ASPItest

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REF		CONTENT	SYSTEM
08847533190	08847533500	3 x ⟶ 1.0 mL	Multiplate

English

Intended use

Assay for the quantitative in vitro determination of platelet function triggered by arachidonic acid. The reagent is intended for use in platelet function testing with whole blood samples on the Multiplate analyzer.

Summary

Arachidonic acid is an omega-6 fatty acid that is found in many of the body's cell membranes. The conversion of arachidonic acid to thromboxane is regulated by the enzyme cyclooxygenase (COX). This enzyme exists in two forms: COX1 the constitutive form found in all tissues and COX2 which is induced during inflammatory states. Only COX1 is present in platelets and is easily inhibited by low dose acetylsalicylic acid that inactivates a key enzyme involved in arachidonate metabolism. Acetylsalicylic acid acetylates serine residue 529 in the polypeptide chain of the prostaglandin H synthase (PGH-synthase), thus inhibiting the production of PGH2 which is the direct precursor of thromboxane. Because platelets are anucleate they do not readily regenerate protein. The effect of acetylsalicylic acid on COX1 is irreversible and lasts for the lifetime of the platelet thereby relying on the generation of new platelets to recover cyclooxygenase activity at a rate of approximately 10 % per day in normal healthy individuals. Low dose acetylsalicylic acid is sufficient to suppress greater than 95 % of thromboxane production by COX1 and such suppression is capable of inhibiting platelet aggregation. However, acetylsalicylic acid affected platelets may still aggregate in the presence of potent platelet agonists such as collagen and thrombin.[1] Arachidonic acid does not itself activate platelets, thereby providing the ideal method to determine the extent of COX1 activity in platelets. If COX1 activity is inhibited by acetylsalicylic acid or other nonsteroidal like agents, resulting aggregation will be reduced or absent.[2] Because platelet aggregation is achieved through platelet-fibrinogen binding at the glycoprotein IIb/IIIa receptor (GpIIb/IIIa) sites, arachidonic-acid-induced platelet aggregation may be reduced or absent in the presence of GpIIb/IIIa receptor antagonists or a deficiency in GpIIb/IIIa receptors such as encountered in Glanzmann thrombasthenia.

Test principle

The ASPItest reagent contains arachidonic acid, which is converted to thromboxane A2 by the platelet cyclooxygenase.

In the Test Cells activated platelets adhere to and aggregate on the sensor wires. This leads to an increased resistance between the sensor wires, which is continuously recorded and expressed via the area under the curve (AUC) in arbitrary units (AU*min or U; conversion: 1 U = 10 AU*min).[3] The ASPItest assay is sensitive to an inhibition of the platelet cyclooxygenase[2,4,5] as well as an inhibition or absence of the GpIIb/IIIa receptor.[6,7]

Reagents - working solutions

SR[a]: Lyophilized reagent containing arachidonic acid: 15 mmol/L; 3 vials, each for 1.0 mL

a) Start reagent

Precautions and warnings

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H411	Toxic to aquatic life with long lasting effects.
EUH 019	May form explosive peroxides

Prevention:

P261	Avoid breathing dust.
P264	Wash skin thoroughly after handling.
P273	Avoid release to the environment.
P280	Wear protective gloves/ eye protection/ face protection.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P391	Collect spillage.

Hazardous components:

- reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

Carefully reconstitute the contents of 1 vial of SR by adding 1.0 mL of distilled or deionized water. Gently swirl SR and allow vial to stand closed for a minimum of 10 minutes at 15-25 °C. Swirl the vial carefully to produce a homogeneous solution before use. Avoid the formation of foam.

Note: The vials are filled with an inert gas instead of a vacuum. After reconstitution you can pipette ≥ 100 µL aliquots of the reagent into micro test tubes for daily use.

The solution is slightly yellow. The color does not indicate a reduction in function.

Storage and stability

Store at 2-8 °C.

The lyophilized reagents are stable up to the stated expiration date. Store the reconstituted reagent (aliquoted or in the original vial) in an upright position.

Stability of the reconstituted reagent either:	
at 15-25 °C	24 hours
at 2-8 °C	24 hours
at -20 °C (±5 °C)	28 days
after 1 time thawing at 15-25 °C	24 hours

Protect reagent from exposure to light, air and elevated temperature ranges.

Do not re-freeze.

Thaw the reagent at 15-25 °C for at least 1 hour while kept in an upright position. Avoid the contact of the reagent with the rubber stopper. Swirl gently the original vial or micro test tube to homogenize the solution prior to use.

Specimen collection and preparation

Only the specimen listed below was tested and found acceptable: venous whole blood.

Use standard sampling tubes to collect the sample and follow the instructions for use from the appropriate manufacturer. Blood collection should be performed with caution to avoid prolonged venous stasis and using a large-bore needle during draw. Avoid foam formation in the blood collection tube.

Gently invert the collection tube to ensure complete mixing of the contents.[8]

Do not store the blood sample on a roll mixer.

Do not freeze or refrigerate samples. Do not preheat the blood before analysis.[9,10]

The anticoagulant used for blood sample collection significantly affects the results of the assay.[9,11] The use of commercial hirudin blood collection tubes is recommended.[12]

Alternatively standard citrated tubes (3.2 % citrate) may be used.[13,14,15] Always ensure citrated blood collection tubes are filled to the indicated fill volume in order to avoid excessive citrate levels.

