SIEMENS Healthineers

Dade® Thrombin Reagent

THROMBIN **REAGENT**

Revision bar indicates update to previous version.

Intended Use

For use in the quantitative determination of fibrinogen in plasma and to accelerate coagulation of anticoagulated samples for immunohematology studies.

Summary and Explanation

Depressed levels of fibrinogen are found in acquired and congenital hypo- and afibrinogenemias.

Acquired fibrinogen deficient conditions occur especially as a result of intravascular proteolysis of fibrinogen by thrombin (consumption coagulopathy e.g. in obstetrics, following surgical intervention), snake venom or plasmin (primary hyperfibrinolysis following therapy with streptokinase, urokinase or tPA). Furthermore, moderate hypofibrinogenemias may occur in cases of diminished production (in acute or chronic liver diseases), loss into the intravascular space (e.g. in ascites or acute hemorrhage and burns) or increased degradation (in shock or carcinoma).

Temporarily elevated levels of fibrinogen are found as a result of the behavior of fibrinogen as an "acute-phase protein":

a. Transitory hyperfibrinogenemias may occur after operations, traumas, myocardial infarction and infections[1].
b. Persistent hyperfibrinogenemias may be found in patients with neoplasias and chronic inflammatory diseases.

Levels of fibrinogen are found to increase slightly with age.

Elevated fibrinogen levels are a risk factor for cardiovascular disease[2].

Blood samples collected from patients who have received heparin may not clot and units of plasma may need to be converted to serum prior to laboratory use. Thrombin can be used to accelerate clotting of anticoagulated samples and units of plasma for use in immunohematologic tests[3].

Principles of the Procedure

The enzyme thrombin converts the soluble plasma protein fibrinogen into its insoluble polymer, fibrin. The clotting time for diluted plasma is inversely proportional to the fibrinogen concentration of the plasma[4–5]. By using this principle, Clauss[4] developed a simple procedure for determining fibrinogen based on measuring the clotting time of diluted plasma after the addition of thrombin. The clotting time obtained in this manner is then compared with that of a standardized fibrinogen preparation.

Reagents

Note: **THROMBIN** **REAGENT** 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 this case, the information contained in the Reference Guides (Application Sheets) supersedes the

information in these Instructions for Use. Please also consult the instruction manual of the instrument manufacturer!

Reagent	Description	Storage	Stability
Dade® Thrombin Reagent THROMBIN REAGENT	Lyophilized reagent containing: • Thrombin, bovine (reconstituted: ~100 IU/mL) • Stabilizer • Buffer	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: reconstituted, 5 days[a]; 15–25 °C: reconstituted, 8 hours[a]

[a] closed original vial

On-board stability
Information regarding 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
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

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.

Preparing Reagents

Dissolve the THROMBIN REAGENT with the amount of distilled or deionized water indicated on the vial label. Close the vial and let stand until the contents have dissolved. Carefully swirl the contents to mix. Do not shake. Mix carefully once more before using.

Note: Do not use any water containing preservatives.

Always store THROMBIN REAGENT in the original vial.

Indication that the reagent cannot be used: Lack of reproducible values.

Specimen Collection and Handling

To obtain the plasma, carefully mix one part sodium citrate solution 0.11 mol/L (3.2 %) with nine parts venous blood, avoiding the formation of foam. Centrifuge as soon as possible for no less than 15 minutes at 1 500 to 2 500 × g and remove the supernatant plasma.

If analysis is to take place immediately, the plasma can either remain on the packed cells or be separated. To separate, transfer the plasma with a plastic pipette into a plastic tube and store at 2 to 8 °C. Do not store on ice.

Although investigations[7] have shown that there is no significant change in fibrinogen values when plasma samples are stored at 4 °C for up to 72 hours, it is advisable to test the samples as quickly as possible after collection.

Please refer to CLSI document H21-A5[6] for detailed information on sample preparation and storage.

