INSTRUCTIONS FOR USE

AGP TEST

TEST FOR THE EVALUATION OF PLATELET AGGREGATION INDUCED BY ADP, ADRENALINE, COLLAGEN AND RISTOCETIN

1 – CLINICAL SIGNIFICANCE AND INTENDED USE

For ***in Vitro*** diagnostic use only

When a blood vessel is damaged, platelets adhere to the wound edges, aggregate, synthesize prostaglandins, and release serotonin, ADP, and ATP. Synthesis of prostaglandins and release products causes further aggregation. Simultaneously, the coagulation cascade begins, thrombin is produced, fibrin is formed, and the platelet plug anchors to the damaged vessel.
Defects in platelet function, due to lack of a cell membrane glycoprotein (Glanzmann thromboasthenia; Bernard-Soulier syndrome), cytoplasmic storage granules (shortage of storage pool), a platelet enzyme (shortage of cyclooxygenase) or a plasma factor (von Willebrand disease) cause frequent post-traumatic bleeding, hematomas, epistaxis or excessive bleeding during menstruation.
Platelets undergo aggregation under different conditions and in the presence of different reactants (stimuli). The term "platelet aggregation" means the adhesion of one platelet to another. This phenomenon can be induced by adding aggregating agents to a platelet-rich plasma (PRP) or to whole blood. Aggregation is dependent on the presence of calcium, fibrinogen, plasma factors, and an aggregating agent. The aggregation reaction varies depending on the aggregating agent used and its concentration. For optical aggregometry (on PRP), ADP, Collagen, Epinephrine (Adrenaline) and Ristocetin are used for screening purposes and are able to provide useful information to make preliminary diagnostic hypotheses.
The choice of these reagents is based on their mechanism of action. ADP and Epinephrine (adrenaline) are contained in platelet granules and are released during the formation of the primary haemostatic plug, thereby potentiating the aggregation reaction. Therefore, the response of platelets to the stimulus generated in vitro with these agonists may be useful in recognizing the nature of the patient's bleeding disorders.
Collagen is not contained in platelets but is found in the connective tissue of blood vessels and is considered the first factor with pro-aggregative activity that platelets encounter following vascular injury. Therefore, the in vitro study of the response of platelets to Collagen is of considerable importance.
Other agonists such as Thrombin, calcium ionophore A23187, Arachidonic Acid, Ristocetin bovine Factor VIII and Serotonin are used to study the platelet response more specifically.
Aggregation is, at present, the most useful in vitro platelet function test. It is the diagnostic tool that can best provide insight that is difficult to obtain with other techniques and can help make a diagnosis and devise appropriate therapy. The use of this technique by experienced personnel has made it possible to describe and diagnose a number of congenital and acquired qualitative platelet dysfunctions. The ability or inability of platelets to respond to different aggregating stimuli is the starting point for identifying the type of platelet disease one is facing.
AGP TEST is a kit for the evaluation of platelet aggregation on PRP and whole blood.

2 - PRINCIPLE OF THE METHOD

The study of the aggregation on PRP is based on the evaluation of changes in transmittance in a plasma sample during the aggregation.
When ADP, Adrenaline and Collagen are added to platelet rich plasma, it stimulates platelets to change shape and to aggregate. The aggregation induced by exogenous ADP is primary and reversible aggregation. The aggregation induced by Adrenaline is primary aggregation. For the collagen there is a lag phase during which the collagen fibrils polymerize for platelet activation. The normal platelets release endogenous ADP from its granules. The release of endogenous ADP occurs in a second wave of aggregation.
The Ristocetin, a concentration of 1.0-1.5 mg/ml, normal platelet aggregates in rich plasma or citrated whole blood through a mechanism in which the release of endogenous ADP plays only a small role. Ristocetin-induced platelet aggregation at concentrations of 1.2 mg/ml is absent or significantly lower in patients with von Willebrand's syndrome. At concentrations of 1.5 mg/ml there is a lower degree of abnormality. The majority of patients with von Willebrand's syndrome shows a negative response, as well as patients with Bernard-Soulier syndrome.
Aggregation study in whole blood is based on to the evaluation of the electric resistance changes. Two electrodes while immersed in the sample are rapidly covered with platelets which, at the very first contact, appear in shape of monolayer. When the aggregating agent is added, additional platelets tend to attach the monolayer previously formed, determining an impedance increase between the two electrodes.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Confezione
AGP TEST	Platelet aggregation test	**3115001** (900 test)	2 glass vials containing lyophilized ADP. Reconstitute each vial with 0,5 mL of Diluent A to have a solution 0,1 mM (2 x 0,5 mL) 5 glass vials containing lyophilized adrenaline. Reconstitute each vial with 0,5 mL of bidistilled water to have a solution 5 mM. (5 x 0,5 mL) 1 glass vial containing Collagen. The vial contains a suspension with 1 mg/mL of Type 1 collagen fibrils sourced from equine tendon, type I (1 x 0,5 mL) 1 glass vial containing Ristocetin A sulphate lyopilised form; antibiotic isolated from Nocardia lurida, containing in excess of 90% Rsitocetin A (1 x 0,5 mL) 1 glass vial containing Diluent A: buffer for dilution with TRIS, pH 7,3 (1 x 50 mL) 1 glass vial containing Diluent B: solution to dilute the collagen (1 x 4 mL) Secondary packaging: cardboard box.

