Dade® Fibrinogen Determination Reagents

FIBRINOGEN **DETERMINATION**

Revision bar indicates update to previous version.

CE 0197

Intended Use

FIBRINOGEN **DETERMINATION** are in vitro diagnostic reagents for the quantitative, WHO-standardized determination of fibrinogen as an aid to diagnosis of congenital or acquired fibrinogen deficiency or dysfunction in patients with bleeding disorders or at risk for fibrinogen deficiency in human sodium citrated plasma by means of automated, semi-automated and/or manual coagulometric methods.

In addition, **FIBRINOGEN** **DETERMINATION** can be used as an aid in diagnosis and monitoring of fibrinogen consumption in patients at risk or with signs of disseminated intravascular coagulopathy (DIC).

Summary and Explanation

Fibrinogen, a 340 kDa glycoprotein synthesized in the liver, is essential for the formation of a fibrin clot. Cleavage of fibrinogen by thrombin generates fibrin monomers, which spontaneously polymerize, forming a first unstable fibrin clot, which is stabilized by cross-links induced by FXIIIa activity. Decreased or dysfunctional fibrinogen often causes an increased risk for bleeding[1–4].

The function and quantity of fibrinogen in plasma can be altered by both inherited and acquired disorders:

- Inherited defects can lead to a decreased fibrinogen concentration (hypofibrinogenemia) or dysfunctional protein (dysfibrinogenemia). Dysfibrinogenemia can be associated with bleeding or thrombosis, or both[1–3,5].
- Acquired fibrinogen deficiency states can occur as a result of increased consumption (e.g. disseminated intravascular coagulopathy[6], fibrinolytic therapy), reduced synthesis in severe liver disease, or hemodilution[4].
- **FIBRINOGEN** **DETERMINATION** are reagents for the quantification of functional fibrinogen according to the Clauss method. The determination of fibrinogen in plasma is indicated in the following cases:
  - diagnosing congenital or acquired fibrinogen deficiency states,
  - monitoring fibrinogen substitution therapy
- As fibrinogen reacts as "acute-phase" protein, plasma levels increase in response to acute and chronic inflammation, like infections, trauma, surgery, acute cardiac events or cancer. Elevated fibrinogen levels were shown to be associated with an increased risk for major cardiovascular events as well as nonvascular mortality[1,7].

Principles of the Procedure

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[8,9,10]. Using this principle, Clauss[8] 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

Note: FIBRINOGEN DETERMINATION can be used manually or on automated coagulation analyzers. Sysmex 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® Fibrinogen Determination Reagents FIBRINOGEN DETERMINATION			
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.	15–25 °C: reconstituted, 8 hours[a]; 2–8 °C: reconstituted, 5 days[a]
FIBRINOGEN STANDARD	Lyophilized reagent containing: • human plasma • HEPES • Stabilizer	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 4 hours[b]
OV BUFFER	Ready to use liquid containing: • Sodium barbiturate (0.0285 mol/L) • Sodium chloride (0.125 mol/L) pH 7.35 ±0.1	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 8 weeks

[a] closed original vial
[b] closed vial

The fibrinogen content is tested with a method for determining coagulable proteins.

The concentration of the Standard is given on the enclosed Table of Assigned Values.

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.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.

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.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed). In case Eudamed is not available, SSP can be delivered by the manufacturer on request.

Preparing Reagents

Reconstitute THROMBIN REAGENT with 1.0 mL distilled or deionized water. Restopper the vial and allow to stand until the content is dissolved. Invert gently to mix. Do not shake. Mix carefully once more before using.

Note: Do not use water containing preservatives. Always keep THROMBIN REAGENT in the original vial during use and storage.

Reconstitute FIBRINOGEN STANDARD with 1.0 mL distilled or deionized water and dissolve by agitating carefully (without foam formation). Allow to stand at least 15 minutes at 15 to 25 °C. Do not shake. Mix carefully once more before use.

OV BUFFER is ready to use.

Indication of deterioration: inability to obtain reproducible values.

Specimen Collection and Handling

Collecting the Specimen

Note: For patient conditions which may affect test results, see "Limitations", page 6.

