Pathromtin® SL

Revision bar indicates update to previous version.

CE 0197

Intended Use

Pathromtin® SL is an in vitro diagnostic reagent for the quantitative determination of activated partial thromboplastin time (APTT) as an aid to diagnosis, screening for hemostasis disorders and monitoring of unfractionated heparin in human sodium citrated plasma by means of automated, semi-automated and/or manual coagulometric methods.

For APTT testing no international reference preparation or method is available.

Summary and Explanation

Pathromtin® SL is a liquid reagent based on plant phospholipids with silicon dioxide particles for plasma activation.

The APTT, a global screening procedure[1,2] used primarily to evaluate coagulation abnormalities in the intrinsic pathway, will also detect severe functional deficiencies in factors: FII,FV, FX, or fibrinogen. The APTT has also been widely advocated[3–6] as a means to monitor the effectiveness of unfractionated heparin therapy where the clotting time is prolonged in proportion to the level of heparin. In patients receiving oral anticoagulants, the circulating levels of factors: FII, FVII, FIX, and FX are depressed therefore the APTT can be expected to be prolonged. The presence of non-specific inhibitors, such as the lupus-like anticoagulant[1,7], may prolong the APTT but this effect is variable and generally recognized as being related more to the nature of the APTT reagent employed.

In summary, the APTT is a clinically important screening test with wide applicability for the diagnosis of coagulant disorders and therapeutic monitoring of both, hemorrhagic and thrombotic disease[8–10], and is specifically used

- In pre-surgery bleeding risk assessment
- In screening for bleeding disorders, e.g. in case of suspected intrinsic factor deficiency or inhibitors to intrinsic coagulation factors
- As an aid to diagnosis of lupus anticoagulants (inhibitors) in patients with thrombophilia
- For monitoring of therapy in patients receiving unfractionated heparin

Furthermore, Pathromtin® SL can be used in combination with the respective factor: FVIII, FIX, FXI or FXII deficient plasma for the quantification of the coagulation factors: FVIII, FIX, FXI and FXII.

Principles of the Procedure

Incubation of plasma with the optimal quantity of phospholipids and a surface activator leads to activation of factors of the intrinsic coagulation system. The addition of calcium ions triggers the coagulation process; the time to formation of a fibrin clot is measured.

Reagents

Note: Pathromtin® SL can be used 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
Pathromtin® SL	Ready to use liquid containing: • Silicon dioxide (1.2 g/L) • soy lecithin (0.25 g/L) • Sodium chloride • HEPES, pH 7.6 • Preservative: • Sodium azide (<1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–25 °C: once opened, 2 weeks[a]

[a] closed original vial

Signs of expiry: Deviations from the normal laboratory value in the determination of normal plasma or controls.

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.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

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

Pathromtin® SL must be gently inverted (5 to 8 times) to mix before first use.

Pathromtin® SL must be used at room temperature (15 to 25 °C).

Every 24 hours of use, the reagent must be inverted to resuspend any sediment.

$CaCl_2$ SOLUTION 0.025 mol/L: warm to 37 °C.

Specimen Collection and Handling

Collecting the Specimen

To obtain the plasma, carefully mix one part of 0.11 or 0.13 mol/L (3.2 % or 3.8 %) sodium citrate solution with nine parts of freshly collected patient blood, avoiding the formation of foam. It is recommended that blood specimens for plasma-based coagulation testing should be collected by venipuncture using a blood collection system that collects the specimen directly into a glass or plastic evacuated tube containing the appropriate additive. Immediately centrifuge for no less than 15 minutes at 1 500 × g, remove the supernatant plasma and keep at 15 to 25 °C until use in the test (max. 4 hours).

Procedure

Materials Provided

REF	Contents		
OQGS29	Pathromtin® SL	10 ×	5 mL
OQGS35	Pathromtin® SL	20 ×	5 mL

Materials Required but not Provided

Item	Description
REF ORHO37	$CaCl_2$ SOLUTION, Calcium Chloride Solution, (0.025 mol/L)
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, as control for the normal range
REF 291071 REF B4244-20	Dade® Ci-Trol® 2, or Ci-Trol CONTROL 2, Dade® Ci-Trol® Coagulation Control Level 2, as control for the pathological/therapeutical range
REF 291072 REF B4244-30	Dade® Ci-Trol® 3, or Ci-Trol CONTROL 3, Dade® Ci-Trol® Coagulation Control Level 3, as control for the pathological/therapeutical range
REF B4224-50	Ci-Trol HEPARIN CONTROL 1, Dade® Ci-Trol® Heparin Control, Low
REF B4224-60	Ci-Trol HEPARIN CONTROL 2, Dade® Ci-Trol® Heparin Control, High
–	Pipettes for precise measurement of 0.1 mL
–	Plastic test tubes
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

