruction manual of the instrument manufacturer.

Dade® Thrombin Reagent: a lyophilized preparation of bovine thrombin (≤ 100 NIH IU/mL) with stabilizers and buffers.

- Reconstituted material is stable stored stoppered for 8 hours at 22 to 28 °C or 5 days at 2 to 8 °C.

Note: Always keep thrombin in original vial during testing and for storing reconstituted material.

Dade® Fibrinogen Standard: Citrated plasma (human), with HEPES buffer and stabilizers. Lyophilized, assayed for fibrinogen using a clottable protein method. The concentration of the Standard is given on the enclosed Table of Assigned Values.

Note: This standard cannot be used with the Ratnoff-Menzie or biuret procedures because cloudiness occurs when the fibrinogen clot is dissolved in strong alkali.

- Reconstituted material is stable for 4 hours stored stoppered at 2 to 8 °C when not in use.

Dade® Thrombin Reagent and Dade® Fibrinogen Standard must be:

- Stored at 2 to 8 °C.
- Reconstituted with 1.0 mL distilled or deionized water. Restopper vial and allow to stand until dissolved. Invert gently to mix. Do not shake.

Note: Do not use water containing preservatives.

Indication of deterioration: Inability to obtain reproducible values.

Dade® Owren's Veronal Buffer: 2.84 x 10^{-2} M sodium barbital in 1.25 x 10^{-1} M sodium chloride, pH 7.35 ± 0.1.

Caution: Not for internal or external use by humans or animals.

Store at 2 to 8 °C.

Exercise care during multiple pipettings to avoid contamination. Physical signs of deterioration are limited to visible microbial contamination.

Information about on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

Safety data sheets (MSDS/SDS) available on siemens-healthineers.com/sds.

CAUTION!
Federal (USA) law restricts this device to sale by or on the order of licensed healthcare professionals.

Danger! THROMBIN REAGENT
Hazardous ingredient: Thrombin, bovine (≤ 5 % [w/w]).
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261: Avoid breathing dust. **P304 + P340**: IF INHALED: Remove person to fresh air and keep comfortable for breathing. **P342 + P311**: If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.

CAUTION! POTENTIAL BIOHAZARD
FIBRINOGEN STANDARD contains human source material.
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests that are CE marked or FDA approved for this purpose. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Caution
THROMBIN REAGENT
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

OV BUFFER is not intended for either internal or external use with humans or animals. Avoid contamination of the preparation during multiple pipettings. A visible microbial contamination is a sign that the preparation cannot be used.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Specimen Collection and Preparation

Note: For patient conditions which may affect test results, see *Limitations of Procedure*.

Mix nine parts of freshly collected patient blood with one part of 0.13 mol/L (3.8 %) sodium citrate. Evacuated tubes containing the desired anticoagulant are commercially available and may be used with caution in blood coagulation studies. For special studies, syringe technique may be preferred.

Centrifuge the blood specimen for a minimum of 15 minutes at 1500 x g as soon as possible after collection. For alternative blood collection procedure see the CLSI document H21-A5[7]. If immediate testing is to be done, the plasma may remain on the packed cells or separated. To separate the plasma, use a plastic transfer pipette, remove the plasma to a plastic tube and keep refrigerated until ready to test. Do not store on ice.

Although studies[4] have demonstrated that there is no significant change in fibrinogen values on plasma samples stored up to 72 hours at 4 °C, it is good laboratory practice to test samples as soon as possible after collection.

