Thromborel® S

Revision bar indicates update to previous version.

CE 0197

Intended Use

Thromborel® S is an in vitro diagnostic reagent for the quantitative determination of prothrombin time (PT) as an aid to diagnosis, screening for hemostasis disorders and monitoring of oral anticoagulation therapy with vitamin K antagonists in human sodium citrated plasma by means of automated, semi-automated and/or manual coagulometric methods.

For monitoring vitamin K antagonist anticoagulation therapy PT is reported in WHO-standardized INR (International Normalized Ratio).

Summary and Explanation

Thromborel® S is a lyophilized human placental thromboplastin reagent. The reagent initiates clotting via the extrinsic and common pathways in a global screening test, the prothrombin time (PT).

The PT is a clinically important test for the detection of coagulation abnormalities and can be used[1–3]:

- for monitoring of oral anticoagulant therapy in patients receiving vitamin K antagonists,
- in pre-surgery bleeding risk assessment,
- in screening for bleeding disorders like suspected extrinsic factor deficiency,
- as aid in diagnosis for hemostasis disorders like liver disease or DIC
- to derive the fibrinogen concentration (when in the normal range) from the PT by photo-optical coagulation analyzers.

The sensitivity of Thromborel® S for monitoring oral anticoagulant therapy with vitamin K antagonists is very similar to the first WHO human brain reference thromboplastin and make it beneficial in monitoring oral anticoagulant therapy with vitamin K antagonists. In addition, its high sensitivity (i.e. the responsiveness of the reagent to moderately depleted factor activity) allows differentiation of abnormal plasmas, even in the mildly pathological range[4,5].

Furthermore, Thromborel® S can be used in combination with the respective factor FII, FV, FX or FVII deficient plasma for the quantification of the coagulation factors: FII, FV, FX and FVII.

Principles of the Procedure

The coagulation process is triggered by incubation of plasma with the optimal amount of thromboplastin and calcium. The time to formation of a fibrin clot is then measured.

Reagents

Note: Thromborel® S 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
Thromborel® S	Lyophilized reagent containing: • placenta dry (reconstituted: ≤60 g/L) • Calcium chloride (reconstituted: ~1.5 g/L) • Stabilizer • Preservatives	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 8 hours[a]; 15–25 °C: reconstituted, 2 days[a]; 2–8 °C: reconstituted, 5 days[b]

[a] opened vial
[b] closed vial

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

Note on reagent expiration: Control values outside the assigned range for the control used (e.g., CONTROL N) are an indicator of reagent expiration.

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.

Warning! Thromborel® S
Hazardous ingredient: Gentamicin sulfate (0.115 % [w/w]), reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (0.0239 % [w/w]).
H317: May cause an allergic skin reaction. **H412**: Harmful to aquatic life with long lasting effects. **P261**: Avoid breathing dust. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P302 + P352**: IF ON SKIN: Wash with plenty of soap and water. **P333 + P313**: If skin irritation or rash occurs: Get medical advice/attention. **P362 + P364**: Take off contaminated clothing and wash it before reuse. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

CAUTION! POTENTIAL BIOHAZARD
The reagent is prepared from human placenta. During the manufacturing process, steps are taken to remove and/or inactivate any viruses which may be present.
Nevertheless, since absence of infectious agents cannot be proven, all materials obtained from human tissue or body fluids should always be handled with due care, observing the precautions recommended for biohazardous material.

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 Thromborel® S Reagent with the amount of distilled or deionized water stated on the vial label and mix well by inverting the vial 8 to 10 times, then warm the reagent to 37 °C before use. Please note: after reaching 37 °C the reagent must be incubated at this temperature for 30 minutes. If a water bath is used a total incubation time of 45 minutes is recommended. Before use the reagent should be mixed carefully.

Specimen Collection and Handling

Please refer to CLSI document H21-A5 for detailed information on sample preparation and storage[8].

Collecting the Specimen

Mix nine parts of freshly collected patient blood with one part of 0.11 or 0.13 mol/L (3.2 % or 3.8 %) sodium citrate solution. An evacuated tube system or syringe may be used.

Storing the Specimen

Store in an unopened tube at room temperature. Do not store on ice or at 2 to 8 °C as cold activation of FVII may alter results.

