CE
In Vitro
Diagnostic

Behnk Elektronik

LTA KIT (KIT 1 - 5)

Reagents for the determination of light transmission aggregometry in platelet rich plasma

REF 057301 REF 057302 REF 057303
REF 057304 REF 057305

INTENDED USE

This reagent kit is designated for professional in vitro diagnostics in the laboratory. It is used for the analysis of platelet function based on light transmission aggregometry (LTA) in platelet-rich human plasma on the fully automated system Thrombomate® XRA.
The tests assist in the diagnosis of platelet dysfunction and can be used as an aid in the management of patients with known dysfunction. Examination and evaluation should always include multiple agonists, which are considered in context.
These tests should be used in conjunction with other clinical and diagnostic information to diagnose and treat patients.

PRINCIPLE

After automated homogenization, platelet rich plasma (PRP) in segments of a cuvette strip is mixed with the reagents **ADP, AA, Col, Epi, TRAP** or **Ris** (see below) by an automated pipette. Platelet aggregation (or agglutination in the case of Ris) is measured under constant mixing with a steel ball at two wavelengths. This leads to a change of light transmission. Evaluated are the time from adding the reagent until the onset of the shape change or the aggregation, respectively, the velocity of aggregation (slope) and the maximum aggregation (in percent). See the manual or the literature for further details.

CLINICAL SIGNIFICANCE

Several receptors are involved in platelet aggregation by **ADP** (adenosine 5`-diphosphate), specifically P2Y1 and P2Y12. ADP induced stimulation triggers intracellular reactions via G-proteins, leading to release of substances from various platelet granules, including secretion of serotonin, thromboxane A2, and ADP. This induces platelet aggregation. Disorders in the function of ADP receptors can have various causes. Frequently they are induced by drugs directed against P2Y12. Excessive inhibition or other congenital or acquired disorders of ADP receptors may be associated with bleeding.
The most frequent cause for pathological aggregation with ADP is the influence of drugs that inhibit the function of the Gi-protein coupled purinergic P2Y12 receptor, specifically clopidogrel, prasugrel, ticagrelor,or Cangrelor. (7) In some patients the inhibitory effect of such drugs, especially clopidogrel, may be reduced or undetectable. (8),(9),(10) Aggregation with ADP may be absent in patients with Thrombasthenia Glanzmann while normal values will be obtained in Bernard-Soulier syndrome of in VWF deficiency. In storage pool disease, deficiency of thromboxane synthetase or cyclooxygenase 1 (COX-1), or after ingestion of aspirin often only the first wave of ADP aggregation is affected.
Aggregation of platelets by **AA** (arachidonic acid) is induced via stimulation of the biosynthesis of thromboxane A2 (TXA2). In platelets, AA is transformed via several steps into prostaglandin H2 by cyclooxygenase 1 (COX-1) and is further processed into TXA2 by thromboxane synthetase. Binding of TXA2 to the thromboxane prostanoid receptor α (TPα) induces signaling and a conformational change of the fibrinogen receptor GPIIB/IIIa, resulting in platelet aggregation. The most important cause for pathological values in AA induced aggregation is ingestion of aspirin or aspirin containing drugs which inactivate COX-1. (1)
The most important cause for pathological results with AA is the ingestion of aspirin or aspirin-containing drugs, less frequently are cases of "aspirin-like defects", thrombasthenia Glanzmann, deficiency of the enzyme COX-1 or thromboxane synthase, a deficiency of the thromboxane prostanoid receptor α, or storage pool disease. In some cases, acquired functional defects may give abnormal results with AA. This includes acquired or congenital forms or cardiac valve disease, use of on pump coronary bypass systems, uremia, hemolytic uremic syndrome (HUS), dialysis, glomerulonephritis, rejection after kidney transplant, sepsis / DIC, acute thrombosis, vascular disorders (cavernous hemangioma or aortic aneurysm), after burns, or at very low body temperature, sickle cell anemia, or splenectomy.
Col (collagen) activates several different receptors on the platelet surface, primarily GP IV and GP Ia/IIa. (2) A deficiency of collagen receptors may lead to a hemorrhagic diathesis and can enhance the reactivity towards drugs such as aspirin. The activation by collagen induces platelet aggregation, typically after a lag phase.
Abnormal results are found after ingestion of aspirin, in storage pool disease, in patients with deficiency of COX.1 or thromboxane synthase, and in patients with thrombasthenia Glanzmann, where the COL aggregation response is often totally absent. (11), (12)
Epi (epinephrine) activates the G-protein coupled α2-adreno receptor. This induces direct aggregation without "shape change" by exposure of the fibrinogen receptors, release of calcium ions from the endoplasmatic reticulum and inhibition of adenylate cyclase, inducing a reversible formation of aggregates. A second aggregation wave can be induced by release of ADP and thromboxane A2 from platelet granules. But this does not occur in all cases.
Healthy persons often show a weak reaction on EPI, specifically the absence of a second wave. But also, hyper reactivity is found (13), probably by genetic causes. (14), (15) Defects of the platelet α2-adreno receptor may lead to bleeding. (18) In other congenital platelet function defects the reactivity against EPI may be heterogeneous. Abnormal results can be found often in Bernard-Soulier syndrome, in storage pool defect, and in thrombasthenia Glanzmann, though not in all cases. Also, reactivity after aspirin ingestion may be heterogeneous.
The synthetic hexapeptide **TRAP** (thrombin receptor activating peptide) activates the thrombin receptor PAR-1 without cleavage of the molecule, hence resembling the activity of thrombin, the most potent activator of platelets. Thrombin activates several receptors on platelets, especially PAR-1, and induces intracellular signalling via G-proteins. This leads to formation of thromboxane A2 (TXA2), release of ADP, serotonin, epinephrine, and adhesive proteins such as p-selectin and CD40. This induces platelet aggregation and stimulation of the procoagulant function of platelets. (3) The effect of TRAP is only weakly inhibited by antiplatelet drugs such as aspirin or P2Y12 antagonists. Therefore, TRAP is a useful replacement for thrombin as platelet activator and can be used to detecting effects of GPIIb/IIIa antagonists or for general platelet function assessment.
Normal results with10 µM TRAP in PRP of healthy subjects Are typically at >70 % aggregation. Platelet drugs directed against GIIIb/IIIa (aggrastat, abciximab, integrilin) show a dosage dependent inhibition. (17), (18), (19)
P2Y12 antagonists show a weak inhibition. (20) In patients with peripheral arterial disease after angioplasthy a reduced inhibition of dual antiplatelet therapy was observed with TRAP. (21) PAR-1-antagonists (voraxapar) induce a concentration dependent inhibition of platelet aggregation with TRAP. (22, 23)
Ris (ristocetin), an antibiotic from Nocardia lucida, induces as a cofactor of VWF (von Willebrand -Factor) aggregation (or agglutination) of platelets via the VWF receptor (GPib-V-IX complex) in the platelet membrane. In patients with VWF syndrome, the following results are found in many but not in all cases:

VWF deficiency type	**Agglutination**
Type 1	No or lowered
Type 2A	No or lowered
Type 2B	Enhanced
Type 3	No or very low

In patients with Bernard-Soulier syndrome there is no aggregation/ agglutination, or it is drastically reduced. In Storage-Pool disease and thrombasthenia Glanzmann the first phase of agglutination is frequently affected. Defects of cyclooxygenase or thromboxane synthase or of aspirin are not detected. To some extent, results depend on platelet counts.

REAGENTS

Two plastic reagent trays with reagents of the following composition(s)

REF	Name	Reagent and concentration in the test					
		ADP	AA	Col	Epi	Ris	TRAP
057301	LTA 1	2.5 µM	1 mM	2 µg/ml	5 µM	-	10 µM
057302	LTA 2	5 µM	1 mM	2 µg/ml	-	-	-
057303	LTA 3	-	-	-	-	0,6 / 1,2 mg/ml	-
057304	LTA 4	2.5 M / 5 µM	1 mM	2 µg/ml	-	0,6 mg/ml	-
057305	LTA 5	2.5 M / 5 µM	1 mM	2 µg/ml	-	1,2 mg/ml	-

The reagent tray contains the following colour coded vials:

RE **ADP** **100 µM**
Adenosine-5'-diphosphate, stabilizers

RE **AA** **20 mM**
Arachidonic acid, stabilizers

RE **Col** **50 µg/ml**
Collagen (fibrillar, from horse tendon), stabilizers

RE **Epi** **100 µM**
Epinephrine ((R)-1-(3,4-Dihydroxyphenyl)-2-(N-methylamino)-ethanol), as bitartrate, stabilizers

RE **TRAP** **500 µM**
Thrombin Receptor PAR-1 activating peptide (S-F-L-L-R-N), stabilizers.

