Dade® Thrombin Reagent

THROMBIN **REAGENT**

Revision bar indicates update to previous version.

CE 0197

Intended Use

THROMBIN **REAGENT** is an in vitro diagnostic reagent 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, **THROMBIN** **REAGENT** 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].

THROMBIN **REAGENT** is a reagent 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

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[8–9]. By using this principle, Clauss[8] 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. 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® 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

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
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.

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

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[11] 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[10] 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 ORKE41 REF 291070 REF B4244-10	CONTROL N, Control Plasma N, or Dade® Ci-Trol® 1 Ci-Trol CONTROL 1, Dade® Ci-Trol® Coagulation Control Level 1
REF OUPZ17	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 ORKL17	STANDARD PLASMA, Standard Human Plasma
–	Sodium citrate solution for blood collection for coagulation tests
Coagulation analyzers[b], 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)

[b] 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.

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[c]
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

[c] 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

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.

Direct thrombin inhibitors may interfere with fibrinogen assays according to Clauss Method. Test results from patients under DTI therapy should be interpreted with caution[16].

Blood plasma substitutes that contain 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

1.8 to 3.5 g/L[13]

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[14].

Performance Characteristics

The data obtained with the thrombin clotting time method correlated excellently with other methods often used for the quantitative determination of fibrinogen[11,15].

Measuring Range

The measuring range depends on the individual application of the assay due to instrument related conditions. Application specific performance data is listed in the respective Reference Guides of the instruments.

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 CA-1500 System.

Sample	n	Mean [g/L]	Within-Run CV [%]	Run-to-Run CV [%]	Total CV [%]
CONTROL N	40	2.6	5.9	0.0	5.9
CONTROL P	40	0.89	4.8	0.0	4.8
Plasma pool (low)	40	0.95	7.1	2.3	7.4
Normal plasma pool	40	2.8	3.5	0.0	3.5
Data-Fi FIBRINOGEN CONTROL	40	1.1	3.8	2.4	4.5

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

The reproducibility was assessed by the manufacturer for fibrinogen with THROMBIN REAGENT based on publicly available proficiency testing information in 2019. The overall reproducibility median CV % was found to be <7 % including lot, instrument, laboratory and operator variability factors.

Method Comparison

THROMBIN REAGENT was compared to FIBRINOGEN DETERMINATION, using the CA-1500 System, 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:

	n	Slope	Intercept	Correlation Coefficient
THROMBIN REAGENT	80	1.03	−0.063 g/L	0.995

Interference

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

Substance	Test concentration
Bilirubin	6 mg/dL
Hemoglobin (free)	100 mg/dL
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[12].

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

Direct thrombin inhibitors may interfere with fibrinogen assays according to Clauss Method. Test results from patients under DTI therapy should be interpreted with caution[16].

Technical Assistance

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

Current Version of Application Sheets

THROMBIN REAGENT 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 3 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

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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

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