Plasma should be tested within 24 hours of blood collection. Samples should not stand at 37 °C for more than 5 minutes. If the patient is on both heparin and coumarin-based anticoagulant therapy, the results may vary with time of storage.

Procedure

Materials Provided

REF	Contents	
OUHP29	Thromborel® S Table of lot- and analyzer-specific ISI Values	10 × → 4 mL
OUHP49	Thromborel® S Table of lot- and analyzer-specific ISI Values	10 × → 10 mL

Materials Required but not Provided

Item	Description
REF ORKE41 REF B4244-10 REF 291070	CONTROL N, Control Plasma N, Ci-Trol CONTROL 1, Dade® Ci-Trol® Coagulation Control Level 1 or Dade® Ci-Trol® 1
REF OUPZ17 REF B4244-20 REF 291071	CONTROL P, Control Plasma P, Ci-Trol CONTROL 2, Dade® Ci-Trol® Coagulation Control Level 2 or Dade® Ci-Trol® 2
REF B4244-30 REF 291072	Ci-Trol CONTROL 3, Dade® Ci-Trol® Coagulation Control Level 3 or Dade® Ci-Trol® 3
REF OPAT03	PT-Multi CALIBRATOR, PT-Multi Calibrator (Refer to the Instructions for Use for details on use)
REF ORKL17	STANDARD PLASMA, Standard Human Plasma, or fresh normal plasma[7] for determining the reaction time of normal plasma
–	For blood collection, use sodium citrate (0.11 mol/L / 3.2 %), or
–	Distilled or deionized water without preservatives
–	Plastic test tubes
–	Plastic transfer pipettes
–	Pipettes for precise measurement of 10.0 mL, 1.0 mL, 0.20 mL and 0.10 mL
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.

Manual Testing

Pipette into a test tube pre-warmed to 37 °C:	
Citrated plasma	100 µL
Incubate at 37 °C for 1 minute.	
Thromborel® S Reagent (warmed to 37 °C)	200 µL
On addition of Thromborel® S Reagent start stop-watch or timer on the coagulation analyzer and determine the coagulation time.	

Establishment of the Reference Curve in % of Norm

Refer to the Reference Guide (Application Sheet) for your analyzer for calculation using % of Norm.

Internal Quality Control

Normal range: CONTROL N or Ci-Trol CONTROL 1

Therapeutic range: CONTROL P, Ci-Trol CONTROL 2 or Ci-Trol CONTROL 3

Two levels of quality control material (normal and pathological range) have to be measured at start of the test run, with each calibration, upon reagent vial changes and at least every eight hours on each day of testing. The control material should be processed as a sample. Each laboratory should establish its own QC ranges based on the assigned values and ranges provided by the control manufacturer or based on values determined by the laboratory.

If control values are recovered outside the defined range, check the instrument, reagent and calibration for problems. Do not release patient results until the cause of deviation has been identified and corrected.

Results

The results can be reported in seconds, in % of Norm, or as International Normalized Ratio (INR). These results should be related to the normal range for PT testing in each laboratory. It is suggested that the patient results be reported to the clinician in conjunction with the reference interval. Some instruments enable a technical support in the comparison of PT results in relation to normal results, see Prothrombin Ratio (PR). Monitoring of oral anticoagulant therapy with vitamin K antagonists should only be reported with PT results expressed as INR as recommended in official guidelines and in the literature[7].

To obtain the prothrombin ratio, the reaction time of the sample is divided by the reaction time of the normal plasma pool (e.g. STANDARD PLASMA):

$$PR = \frac{\text{Reaction time of sample (seconds)}}{\text{Reaction time of normal plasma (seconds)}}$$

If the prothrombin ratio is determined using a normal plasma which does not have a PR of 1.0, the PR of this plasma has to be taken into account in the calculation:

$$PR = \frac{\text{Reaction time of sample (seconds) x PR of normal plasma}}{\text{Reaction time of normal plasma (seconds)}}$$

The prothrombin ratio can be converted into internationally comparable values by means of the International Sensitivity Index (ISI). The result obtained is in International Normalized Ratio (INR):

$INR = PR^{ISI}$

Thromborel® S Reagent is calibrated against the international reference thromboplastin preparations in test runs on normal plasmas and plasmas of donors on oral anticoagulants in the stable phase. The ISI value for Thromborel® S Reagent is stated in the lot-dependent Table of Assigned Values.