Adrenaline Lyophile

Danger

H300; H318
EUH031
P305+P351+P338; P310; P264; P330
(Sodium bisulphite; Epinephrine bitartrate)

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Tubes in siliconized glass or plastic, pipettes. Bidistilled water. Centrifuge. Aggregometer.

5 - PRECAUTIONS AND WARNINGS

- AGP TEST is a kit for *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- Do not use after expiration date. The quality of the reagent cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.

- Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations.
- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- This product is classified as dangerous according to current European legislation (view above table and consulting the MSDS).
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human organic districts samples. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

6 - Storage conditions and shelf life

Store the kit at +2 - +8°C. The kit is stable until expiration date printed on the package label.

7 – Specimens Collection

Collect the sample from an antecubital vein without stasis by slowly drawing up the blood with the syringe and slowly expelling it (after having removed the needle), into the collection tube; avoid haemolysis. Carry out the venepuncture with a plastic syringe and mix 9 volumes of blood with 1 volume of trisodium citrate 3.8% in a plastic or siliconized tube.
If the aggregation is carried out onto PRP, respect the following instruction:
Centrifuge the blood *100-170 x g for 15*minutes, carefully draw off the supernatant (PRP) and carry out a platelet count on this. Re-centrifuge the remaining citrated blood at 2000 g for *20* minutes and decant the supernatant (PPP). Maintain the PRP at room temperature and carry out the test within 3 hours.

8 – Preparation of working solutions

ADP - Reconstitute one vial with 0,5 mL of **Diluent A**. The lyophilized reagent once reconstituted is stable for 8 hours at RT, 2 weeks at +2 - +8°C and 1 month at -20°C. To avoid repeated thawing and freezing it is advised to subdivide the solution into aliquots of 0,1 mL and freeze. With the work scheme proposed here, the reagent is sufficient to carryout **70 aggregation test/vial** (generally about 35 patients/vial).
Adrenaline – Reconstitute one vial with 0,5 mL of distilled water. After reconstitution and taking of the amount required for the test, the vials of adrenaline should be removed. Before use dilute two a 0,1 mL aliquots of **Adrenaline** (previously reconstitute with 0,5 mL of bidistilled water) with 4,9 mL and 0,9 mL of Diluent A to obtain two working solutions at concentrations of 0,1 mM and 0,5 mM respectively. Working solution is stable for 60 minutes at RT. Each vial contains sufficient reagent to carry out **100 aggregation test**.
Collagen - Mix by inverting the collagen and dilute 0.1 mL with 0.4 mL of **Diluent B**. Concentration of work solution: 200 µg/mL. This solution is stable few hours in a bath of melting ice.
Ristocetin - reconstitute a vial of Ristocetin with 0,5 mL of **Diluent A**. Concentration of work solution: 50 mg/mL. This solution is stable one month at -20°C. To avoid repeated thawing and freezing it is advised to subdivide the solution into aliquots of 0,1 mL and freeze. With the work scheme proposed here, the reagent is sufficient to carryout **33 aggregation test/vial**.