The blood collection system must be standardized at each center. It is only possible to compare the results of an individual sample with reference ranges when the same sample anticoagulant is employed. Sample collection systems from various manufacturers may contain differing materials which could affect the test results. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Analyze blood samples within the period of 0.5 to 3 hours after blood collection.

Visually check the blood samples for clots before measurement. If clots are found the blood sample has to be refused. Microthrombi in the sample could adversely affect the test results. Mix the blood sample by inverting the tube gently just before measurement.

Materials provided

- See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 06675590001, Test Cells, 6 x 10 pieces
- Saline solution 0.9 % (without additives, e.g. methyl ester)
- Multiplate analyzer. See operator’s manual and Quick Start Guide of the analyzer for additionally required materials.
- Distilled or deionized water
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Place SR and Test Cells in the designated position.

Test procedure for hirudinized and citrated blood:	
Saline solution 0.9 % (prewarmed to 37 °C)	300 µL
Sample (15-25 °C)	300 µL
Incubation	180 seconds
SR	20 µL
Measuring time	6 minutes

Final concentration: 0.5 mmol/L arachidonic acid

Temperature conditions and incubation times must be precisely observed. When using the electronic pipette follow the software instructions displayed by the Multiplate analyzer.

Quality control

A normal blood sample can be used as a control of the activity and stability of the reagent.

Follow the applicable government regulations and local guidelines for quality control.

Limitations - interferences

Impaired platelet function has been reported after the ingestion of various drugs and herbal medicines.[16,17] Aggregation may be abnormal in patients with thrombocytopenia.[18,19]

The presence of GpIIb/IIIa antagonists in the sample leads to weak aggregation.[3]

Certain fatty acids found in various human diets are widely known to inhibit platelet function.[20,21]

No significant effect on the test result has been observed with hematocrit values between 34 to 55 %.

Hemolysis may interfere with test results. Therefore, the use of hemolyzed blood samples is not recommended.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested.

Endogenous substances: No impact on results was observed up to the listed concentrations.

Compound	**Concentration**
Conjugated bilirubin	1 mg/dL
Unconjugated bilirubin	33 mg/dL

Pharmaceutical compounds: Interference was observed at listed therapeutic concentrations.

Compound	**Drug category**	**Therapeutic concentration**	**Effect on ASPItest**
Dabigatran	Anticoagulant	17.5 mg/dL	Decrease
Dextran 40	Replacement agent	20 g/L	Decrease
Diclofenac	Anti-inflammatory drug	8.45 µmol/L	Decrease
Ethanol	Local anesthetic	43.4 mmol/L	Decrease
Fenoprofen	Nonsteroidal anti-inflammatory drug	268 µmol/L	Decrease
Hydroxyethyl starch	Replacement agent	45.3 g/L	Decrease
Ibuprofen	Nonsteroidal anti-inflammatory drug	340 µmol/L	Decrease
Indomethacin	Nonsteroidal anti-inflammatory drug	50.5 µmol/L	Decrease
LMWH (Heparin)	Anticoagulant	1000 IU/L	Increase
Penicillin G	β-lactam antibiotic	10000 IU/mL	Decrease
Streptokinase	Thrombolytic agent	100000 IU/L	Decrease
Ticagrelor	Anticoagulant	27.7 mg/L	Decrease

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Expected values

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Expected values have been established in a study with 157 healthy donors using Sarstedt S-Monovette 1.6 mL hirudin tubes.

Result (2.5th to 97.5th percentile):

Tube type	AUC [U]
Hirudin tubes	86-162

Expected values have been established in a study with 156 healthy donors using BD Vacutainer 9NC 0.109 M Buff. Na_3 Citrate Tubes.

Result (2.5th to 97.5th percentile):

Tube type	AUC [U]
Citrated tubes (buffered)	51-112

Note: For establishing reference values the normal donor must not take nonsteroidal anti-inflammatory drugs (NSAIDs) or ADP-receptor antagonists within 10 days prior to testing.

Specific performance data

Representative performance data on the Multiplate analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Repeatability was determined for 3 reagent lots by measuring 12 hirudinized blood samples on 6 devices on all channels with 2 runs (i.e. 360 measurements in total). Mean, coefficient of variation (CV) and standard deviation (SD) were calculated for every blood sample. The pooled mean, pooled coefficient of variation (CV_{pool}) and pooled standard deviation (SD_{pool}) were calculated for every reagent lot.

ASPItest reagent lot	n (native)	$Mean_{pool}$ [U]	CV_{pool} [%]
1	360	110	6
2	360	106	6
3	360	105	7

ASPItest reagent lot	n (low)	$Mean_{pool}$ [U]	SD_{pool} [U]
1	360	20	4
2	360	20	3
3	360	20	2

Method comparison

A comparison of the ASPItest on a Multiplate analyzer with an automated platelet function test gave the following conformity between results in the normal and in the low range:

Number of samples measured: 114

Concordance (%): 90

Results of the ASPItest were between 6 U and 189 U.

References

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For further information, please refer to the appropriate operator's manual and quick start guide for the Multiplate analyzer, the respective applications sheets, the product information, and Method Sheets of all necessary components.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

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