Derived Fibrinogen

Using Thromborel® S Reagent and the appropriate assay on coagulation analyzers, the fibrinogen concentration may be derived by analyzing the change in optical signal during prothrombin time

determinations, using a derived fibrinogen calibration curve. This calibration curve (master curve) is provided in the lot-dependent Table of Assigned Values.

Limitations

Normal samples spiked with heparin concentrations exceeding 0.6 U/mL produced abnormal results. However, Thromborel® S Reagent may be used to monitor the administration of overlapping dosages of heparin and oral anticoagulants. Inhibitors of the Lupus type anticoagulant can influence prothrombin time and lead to INRs that do not accurately reflect the true level of anticoagulation[9].

Lipoglycopeptide antibacterial drugs (such as oritavancin or telavancin) may interfere with PT based assays. Consult Instructions for Use of respective drugs.

The choice of anticoagulant (i.e. oxalate instead of citrate) and the condition of the specimen (e.g hemolyzed, lipemic, parenteral feeding, etc.) may affect results of PT and derived Fibrinogen. The latter is particularly true for PT measurements done with optical instruments. Hirudin or other direct thrombin inhibitors in therapeutic dose result in prolonged prothrombin times[10–11].

Derived fibrinogen results within the reference interval can be directly reported. Results outside the reference interval should be re-measured by a standard fibrinogen determination method, e.g. Fibrinogen method with Dade® Thrombin Reagent or with Multifibren® U reagent. Derived fibrinogen testing is not suitable in patients with dysfibrinogenemia[12] or patients with prolonged PT, e.g. under oral anticoagulation or with PT% below 25 % of Norm[13,14]. In thrombolysis therapy, derived fibrinogen and fibrinogen determination according to Clauss may deviate and should be considered in therapy control. Blood plasma substitutes that contain hydroxyethyl starch (HES) may interfere with the analysis. Therefore, it is advised that plasma samples that contain such substitutes should not be analyzed with the PT derived fibrinogen method.

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

Expected Values

Values for healthy individuals vary from laboratory to laboratory depending on the technique used. Therefore, each laboratory should establish its own reference intervals based on the procedure and coagulation analyzers used.

In studies on the CA-7000 System with ostensibly healthy subjects the following reference intervals (2.5^{th} to 97.5^{th} percentile) were determined:

		% Reference Interval	
	n	**2.5^{th} Percentile**	**97.5^{th} Percentile**
PT	158	9.8 seconds	12.1 seconds
Derived Fibrinogen	124	1.7 g/L	3.2 g/L
PT (% of Norm)[15]		70 %	130 %

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.

Therapeutic Ranges

Therapeutic ranges for INR may vary depending on the indication of oral anticoagulant therapy[6].

Performance Characteristics

Measuring Range

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

Sensitivity

Factor Sensitivity of Thromborel® S

According to CLSI H47-A2, the PT reagent/instrument combination used should provide abnormally prolonged results for plasmas that have less than 30 % factor activity of the coagulation factors: FII, FV, FVII and FX. CLSI H47-A2 recommends to determine sensitivity levels by serial dilution of normal plasma into deficient plasma. Sensitivity levels determined by this method should ideally be within 30 and 45 %. However, the factor sensitivity levels determined by this method is strongly dependent on deficient plasma used[16].

Precision

The precision of the PT determination is highly dependent on the method used. The precision of Thromborel® S Reagent on the BCT System was estimated by assaying normal and pathological control plasmas over a five day period, one run per day in replicates of eight. In a study the within-run precision ranged from 0.7 to 1.2 %, and the inter-assay precision ranged from 1.5 to 2.2 %.

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

The reproducibility was assessed by the manufacturer for Thromborel® S based on proficiency testing information in 2018/2019. The overall reproducibility median CV% was found to be

- PT % <8 %
- PT seconds <5 %
- PT INR <7 %
- Derived Fibrinogen <11 %

including lot, instrument, laboratory and operator variability factors.

Method Comparison

A comparison of Thromborel® S Reagent with the British Comparative Thromboplastin yielded a correlation coefficient of 0.979, with good numerical agreement of the values in % of Norm[4].

Technical Assistance

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

Current Version of Application Sheets

Thromborel® S 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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