9 – Test procedure

The following working plan is for a volume of 0,5 mL of PRP (in agreement with the type of aggregometer) or 1 mL of a blood.
Transfer 0,5 mL of PRP and 0,5 mL of PPP into two cuvettes of the aggregometer, previously prepared for the test according to the manufacturer's instructions and set each channel to 90% and 10% trasmittance respectively with the cuvettes of PPP and PRP. The setting must be repeated for each plasma and for each cuvette.
The operating procedures proposed here for the various aggregant agents are based on the results reported in the literature for normal subjects. It is therefore advisable that each laboratory establish its own normal range and follow a work scheme for carrying out the test which takes into account the obtained values.
ATTENTION: with the new instruments it is possible carry out a fast and simple process of auto-calibration (see the operatives manual for each instrument). This procedure allows a better standardization of the used method.

PROCEDURE ON PRP

1. Prepare PRP and PPP as described in section 7.
2. Add 500 µL (250 µL*) of PRP to an aggregation cuvette containing stirring bar and incubate at 37°C for 3 minutes.
3. Add 500 µL (250 µL*) of PPP to an aggregation cuvette without stirrer.
4. Place PRP and PPP cuvettes in corresponding instrument sample wells and follow manufacturer's instruction for setting base lines.
5. Bring aggregating reagents to room temperature and swirl to mix.
6. For each aggregating reagent add the quantity indicated in the table to PRP cuvette to obtain different aggregation curves.
7. Record platelet aggregation response for a minimum of 5 minutes.

Parameter	Concentration	Aggregation type	volume to add at PRP	Notes
ADP	0.8 µM	biphasic	4.0 µL (2.0 µL) *	Routine test
	2 µM	irreversible monophasic	10.0 µL (5.0 µL) *	
Adrenaline	1.0 µM	biphasic	5.0 µL (2.5 µL) * og Adrenaline 0.1 mM	Routine test
	10 µM	irreversible monophasic	10.0 µL (5.0 µL) * of Adrenaline 0.5 mM	
Collagen	200 µg/mL		10.0 µL (5.0 µL) *	
Ristocetin	1.5 mg/mL		15.0 µL (7.5 µL) *	
If an aggregation is not obtained or is markedly reduced, (von Willebrand's disease and Bernard-Soulier's syndrome) repeat the test, in order to have a confirmation of the diagnosis, operating as follows: - to 0,4 mL of PRP add 0,1 mL of normal pooled plasma; - add 15 µL of Ristocetin to obtain a final concentration of 1,5 mg/mL; If an increase in aggregation is recorded, the diagnosis of von Willebrand's disease is confirmed.				

*The figures in parentheses are half volumes that a lot of aggregometers can now handle; if it was necessary to use the proper rubber adhesive spacers.

WHOLE BLOOD PROCEDURE

1. Add 500 µL of saline solution and 500 µL of whole blood with anticoagulant in a 1 mL plastic cuvette containing stirring bar and incubate at 37°C for 5 minutes.
2. After connecting the electrode to the socket, put incubated at 37°C for 5 minutes.
3. After incubation, place it in the vial containing the diluted blood. (Place the filaments to the back of aggregometer).
4. Place the cuvette into the reaction well and incubate 2 minutes, holding the door closed. Open the door and pipette the amount of collagen careful pipette on the bottom, rinse several times or 20 µL of reconstituted Ristocetin (Warning: avoid formation of air bubbles).
5. Record platelet aggregation.

Parameter	Finale concentration	volume to add at whole blood	Notes
Collagen	2 µg/mL	10 µL	It is recommended to work at two different concentrations of collagen: 2 µg/mL and 4 µg/mL
	4 µg/mL	20 µL	
	5 µg/mL	25 µL	
Ristocetin	1 mg/mL	20 µL	

10 – INTERPRETATION OF RESULTS

Normal values

As the normal absolutes values are not available yet, for whole blood aggregation, it is recommended for each laboratory to establish their own normal ranges in order to compare with aggregation curves taken from pathological subjects.

NOTE:The following Normal Ranges were obtained from various laboratories and pubblications. They should be used as a guideline only.