RE **Ris** **15 mg/ml**
Ristocetin, an antibiotic that agglutinates platelets in the presence of von Willebrand-Factor.

2 vials *LTA Diluent* 20 ml
DIL **LTA Diluent**
Dilution buffer

2 vials *Clean Pro* 15 ml
CL **Clean Pro**
Cleaning solution;
Sodium hydroxide solution (NaOH), 1 mol/L

Danger
H290, H314

According to 1272/2008 regulation, this reagent is classified as dangerous:
H290: May be corrosive to metals.
H314: Causes severe skin burns and eye damage.
Precautionary statements:
P280: Wear protective gloves/protective clothing/eye protection.
P301+**P330**+**P331**: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303+**P361**+**P353**: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P304+**P340**: IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305+**P351**+**P338**: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310: Immediately call a poison center/doctor.
Substances that contribute to the classification: sodium hydroxide

Additional Kit Content:
Balls 2 mm, *Silicone Caps* (Prevent evaporation) and *LTA Cuvette Bars*.
All reagents are only for use with Thrombomate® XRA.

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SAFETY CAUTIONS

Behnk reagent kits are designated for professional in-vitro-diagnostic use.
Good Laboratory Practices must be applied during use of reagents, reference or control plasmas, and human samples and should be handled as potentially infectious.
For further information, Material Safety datasheet is available upon request.
Dispose of waste in accordance with the local regulations.

PREPARATION OF REAGENTS

RE Remove the screw cap. First carefully lift the stopper of the bottle to release the bottle vacuum caused by production, then remove the stopper.
Reconstitute the lyophilizate in each vial with exactly 1 ml *LTA Diluent*.

DIL Ready to use.

CL Ready to use.

Fixing the silicone caps against evaporation: Lock each reagent vial in the and the Clean Pro bottle with a silicone cap. Let the reconstituted vial rest for 10 min and gently slew it prior to use in a circular motion.

Inserting into the instrument: Read the barcodes on the *Reagent Tray*, *LTA diluent*, and *Clean Pro* respectively in the reading position of the Thrombomate® XRA and insert them into the specific position in the instrument. Reading of the barcode initiates the automated stability monitoring.

Notice: The silicone caps should remain on the bottles for their entire lifetime. They are pierced by the pipetting needle during operation.

STABILITY AND STORAGE

Storage at 2-8 °C.
The un-opened reagents are stable until the declared expiration date.
Storage after reconstitution
The reconstituted reagents shall be stored capped with the silicon cap at 2-8 °C in the original vial. Do not freeze.
Stability after reconstitution
Reagent is stable in the original vial with the silicone cap:

Storage conditions	LTA 1 / 2 / 4 / 5	LTA 3
At 2-8 °C	28 days	28 days
Laboratory mode*	10 days	21 days
At 15-25 °C	7 days	14 days
All time onboard	7 days	14 days

** Laboratory mode* = 8 h on board, 16 h 2-8 °C
Reagents after the expiration date will not be accepted by the instrument.

SAMPLES COLLECTION AND HANDLING

Blood collection for aggregation testing should be made as gentle as possible into commercially available collection tubes. Use 0.11 M sodium citrate for anticoagulation.
Blood collection should be made by very gentle venous puncture, preferably without or with just minimum stasis of the vein. Mix blood and anticoagulant well by gentle inverting. Avoid foam formation. Store the sample at 15-25 °C. Do not expose the blood to lower temperatures than 15 °C and avoid mechanical stress through shaking during transportation and storage because this may lead to platelet activation. Pneumatic transportation systems are not recommended unless carefully validated.

Sample stability:
Maximum 4 hours after blood collection until completed analysis.