Reagents Provided

FIBRINOGEN DETERMINATION, REF B4233-17SY *with*

6 x → 1 mL, THROMBIN REAGENT, Dade® Thrombin Reagent

1 x → 1 mL, FIBRINOGEN STANDARD, Dade® Fibrinogen Standard

3 x 15 mL, OV BUFFER, Dade® Owren's Veronal Buffer

Table of Assigned Values

Materials Required but Not Provided

1. 0.13 mol/L sodium citrate.
2. Normal Control such as Dade® Ci-Trol® Coagulation Control, Level 1
3. Abnormal Control such as Dade® Data-Fi® Abnormal Fibrinogen Control
4. Preservative-free distilled or deionized water
5. 12 x 75 mm plastic test tubes
6. Pipetting devices capable of accurately measuring 5.0 mL, 1.0 mL, 0.2 mL and 0.1 mL

Procedure Fibrometer*

Preparation of Dade® Fibrinogen Standard for Standard Curve: Make dilutions of Dade® Fibrinogen Standard from 1:4 to 1:32 in Dade® Owren's Veronal Buffer using 12 x 75 mm plastic tubes. Use a clean pipette tip to mix each tube thoroughly and discard the pipette tip after transferring contents.

See example below:

Test Tube	Owren's Veronal Buffer	Fibrinogen Standard	Transfer from Tube 1	Resultant dilution	Concentration factor[+]
1	1.5 mL	0.5 mL	---	1:4	x 2.5
2	0.4 mL	---	0.6 mL	1:6.67	x 1.5
3	0.6 mL	---	0.4 mL	1:10	x 1
4	0.3 mL	---	0.1 mL	1:16	x 0.625
5	0.7 mL	---	0.1 mL	1:32	x 0.312

[+] The equivalent fibrinogen level of each standard dilution referenced to a dilution of 1:10 is determined by multiplying the assay value of the Fibrinogen Standard by its concentration factor.

Assay Procedure

1. Place cups into the heat block for the number of tests to be performed.
2. Pipette 0.2 mL of each dilution of Dade® Fibrinogen Standard into the number of cups.
3. Incubate the dilution cup at 37 °C for 2 minutes (not longer than 5 minutes).
4. Add 0.1 mL Dade® Thrombin Reagent (stored at room temperature) into the cup and start the Fibrometer timer.
5. Record the clotting time when Fibrometer timer stops.
6. For each of the five dilutions, plot the mean clotting time on furnished graph paper. Connection of the points usually approximates a straight line. This calibration curve may be extended beyond the 1:4 and 1:32 dilution to a maximum of 800 mg/dL and a minimum of 50 mg/dL.

Patient Sample

Dilute patient sample and control plasma 1:10 with Dade® Owren's Veronal Buffer. Repeat steps 2 to 5 above, substituting a 1:10 dilution of patient plasma or control for the Dade® Fibrinogen Standard dilution.

Note: It is recommended to run duplicate determinations on each dilution of Dade® Fibrinogen Standard as well as on each patient and control plasma.

Note: Calibration curves are valid as long as the reagent used in the determination has the same lot number as that used in making your curve. New curves should be produced with each change of reagent lot or any change of instrument.

Results

Plot the results of the fibrinogen standards on 2-cycle log-log graph paper with the fibrinogen concentration on the x-axis and the time in seconds on the y-axis. Connected points should produce a smooth curve. Determine the fibrinogen concentration in mg/dL from the standard curve by reading the clotting time obtained on 1:10 plasma dilutions.

For fibrinogen values greater than 800 mg/dL: If the time on any individual patient is extremely short, dilute the plasma 1:20 (0.1 mL + 1.9 mL buffer) instead of 1:10. Read the value from your curve and multiply by the dilution factor of 2.

Example:

A clotting time of 6.0 seconds on a given calibration curve might indicate a fibrinogen concentration of approximately 400 mg/dL. If a 1:20 dilution was used, the reading would then be multiplied by a factor of 2:

400 mg/dL x 2 = 800 mg/dL

For fibrinogen values lower than 50 mg/dL: If a prolonged clotting time is obtained using the 1:10 dilution of the patient's plasma, test a 1:5 (0.2 mL + 0.8 mL buffer) or a 1:2 (0.4 mL + 0.4 mL buffer) dilution. Read the value from your curve and divide by the dilution factor (2 for 1:5 dilution, 5 for 1:2 dilution).