Procedure

Materials Provided

REF	Contents		
B4233-25	Dade® Thrombin Reagent THROMBIN REAGENT	10 × →	1 mL
B4233-27	Dade® Thrombin Reagent THROMBIN REAGENT	10 × →	5 mL

Materials Required but not Provided

Item	Description
REF ORKE45 REF B4244-10	CONTROL N, Control Plasma N, or Ci-Trol CONTROL 1, Dade® Ci-Trol® Coagulation Control Level 1
REF OUPZ19	CONTROL P, Control Plasma P
REF B4233-22	Data-Fi FIBRINOGEN CONTROL, Dade® Data-Fi® Abnormal Fibrinogen Control Plasma
REF B4234-25	OV BUFFER, Dade® Owren's Veronal Buffer
REF ORKL19	STANDARD PLASMA, Standard Human Plasma
–	Sodium citrate solution for blood collection for coagulation tests
Coagulation analyzers, such as:	• SYSMEX CA-600 series • SYSMEX CA-1500 System • SYSMEX CS-2500 System • SYSMEX CS-5100 System

Manual Testing

Fibrinogen Determination

Dilute patient and control plasma 1:10 with OV BUFFER.

Pipette into prewarmed coagulation tubes as follows:

	Patient Plasma	Control Plasma
Plasma sample (diluted 1:10)	0.2 mL	–
Control Plasma (diluted 1:10)	–	0.2 mL
	Incubate in waterbath at 37 °C for 1–2 minutes or in heatblock at 37 °C for 2–4 minutes (no longer than 5 minutes).	
THROMBIN REAGENT (stored at 15–25 °C)	0.1 mL	0.1 mL
	Start stopwatch simultaneously with addition of THROMBIN REAGENT	

Always test samples and controls in duplicate.

Performing Calibration

Prepare five dilutions of STANDARD PLASMA with OV BUFFER ranging from 1:4 to 1:32.

Carefully mix the contents of each tube with a clean pipette for each tube.

Example:

Test Tube	OV BUFFER	STANDARD PLASMA	Transfer from Tube 1	Dilution	Conversion Factor[b]
1	1.5 mL	0.5 mL	–	1:4	× 2.5
2	0.4 mL	–	0.6 mL	1:6.67	× 1.5

Test Tube	OV BUFFER	STANDARD PLASMA	Transfer from Tube 1	Dilution	Conversion Factor[b]
3	0.6 mL	–	0.4 mL	1:10	× 1.0
4	0.3 mL	–	0.1 mL	1:16	× 0.625
5	0.7 mL	–	0.1 mL	1:32	× 0.312

[b] The corresponding fibrinogen content of each standard dilution in relation to a 1:10 dilution is determined by multiplying the indicated STANDARD PLASMA concentration with the respective conversion factor.

These dilutions are used for establishing the reference curve rather than the 1:10 dilutions used in the assay for patient or control sample. Plot the average clotting time for each of the 5 points on double logarithmic paper. Record the fibrinogen concentration on the x-axis and the time in seconds on the y-axis. The reference line is produced by connecting the points.

A new reference curve must be established each time there is a change in equipment or a new lot of THROMBIN REAGENT is used.

Internal Quality Control

Normal range: Ci-Trol CONTROL 1, or CONTROL N

Pathological range: Data-Fi FIBRINOGEN CONTROL, or CONTROL P

Two controls (one in the normal range and one in the pathological range) have to be measured at least once every 8 hours for assays run for patient testing during that interval. Controls should be run after each new calibration curve and after each change of reagent vial. Recalibration may be necessary if control values are outside the target range. Do not release patient results until the cause of deviation has been identified and corrected.

Calculating the Analytical Results (manual method)

Determine the fibrinogen concentration of the patient plasmas in g/L by using the calibration curve and the clotting time obtained with the 1:10 plasma dilutions.

1. If very short times are obtained (high concentration of fibrinogen), dilute the plasma 1:20 (0.1 mL + 1.9 mL buffer) and analyze again. Then multiply the value in g/L read from the curve with the dilution factor (2).
2. If very long times are obtained (low concentration of fibrinogen), dilute the plasma only 1:5 (0.2 mL + 0.8 mL buffer) or 1:2 (0.4 mL + 0.4 mL buffer) and analyze again. Then divide the value in g/L read from the curve with the dilution factor 5 or 2 respectively.
3. No clotting in the 1:2 dilution of a patient plasma indicates a fibrinogen concentration of less than 0.15 g/L.

Clotting of Heparinized Patient Samples

To whole blood or separated plasma, add the amount of dry thrombin that adheres to the tips of several applicator sticks, or add 1 to 2 drops (0.1 mL) of reconstituted THROMBIN REAGENT (100 U/mL) per 1 mL of sample. Mix and incubate at 37 °C for 5 to 10 minutes.

Clotting of Units of Plasma

To 250 mL of plasma, add 1 to 2 mL of reconstituted THROMBIN REAGENT (100 U/mL). Mix and incubate at 37 °C for 30 minutes to 1 hour.