Mix nine parts of freshly collected patient blood with one part sodium citrate 0.11 mol/L (3.2 %) (customary blood collection systems).

Centrifuge the blood specimen for a minimum of 15 minutes at 1500 × g as soon as possible after collection. For alternative blood collection procedure, see the CLSI document H21-A5[11].

If immediate testing is to be done, the plasma may remain on the packed cells or be separated. To separate the plasma, use a plastic transfer pipette, remove the plasma to a plastic tube and keep refrigerated at 2 to 8 °C until ready to test. Do not store on ice.

Storing the Specimen

Although studies[12] have demonstrated that there is no significant change in fibrinogen values for plasma samples stored up to 72 hours at 4 °C, it is advisable to test samples as soon as possible after collection.

Procedure

Materials Provided

REF	Contents	
B4233-15SY	Dade® Fibrinogen Determination Reagents FIBRINOGEN DETERMINATION	
	Dade® Thrombin Reagent THROMBIN REAGENT	6 × → 1 mL
	Dade® Fibrinogen Standard FIBRINOGEN STANDARD	1 × → 1 mL
	Table of Assigned Values	
	Dade® Owren's Veronal Buffer OV BUFFER	3 × 15 mL

Materials Required but not Provided

Item	Description
REF ORKE41 REF 291070 REF B4244-10	CONTROL N, Control Plasma N, or Dade® Ci-Trol® 1, or Ci-Trol CONTROL 1, Dade® Ci-Trol® Coagulation Control Level 1
REF OUPZ17 REF B4233-22	CONTROL P, Control Plasma P, or Data-Fi FIBRINOGEN CONTROL, Dade® Data-Fi® Abnormal Fibrinogen Control Plasma
–	Sodium citrate solution for drawing blood for coagulation tests
Coagulation analyzers[c], such as:	• Automated Blood Coagulation Analyzer CA-600 series (CA-600 series) • AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

[c] Availability of analyzers may vary by country.

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Semi-automated or manual testing is possible but not validated by the manufacturer.

Manual Testing

Dilute patient and control plasma 1:10 with Dade® Owren's Veronal 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 timer immediately upon adding the THROMBIN REAGENT	

Performing Calibration

Dilute FIBRINOGEN STANDARD in OV BUFFER from 1:4 to 1:32. Use a clean pipette tip each time to mix the tube carefully and then discard the pipette tip.

Example:

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

[d] The corresponding fibrinogen content of each standard dilution in relation to a 1:10 dilution is determined by multiplying the FIBRINOGEN STANDARD concentration given with the appropriate conversion factor. The standard dilutions are used in the test instead of the 1:10 dilution of the patient or control plasma. For each of the 5 points, plot the average clotting time on double logarithmic paper. Record the fibrinogen concentration on the x-axis and the time, in seconds, on the y-axis. A reference curve is created by connecting the points.

Note: Double determinations are recommended for every dilution of the Fibrinogen Standard as well as for every patient and control plasma.

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

For each calibration and at least every 8 hours on each work day, two controls (one in the normal range and one in the pathological range) should be measured. The control material should be handled the same as a sample. Each laboratory should determine its own quality control range either with the target values and ranges indicated by the manufacturer or by means of the acceptance range determined in the laboratory. If the measured control values lie outside the previously determined range, then the reagents, the calibration curve and the coagulation analyzer should be examined.

Documentation of the steps undertaken to identify and eliminate the problem is recommended before releasing patient results.

Results

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

For fibrinogen values over 8 g/L: 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 4 g/L. If a 1:20 dilution was used, the reading would then be multiplied by a factor of 2:

4 g/L × 2 = 8 g/L

For fibrinogen values lower than 0.5 g/L: 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 appropriate 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:

0.9 g/L ÷ 2 = 0.45 g/L

No clotting with a 1:2 dilution of patient plasma suggests a fibrinogen concentration below 0.15 g/L or a fibrinogen abnormality.

Limitations

The manufacturer has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by the manufacturer 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 Application Sheets or these Instructions for Use.

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[13].

Direct thrombin inhibitors may interfere with fibrinogen assays according to Clauss Method. Test results from patients under DTI therapy should be interpreted with caution[14].