Pre-warm $CaCl_2$ SOLUTION at 37 °C	
Pipette into a test tube pre-warmed to 37 °C:	
Citrated plasma	100 µL
Pathromtin® SL	100 µL Mix well. Incubate at 37 °C for 2 minutes.
$CaCl_2$ SOLUTION (pre-warmed)	100 µL On addition of $CaCl_2$ SOLUTION start stop-watch or timer on the coagulation analyzer and determine the coagulation time.

Monitoring of Unfractionated Heparin Therapy with APTT

When using the APTT for this purpose, the factors influencing the test should be kept in mind. General considerations are listed below.

A. Time of collection is important since the *in-vivo* half-life of unfractionated heparin is approximately 1.5 hours[5]. When it is administered, it has an immediate anticoagulant effect

but the degree of this effect decreases rapidly with time. This is especially apparent with intermittent single intravenous injections.

B. The anticoagulant used for sample collection can alter test results.

C. Platelet factor 4, a heparin neutralizing factor in platelet alpha-granules, can be released by platelet aggregation or damage. To prevent this occurrence *in-vitro*, the specimen should be collected with a minimum of trauma. Cold temperatures are known to induce platelet aggregation and release platelet factor 4; therefore, centrifugation at room temperature is recommended for heparin testing.

D. Using APTT to monitor unfractionated heparin therapy is time-dependent. Delay in testing samples will result in prolonged APTT determinations. Therefore, it is imperative that the testing on all samples be performed as soon as possible.

E. Increased contact activation times may result in prolonged APTT in plasma containing heparin. It is imperative that the optimal heating-activation time of the Pathromtin® SL-plasma mixture be rigidly standardized[12].

F. Different test systems (i.e. manual, photo-optical, etc.) will exhibit variable heparin sensitivity. Interchanging of test systems should be avoided.

G. Baseline data on the APTT of each patient before the start of therapy should be established where feasible to determine the respective patient APTT as it relates to the reference range established for the test in that laboratory.

H. Studies have shown variability in original estimates of the quality of unfractionated heparin from different sources and different manufacturers. *In-vivo* reactivity varies with the type of heparin administered, the metabolism of the individual and other coadministrated medications[5,12].

I. Because the APTT can vary with technique, method, equipment, reagent lot and heparin used, each laboratory must establish its own therapeutic ranges, or verify them whenever one or more of the aforementioned variables is changed. This can be done by simultaneously determining the APTT and the heparin concentration for samples from patients receiving heparin therapy. A dose response curve can be calculated from the data using regression analysis, and the APTT range corresponding to a heparin concentration of 0.3 to 0.7 U/mL (by a factor Xa inhibition assay) can be derived[5,13,4].

Internal Quality Control

Normal range:	Dade® Ci-Trol® 1, Ci-Trol CONTROL 1, or CONTROL N
Pathological range:	Dade® Ci-Trol® 2, Ci-Trol CONTROL 2, or Dade® Ci-Trol® 3, Ci-Trol CONTROL 3, or CONTROL P
Heparin monitoring:	Ci-Trol HEPARIN CONTROL 1 Ci-Trol HEPARIN CONTROL 2

Two controls (one in the normal range and one in the pathological/therapeutic range) must be measured at the start of the test run, after each change of reagent vial, and at least once during an 8-hour shift. The control material should be prepared and processed in the same manner as the patient samples. Each laboratory should establish its own confidence intervals for the controls. This interval is generally ±2 to ±2.5 standard deviations from the mean control value. If the control values are outside of the confidence interval, the controls, reagents and instrument must be checked. Before reporting the patient values, it is recommended that all steps should be documented that were taken to identify and rectify the problem. New control ranges should be defined for each new lot of reagents or controls.

Results

The result is given in seconds.

Limitations

It should be noted that APTT testing may be affected by several commonly administered drugs. Therapeutic doses of hirudin or other direct thrombin inhibitors may prolong clotting times[18,19].

Lipoglycopeptide antibacterial drugs (such as oritavancin or telavancin) may interfere with APTT based assays. Consult Instructions for Use of respective drugs[20,21].