For **preparing platelet rich plasma (PRP)** the blood is centrifuged at room temperature for 150 x g. Optionally centrifuge again for 5 min (at 150 g) if there are erythrocytes visible in the supernatant.
Notice: Switch off the automatic brake function.
For better standardization and comparability of results use always the same centrifuge.
PRP is gently transferred with a disposable plastic pipette or a pipette with disposable plastic tip into a Thrombomate sample tube. Close this with the red cap piercing stopper.
Let the PRP rest for 30 min prior to analysis. The platelet count should be determined because a certain minimum quantity of platelets is required for a reliable measurement.

Adjustment of the platelet count by mixing of PRP with autologous platelet free plasma is not longer recommended, except in extremely low platelet numbers. (4),(5)
After filling the sample into the sample tube, a bar code label is fixed, read in the instrument, and the tube is inserted into a sample position in the instrument. Refer for further details the user manual f.e. if no barcode system is used.
For **preparation of platelet free plasma (PPP)** the residual blood of the sample or from a different sample of the patient is centrifuged for 20 min at 1500x g. Transfer the supernatant gently into a different sample tube.
Notice: Avoid formation of foam. Do not invert or place the tube into a horizontal position because this may induce bubble formation.

LIMITS

Several **preanalytical factors** (conditions of venous stasis, puncture of the vein, canula, anticoagulant, type of the tube, type of tub, conditions for sample transportation, residual number of erythrocytes, resting time after venepucture and others) may cause variable deviations.Therefore each lab should determine their own reference ranges.
For interpretation of results in patient samples results obtained with other lab tests should be considered (for example von Willebrand Factor, fibrinogen, whole blood count, aggregometry with other reagents or concentrations).
Many dietetic factors or drugs may influence platelet function (6). See the literature for further information.
Lipemia, bilirubin or hemolysis may influence results. Results may also be influenced by the platelet count. Platelet counts < 75/nl may lead to lower results.

Low concentrations of **ADP** (<0.5 – 2.5 µM) induce only a primary or reversible aggregation with unstable aggregates that may disaggregate. At higher ADP concentrations an irreversible second aggregation wave may lead to generation of thromboxane A2 and release of the contents of platelet α-granules.
Aggregation with **COL** starts after a lag-phase, in most cases in a single large wave. Abnormalities in the aggregation with COL my affect the lag-phase, the velocity of aggregation (slope), and the maximum aggregation.

MATERIAL REQUIRED BUT NOT PROVIDED

- Behnk Thrombomate® XRA
- General equipment for a medical laboratory
- *Sample tubes* (REF 057400)

PROCEDURE

Automatic method on Behnk Thrombomate® XRA
Running a test is fully automated after reagents and samples have been inserted into the instrument. See the Thrombomate® XRA manual for further details.

CALIBRATION

Not required

CALCULATION

Relative % representation of PPP to PRP sample.

QUALITY CONTROL

For quality control it is recommended to analyse with each series of patient samples also a sample from a known healthy donor without medication. This sample should be processed and analysed like the patient samples.

PERFORMANCES

Precision was determined on the Thrombomate® XRA compared with PAP-8 (manual system) for the maximum value of aggregation (%).
Analysis of precision was performed by 5-fold analysis of PRP from 5 different individuals.
Notice: All measurements with PAP-8 were performed by the same operator.

		Thrombomate® XRA	Manual System
Reagent	Test concentration	Mean CV	Mean CV
ADP	2.5 µM	**1.6**	**3.7**
AA	1.0 mM	**4.6**	**3.3**
Col	2.0 µg/ml	**1.4**	**3.0**
Epi	5.0 µM	**2.2**	**2.8**
TRAP	10 µM	**2.1**	**2.1**
Ris	1.2 mg/ml	**2.1**	**1.7**

Table 1: Precision analysis (5-fold determination, 5 individuals).
Numbers represent the CV values in %.

EXPECTED VALUES

Notice: Each laboratory should establish its own normal range for each agonist.
Expected results of healthy subjects are listed in the following table:

Reagent	Concentration	% Aggregation
ADP	2,5 µM or 5 µM	>65 %
AA	1 mM	>70 %
Col	2 µg/ml	>70 %
Epi	5 µM	>70 %
TRAP	10 µM	>70 %
Ris	1,2 mg/ml	>80 %
	0,6 mg/ml	<15 %

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Manufacturer	Expiry Date	In vitro Diagnostic	Temperature Range	Reference number	Information	Lot. Number	Keep out of sunlight	Content sufficient for	Reconstitute	Diluent	Corrosive

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