ADP	Concentrations < a 0,3 µM	reversible aggregation
	Conc. between 0,3 and 1,5 µM	biphasic aggregation
	Concentrations > a 1,5 µM	irreversible monophasic aggregation
	Concentration 5 µM	% aggregation 69 – 88 %
	Concentration 10 µM	% aggregation 71 – 88 %
Adrenaline	Conc. < 0,2 µM	reversible aggregation
	Conc. between 0,2 and 5,0 µM	biphasic aggregation with a second wave induced by endogen aggregating agents
	Conc > a 5,0 µM	irreversible monophasic aggregation **(% di aggregation 78-88%)**[1]
Collagen on PRP	Concentration 2 µg/mL	% aggregation 70 - 94%
on whole blood	Concentration 2 µg/mL	aggregation (ohm) 15 – 27
	Concentration 5 µg/mL	aggregation (ohm) 15 – 31
Ristocetin on PRP	Concentration 1,5 mg/mL	% aggregation max 82 - 96%
on whole blood	Concentration 1,0 mg/mL	aggregation (ohm) >5 Ω. <70 sec Lag time

11 – CHARACTERISTICS

This product will perform as described prior to its expiration date when procedural and storage directions are followed.

Linearity, accuracy, precision.

Platelet aggregation induced by common aggregating reagents (ADP, Arachidonic Acid, Collagen and Adrenaline) is a nonlinear test system for some parameters: Lag Phase, Primary Slope, Secondary Slope, biphasic response and disaggregation. The non-linearity is caused by many factors such as the reaction chemistry and instrumentation. Platelet aggregation measures a response rate or activity that is not a quantitative measure of the reactants or their concentration. In platelet aggregation, accuracy is a relative parameter and is dependent on the test system. The limitations of platelet aggregation make it difficult to provide typical precision or reproducibility ranges.

12 – NOTES

• **Epinephrine is not recommended as a standard agonist for whole blood testing clinically.** As a fewer than 50% respond to this very weak agonist.
• To test at the same time optical test on PRP and the release of ATP with **bioluminescent** technique should work on a **lumi-aggregometer**. (Example 700-2). Refer to the Technical Manual and the instructions in User Manual of instrument.

13 – LIMITATIONS OF THE METHOD

- In a study of one hundred and six patients with Storage Pool Desease (SPD), 23% had normal optical aggregation (PRP) responses to ADP, epinephrine, and collagen and 44% had miscellaneous aggregation abnormalities. The authors concluded that SPD is common, heterogeneous and not necessarily associated with optical aggregation (PRP) abnormalities.[2]
- Testing should be performed within 3 hours of collection.
- Many drugs inhibit platelet function.[3,4,5] Unless the purpose of the test is to demonstrate drug-induced inhibition, patients should be drug free for a period ranging from ten (10) days to two (2) weeks before testing.
- Further clinical and laboratory evaluations may be necessary to confirm the diagnosis.
- Red blood cells in PRP may inhibit the ability of the Aggregometer to detect changes in light intensity. This could result in an underestimation of the percentage value of platelet aggregation.[5]
- Lipids in PRP may interfere with the light transmission reading and prevent the recording of aggregation.
- Platelet counts less than 50,000 plt/µL may cause problems with the optical baseline setting, preventing the recording of aggregation.

14 – REFERENCES

1. White M MC, Jennings LK: Platelet Protocols: Research and Clinical Laboratory Procedures. Academic Press, 1999
2. Nieuwenhuis HK, Akkerman J-W N and Sixma JJ: Patients With a Prolonged Bleeding Time and Normal Aggregation Tests May Have Storage Pool Deficiency: Studies on One Hundred Six Patients. Blood 70-3 (620-623) 1987.
3. Kinlough – Rathbone RL : The effects of some other drugs on platelet function. Platelets, Drugs and Thrombosis. Edited by J. Hirsh, J.F. Cade, A.S. Gallus, et al. Basel, S. Karger, 1975, pp 124-131.
4. Packham MA, Mustard JF: Non-steroidal anti-inflammatory drugs, pyrimido-pyrimidine compounds and tricyclic compounds: effects on platelet function. Platelets, Drugs and Thrombosis. Edited by J. Hirsh, J.F. Cade, A.S. Gallus et al. Basel, S. Karger, 1975, pp 124-131.
5. CLSI (Clinical Laboratory Standards Institute), Publication H58-A, Platelet Function Testing by Aggregometry; Approved Guideline; November 2008.

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 6	Updated layout, safety GHS symbols and content	2024/06

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.