Decrease in time of APTT determination in conjugated estrogen therapy in males and oral contraceptive administration in females has been reported[22,23]. Increase in the APTT has been seen in diphenylhydantoin, heparin, warfarin, naloxone and radiographic agent administration[24]. Heparin or low molecular weight Heparin (LMWH) is used for the treatment of thromboembolic diseases followed by prophylaxis of recurrence using oral anticoagulants. The effect of Heparin is monitored by the activated partial thromboplastin time (APTT), the thrombin time (TT) or a dedicated Heparin assay. The discontinuation of heparin depends on whether the therapeutic range of the oral anticoagulation is achieved. Heparinase (Enzyme Commission number EC 4.2.2.7) has been shown to eliminate heparin from plasma[25].

The APTT assay is a functional test which screens for global coagulation disorders of the endogenous coagulation system. It is common knowledge that a low concentration of coagulation factors: FII, FV, FX and high concentration of fibrin(ogen) degradation products also have influence on the assay[26]. The condition of the specimen may affect results. Hemolyzed, icteric and /or lipemic samples might either interfere chemically, biologically or with the physical properties of the detection principle of instruments[27].

The latter is particularly true for optical instrumentation measurements of the APTT. Preactivation of the sample due to poor phlebotomy technique can lead to false results. Referencing the Sample Handling and Collection section of CLSI Document H21-A5[11] it is recommended that test tubes with non-wetable surfaces (e.g., plastic) be used rather than glass test tubes.

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

For the purpose of APTT screening, results in seconds should be related to the reference interval for APTT testing in each laboratory.

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.

In a study of ostensibly healthy individuals using a specific lot of Pathromtin® SL, the following values were obtained:

			90 % Reference Interval	
	n	Median [s]	5^{th} Percentile [s]	95^{th} Percentile [s]
CA-1500 System	100	30.9	26.4	37.5
BCS® System	111	30.2	25.9	36.6

Reference ranges for other populations such as pediatric groups should also be established where warranted.

Note: CLSI Document C28-A3c (cited in H47-A)[29]. states that a parametric approach (mean ± 2 SD) can be applied. The assumption of this approach (Gaussian normal distribution) must however be checked.

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

For characterization of an APTT reagent, determination of heparin sensitivity, factor sensitivity, and Lupus sensitivity is important. These are reagent characteristics which are independent from the analyser used.

Factor Sensitivity

According to CLSI H47-A2, the APTT reagent/instrument combination used should provide abnormally prolonged results for plasmas that have less than 30 % factor activity of the coagulation factors: FVIII, FIX and FXI. 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[28].

Heparin Sensitivity

For determination of heparin sensitivity 167 samples from patients receiving unfractionated heparin were analyzed. By comparing the APTT ratio obtained with Pathromtin® SL and the Heparin activity measured with Berichrom Heparin a correlation coefficient r = 0.709 was observed. Each individual laboratory hospital should determine its own therapeutic heparin range using the ex vivo method according the CLSI guidelines (H57-A2)[29].

Lupus Sensitivity

By testing a panel of LA-negative donors from ostensibly healthy donors and 97 commercially available frozen plasma samples from LA-positive patients (as determined by dRVVT, PT and thrombin time) the following sensitivity was observed:

Sensitivity of Pathromtin® SL[30]

APTT Reagent	Sensitivity (%) 95th cut-off	Sensitivity (%) 99th cut-off
Pathromtin® SL	72.2	59.8

Precision

Using opto-densitometric detection, precision studies were run over a 5 day period, twice per day (n = 8 replicates per day), to total n = 40 for normal and pathological control plasmas as well as a heparin plasma pool. Intra-assay precision was calculated from the individual n = 4 precision values for the daily runs over the 5 days. Intra-assay precision ranged from 0.6 to 2.0 % CV, while the inter-assay precision ranged from 0.3 to 2.8 % CV.

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

The reproducibility was assessed by the manufacturer for APTT with Pathromtin® SL based on publicly available proficiency testing information in 2018/2019. The overall reproducibility median CV% was found to be <5 % including lot, instrument, laboratory and operator variability factors.

Method Comparison

Results of a comparison of APTT determinations using Pathromtin® SL Reagent and another commercially available APTT reagent gave a correlation coefficient of 0.96 and a y-intercept of 2.1 s, and a slope of 0.99.

Technical Assistance

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

Current Version of Application Sheets

Pathromtin® SL 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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