Blood plasma substitutes that contain either dextran or hydroxyethyl starch (HES) may interfere with the analysis.

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

Expected Values

Normal Population

1.8 to 3.5 g/L[15]

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.

Affected Population

Classification of congenital fibrinogen disorders – modified according to ISTH recommendation[16]

Classification	Fibrinogen level (functional fibrinogen)
Afibrinogenemia	Undetectable or reduced amounts
Severe Hypofibrinogenemia	<0.50 g/L
Moderate Hypofibrinogenemia	0.50–0.90 g/L
Mild Hypofibrinogenemia	1.00 g/L – lower limit of normal value

Performance Characteristics

Application specific performance data is listed in the respective Reference Guides of the instruments.

The uncertainty of the calibrator to the reference material is included in the Certificate of Traceability (CoT). CoT is available on sysmex-ifu.com.

Measuring Range

The measuring range depends on the individual application of the assay due to instrument related conditions. The Limit of Quantitation (LoQ) is equal or lower than the lower limit of the measuring range.

Precision

The precision of THROMBIN REAGENT in the CA-1500 System was tested with CONTROL N and CONTROL P over 5 days in 8-fold determinations.

The coefficient of variation for the series was 1.6 % and 6.4 % for CONTROL N and CONTROL P, respectively. From day to day they were 2.7 % and 3.5 %, respectively.

Other system specific results are given in the respective Reference Guides (Application Sheets).

The reproducibility was assessed by the manufacturer for Fibrinogen with FIBRINOGEN DETERMINATION based on publicly available proficiency testing information in 2018. The overall reproducibility median CV% was found to be <7 % including lot, instrument, laboratory and operator variability factors.

Technical Assistance

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

Current Version of Application Sheets

FIBRINOGEN DETERMINATION can be used in combination with various automated coagulation analyzers. Sysmex provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 4 under the dedicated link below:

sysmex-ifu.com/ag

As the manufacturer 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. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.

References

1. Verhovsek M, Moffat KA, Hayward CP. Laboratory testing for fibrinogen abnormalities. Am J Hematol. 2008; 83:928-31.
2. Mackie IJ, Kitchen S, Machin SJ, et al. Guidelines on fibrinogen assays. Br J Haematol 2003;121(3):396-404.
3. de Moerloose P, Schved JF, Nugent D. Rare coagulation disorders: fibrinogen, factor VII and factor XIII. Haemophilia. 2016; 22 Suppl 5:61-5.
4. Besser MW, MacDonald SG. Acquired hypofibrinogenemia: current perspectives. J Blood Med 2016; 7:217-225.
5. Hill M, Dolan G. Diagnosis, clinical features and molecular assessment of the dysfibrinogenaemias. Haemophilia. 2008; 14:889-97.
6. Toh CH, Alhamdi Y, Abrams ST. Current Pathological and Laboratory Considerations in the Diagnosis of Disseminated Intravascular Coagulation. Ann Lab Med. 2016; 36:505-12.
7. Fibrinogen Studies Collaboration, Danesh J, Lewington S, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA. 2005; 294:1799-809.
8. Clauss A. Rapid physiological coagulation method in determination of fibrinogen. Acta Haematol 1957; 17: 237-46.
9. Borgström S. On the prothrombin index in acute affections of the pancreas. Acta Chir Scand. 1945; 90: 419-30.
10. 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).
11. 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.
12. Heil W, Grunewald R, Amend M, et al. Influence of time and temperature on coagulation analytes in stored plasma. Clin Chem Lab Med. 1998;36:459-62.
13. 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.
14. 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.

15. Wagner C, Dati F. Fibrinogen. In: Thomas L, editor. Clinical Laboratory Diagnostics. Frankfurt: TH-Books Verlagsgesellschaft; 1998: 609-12.
16. Casini A, Undas A, Palla R, et al. for the Subcommittee on Factor XIII and Fibrinogen. Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH. J Thromb Haemost 2018;16:1887–90.

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	CH REP	Authorized representative in Switzerland
	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

Ci-Trol, Dade and Data-Fi are trademarks of Siemens Healthineers.
Sysmex is a trademark of SYSMEX CORPORATION.
All other trademarks are the property of their respective owners.

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