Example:

If a 1:5 dilution was used, the reading would be divided by the factor of 2:

90 mg/dL ÷ 2 = 45 mg/dL

No clotting with 1:2 dilution of patient plasma suggests a fibrinogen concentration below 15 mg/dL or a fibrinogen abnormality.

Quality Control

Run a normal control such as Dade® Ci-Trol® Coagulation Control, Level 1 and an abnormal control such as Dade® Data-Fi® Abnormal Fibrinogen Control with each set of fibrinogen determinations. When testing controls, follow the procedure for plasma samples and establish a 95 % confidence limit. If control values are outside the determined range, reagent and equipment should be checked. It is recommended before reporting any patient data to document the actions taken to identify and correct the problem.

Limitations of the Procedure

Results obtained may be affected by the presence of heparin or fibrino(geno)lytic degradation products in the patient's plasma. Significant levels of either of these substances may cause the test to indicate a falsely low fibrinogen level. Because of the high thrombin concentrations used and the dilution of the plasma, heparin concentrations below 1.0 USP units/mL do not significantly alter fibrinogen values[5]. Similarly, levels of fibrino(geno)lytic degradation products below 100 µg/mL do not significantly affect fibrinogen values.

Siemens Healthineers has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens Healthineers as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Healthineers Application Sheets or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values

1.8 – 3.5 g/L (180 to 350 mg/dL)[8]

Each laboratory should determine the reference range on a representative sample population since reference ranges may vary from laboratory to laboratory.

Specific Performance Characteristics

Data obtained using the thrombin clotting time method show excellent correlation with other commonly used fibrinogen quantitative methods[4,6].

Technical Assistance

For customer support, contact your local technical support provider or distributor.

siemens-healthineers.com

Current Version of Application Sheets

FIBRINOGEN DETERMINATION can be used in combination with various automated coagulation analyzers. Siemens Healthineers provides Reference Guides/Application Sheets for coagulation analyzers under the dedicated link below:

siemens-healthineers.com/rg

As Siemens Healthineers continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Siemens Healthineers ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via siemens-healthineers.com/eIFU.

Bibliography

1. Clauss A. Rapid physiological coagulation method in determination of fibrinogen. Acta Haematol 1957; 17: 237-46.
2. Borgström S. On the prothrombin index in acute affections of the pancreas. Acta Chir Scand 1945; 90: 419-430.
3. Jacobsson K. I. Studies on the determination of fibrinogen in human blood plasma. II. Studies on the trypsin and plasmin inhibitors in human blood serum. Scand J Clin Lab Invest 1955; 7: 7-13 (Supplement).
4. Morse EE, Panek S, Menga R. Automated fibrinogen determination. Am J Clin Pathol 1971; 55: 671-6.
5. CLSI Guideline H30-A2: Procedure for the determination of fibrinogen in plasma. Approved Guideline - Second Edition. CLSI, 940 West Valley Road, Suite 1400. Wayne, Pennsylvania 19087-1989 USA, 2001.
6. Okuno T, Selenko V. Plasma fibrinogen determination by automated thrombin time. Am J Med Technol 1972; 38: 196-201.
7. CLSI Guideline H21-A5: Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays. Approved Guideline - Fifth Edition. CLSI, 940 West Valley Road, Suite 1400. Wayne, Pennsylvania 19087-1989 USA, 2008.
8. Wagner C, Dati F. Fibrinogen. In: Thomas L, ed. Clinical Laboratory Diagnostics. Frankfurt: TH Books Verlagsgesellschaft, 1998; 609-12.

Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community		Contains sufficient for <n> tests
	Biological Risks	IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation		Consult instruction for Use
	Non-sterile		CE marking of conformity
CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.	CONTENTS	Contents
	Reconstitution volume	LEVEL	Level
	Keep away from sunlight and heat	WARNING	Warning
DANGER	Danger	RxOnly	Prescription device (US only)
UDI	Device Identification (UDI) barcode	REACH xx/xx/xx	REACH Authorization Number

Legal Information

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