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

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.

Aggregation of platelets by arachidonic acid (**AA**) 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)

Collagen (**Col**) 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.

Epinephrin (**Epi**) 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.

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 signaling 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.

Ristocetin (**Ris**), 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

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

Ref.	Name	Reagent and cocentration in the test					
		ADP	AA	Col	Epi	Ris	TRAP
057301	LTA 1	2.5 µM	1 mM	2 µg/ml	5 µM	-	10µM
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 color coded vials:
ADP = ADP, adenosine diphosphate, stabilizers
AA = Arachidonic acid, stabiliszers
Col = Collagen (fibrillar, from horse tender), stabilizers
Epi = Epinephrin ((R)-1-(3,4-Dihydroxyphenyl)-2-(N-methylamino)-ethanol), as bitartrat, stabilizers.
TRAP = Thrombin Receptor PAR-1 activating peptide (S-F-L-L-R-N), stabilizers
Ris = Ristocetin, an antibiotic that agglutinates platelets in the presence of von Willebrand-Factor, stabilizers.

2 vials *LTA Diluent* 20 ml, and 2 vials *LTA Clean Pro,* 25 ml
LTA Diluent = Diluent (purified water without preservatives)
LTA Clean Pro = Cleaning solution;
Sodium hydroxide solution (NaOH),1 mol/L   *Danger*
  H290: May be corrosive to metals   *'90, H314*
  H314: Causes severe skin burns and eye damage

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

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

Reconstitution of the reagents: Remove screw cap and stopper of the vials in the Reagent Tray. Reconstitute with exactly 1 ml *LTA Diluent.*
LTA Diluent: Ready to use.
LTA Clean Pro: Ready to use.
Fixing the silicone caps against evaporation: Lock each reagent vial in the reagent tray and the LTA 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 LTA Clean Pro* respectively in the reading position of the Thrombomate and insert them into the specific position in the instrument. Reading of the barcode initiates the automated stability monitoring. (for detailed information refer to the User Manua)l
Notice: After the specified stability has expired, the reagent can no longer be used and the device will refuse to use it.
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
- At 2-8 °C   21 days
- At 15-25 °C   7 days
- All time onboard   7 days
- Laboratory mode*   14 days   *Laboratory mode* = 8 h Onboard, 16 h 2-8 °C
Reagents after the expiration date will not be accepted by the instrument.

SAMPLES COLLECTION AND HANDLING [2] [8]

Plasma
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 iverting. 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 throuhj skaing during transportation and storage because this meay lead to plateletactivation. Pneumatic trasportation 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 visisble 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 disposble 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 futher 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 inverst or place the tube into a horizontal position because this may induce bubble formation.

LIMITS

Nothing known so far.

MATERIAL REQUIRED BUT NOT PROVIDED

- General equipment for a medical laboratory
- *Sample tubes* (REF 057400)
- Calibrated pipette (1,0 ml) and disposable pipette tips
- Disposable plastic transfer pipettes
- Blood collection tubes
- Centrifuge
- *Thrombomate XRA*

PROCEDURE

Running a test is fully automated after reagents and samples have been inserted into the instrument. See the *Thrombomate* manual for further details.

CALIBRATION

Not required

CALCULATION

Relative % representation of PPP to PRP sample.

QUALITY CONTROL

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

PERFORMANCES

Pending

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 & 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 %

General aspects:
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, restig 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, aggreometry with other reagents or cocentrations). 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. 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.
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.

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 maxim aggregation. 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)
Healthy persons show often 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.
Normal results with 10 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)

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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