Note: Clotting can be further accelerated by reconstituting the THROMBIN REAGENT in 1 M $CaCl_2$ instead of distilled or deionized water and label accordingly.

Limitations

Levels of the following do not appear to interfere with THROMBIN REAGENT on the SYSMEX CA-1500 analyzer:

	Up to
Bilirubin	6 mg/dL
Hemoglobin (free)	100 mg/dL

	Up to
Triglycerides	284 mg/dL
Heparin (LMW)	0.4 U/mL
Heparin (unfractionated)	0.6 U/mL

The results obtained may be influenced by the presence of heparin or fibrino(geno)lytic degradation products in patient plasma. Significant amounts of each of these substances may lead to a false low value for fibrinogen in the test[8].

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 Reference Guides (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 to 3.5 g/L[9]

Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

For more information on establishing reference intervals see CLSI document EP28-A3C[10].

Performance Characteristics

The data obtained with the thrombin clotting time method correlated excellently with other methods often used for the quantitative determination of fibrinogen[7,11].

Precision

The THROMBIN REAGENT assay was used to measure fibrinogen concentrations in normal and pathological controls and patient pools. Eight determinations per day over 5 days (n = 40) were performed using a SYSMEX CA-1500 analyzer.

	Mean value (g/L)	Within-Run CV (%)	Run-to-Run CV (%)	Total CV (%)
CONTROL N	2.6	5.9	0.0	5.9
CONTROL P	0.89	4.8	0.0	4.8
Plasma pool (low)	0.95	7.1	2.3	7.4
Normal plasma pool	2.8	3.5	0.0	3.5
Data-Fi FIBRINOGEN CONTROL	1.1	3.8	2.4	4.5

Method Comparison

THROMBIN REAGENT was compared to FIBRINOGEN DETERMINATION, using the SYSMEX CA-1500 analyzer, by evaluating 80 plasma samples with concentrations ranging from 0.50 to 8.6 g/L fibrinogen. Regression analysis of the results yielded the following equations:

THROMBIN REAGENT	(n =)	Slope	Intercept	Correlation Coefficient
	80	1.03	-0.063 g/L	0.995

Technical Assistance

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

siemens-healthineers.com

Current Version of Application Sheets

THROMBIN REAGENT can be used in combination with various automated coagulation analyzers. Siemens Healthineers provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 3 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.

References

1. Cooper J, Douglas AS. Fibrinogen level as a predictor of mortality in survivors of myocardial infarction. Fibrinolysis 1991; 5: 105-8.
2. Cook NS, Ubben D. Fibrinogen as a major risk factor in cardiovascular disease. Trends Pharmacol Sci. 1990; 11: 444-51.
3. Technical Manual of the American Association of Blood Banks 9th ed. Arlington: AABB; 1985: 417.
4. Clauss A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol. 1957; 17: 237-46.
5. Jacobsson K. Studies on the determination of fibrinogen in human blood plasma. Scand J Clin Lab Invest. 1955; 7 (Suppl 14): 1-54.
6. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays. Approved Guideline – Fifth Edition. CLSI document **H21-A5** [ISBN 1-56238-657-3]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
7. Morse EE, Panek S, Menga R. Automated fibrinogen determination. Amer J Clin Pathol. 1971; 55: 671-6.
8. CLSI. Procedure for the determination of fibrinogen in plasma; Approved Guideline – Second Edition. CLSI document **H30-A2**. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2001.
9. Wagner C, Dati F. Fibrinogen. In: Thomas L, editor. Clinical Laboratory Diagnostics. Frankfurt: TH-Books Verlagsgesellschaft; 1998: 609-12.
10. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, Approved Guidline – Third Edition. CLSI document **EP28-A3C** [ISBN 1-56238-682-4]. CLSI, 950 West Valley Road, Suite 2500, Wayne, PA 19087-1898 USA, 2010.
11. Okuno T, Selenko V. Plasma fibrinogen determination by automated thrombin time. Amer J Med Tech. 1972; 38: 196-201.
12. Zhang L, Yang J, Zheng X, Fan Q, Zhang Z: Influences of argatroban on five fibrinogen assays. Int J Lab Hematol. 2017 Dec;39(6):641-644. doi: 10.1111/ijlh.12719.

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	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

Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestraße 127
91052 Erlangen
Germany
Phone: +49 9131 84-0
siemens-healthineers.com

Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg
Germany
siemens-healthineers.com