ARACHIDONIC ACID 25 mM

For in Vitro diagnostic use only

Test for the evaluation of platelet aggregation induced by Arachidonic Acid on PRP and whole blood

I. INTENDED USE

Arachidonic acid is a fatty acid present in the granules and membranes of human platelets. It is liberated from phospholipids and, in the presence of the enzyme cyclo oxigenase 1 (COX-1), incorporates oxygen to form the endoperoxide prostaglandin G2 (PGG2). PGG2 is then quickly transformed to prostaglandin H2 (PGH2) which in turn is converted to thromboxane A2 a potent inducer of platelet aggregation. In vitro addition of arachidonic acid to normal platelet rich plasma results in a burst of oxygen consumption, thromboxane formation and platelet aggregation. Ingestion of aspirin or aspirin-containing compounds inhibits COX-1 mediated oxygen consumption, thus precluding all subsequent events leading to platelet aggregation. Arachidonic Acid is an important aggregating agent which is mainly used in the "Aspirin Like Disease" diagnosis, as well as for distinguishing said syndrome from "Storage Pool Disease" and also to evaluate the inhibiting effects of the aspirin (and other anti-inflammatory drugs) onto the platelet aggregation.In addiction the Arachidonic Acid is useful in routine platelet aggregation studies for the evaluation of platelet dysfunction or platelet activation.

II. TEST PRINCIPLE

The platelet aggregation test measures the rate and degree to which dispersed platelets in a sample of platelet rich plasma (PRP) or anticoagulated whole blood forms clumps (aggregates) after the addition of a substance that normally stimulates platelet aggregation (agonist). . It is strictly correlated to the presence of calcium ions and of one or more plasmatic factors. Aggregation study in whole blood is based on the evaluation of the electric resistance changes. Two electrodes immersed in the sample are rapidly covered with a platelets monolayer. When the aggregating agent is added, additional platelets tend to attach the monolayer previously formed, determining an impedance increase between the two electrodes. Aggregation study in PRP sample is based on the decrease of the torbidity of the sample after the aggregation reaction in relation to a Poor Plasma reference. These phenomena are recorded and displayed by an aggregometer which plots the rate and maximum extent of the aggregation reaction.

III. REAGENTS AND MATERIALS

The reagent contains a lyophilized preparation of 5 mg Arachidonic Acid with added buffer and stabilizers.

IV. PREPARATION FOR USE

Reconstitute each vial of Arachidonic Acid reagent with 0.7 mL of distilled or deionized water. Allow to stand for 10 minutes and mix well before use.

V. STORAGE AND STABILITY

The lyophilized product should be stored at 2-8oC and is stable until the expiry date printed on the vial label. After reconstitution, the product is stable for 8 hours at room TEMPERATURE (20-25°C), 2 weeks at 2-8°C or 4 weeks at -20°C. After the freeze the reagent can be thawed for two times without stability loss. It is recommended to aliquot after reconstitution.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

Pipette and tubes
Purified water
Platelet aggregometry system
Trisodium Cytrate 3,8%

VII. SAMPLE COLLECTION AND PREPARATION

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 hemolysis. Carry out the venipuncture with a plastic syringe and mix 9 volumes of blood with 1 volume of trisodium citrate 3.8% in a plastic or siliconized tube.
Whole Blood can be used for impedance aggregation within 3 hours from collection
For optical aggregation centrifuge the blood at 150-200 g for 10-15 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.

VIII. TEST PROCEDURE ON PRP SAMPLE

1- Prepare the sample as described below
2- Pipette the required volume of platelet rich plasma into an aggregation cuvette and add a stir bar. Pre-warm to 37°C for 120 seconds. *(for Chrono-Log instruments add 500 (250) uL of PRP)*
3- Pipette the required volume of platelet poor plasma in to an aggregation cuvette without stir bar. *(for Chrono-Log instruments add 500 (250) uL of PRP)*
4- Place PRP and PPP cuvettes in corresponding instrument sample wells and follow manufacturer's instruction for setting base lines.
5- Add the required volume of Arachidonic Acid directly into the cuvette. Do not allow reagent to run down the wall of the cuvette. (*for Chrono-Log instruments: add 10 µl (5µl) to the PRP sample to obtain a concentration of **0,5mM** and 20 µl (10 µl) to obtain a concentration of **1 mM**).*
6- Allow the aggregation pattern to form for a minimum of 5 minutes.

To work with half volume of sample (in brackets) on Chrono-Log instruments use the the proper rubber adhesive spacers.

IX. WHOLE BLOOD PROCEDURE

Refer to the manufacturers instructions for the correct performance of the test.

For Chrono-Log instruments:

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 it at 37°C for 5 minutes.*
3. *Alfter incubation, put it in the vial containing the diluted blood.*
4. *Put the cuvette into the reaction well and incubate 2 minutes, holding the door closed. Open the door and pipette the amount of AA careful pipette on the bottom, rinse several times (Warning: avoid formation of air bubbles).*
5. *Record platelet aggregation.*

*Add: 10 µl of diluted AA to 1 mL of diluted sample to have a concentration of **0,5 mM**.*
*20 µl of diluted AA to 1 mL of diluted sample to have a concentration of **1,0 mM**.*

X. QUALITY CONTROL

The results of platelet aggregation studies should be interpreted against the results of aggregation profiles of a normal sample tested at the same time. The normal donor should not have ingested aspirin or aspirin containing compounds in the preceding 10 days.

XI. RESULTS INTERPRETATION

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
Arachidonic Acid induces TxA2 and granule release to give a single strong wave of aggregation in normal individuals at concentrations from 0,5 to 1 mM (final concentration in PRP or whole blood).

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

Normal ranges in Platelet Rich Plasma (PRP) ± 1 σ			
Reagent	***Conc.***	***Aggregation (%)***	***ATP (nmole)***
Arachidonic Acid	*0,5 mM*	*74 – 99 (± 2 σ)*	*0,56 – 1,40*

Normal ranges in Whole Blood (WB) ± 2 σ			
Reagent	***Conc.***	***Aggregation (ohms)***	***ATP (nmole)***
Arachidonic Acid	*0,5 mM*	*5 – 17*	*0,6 – 1,40*

The expected response to Arachidonic Acid the most commonly encountered defects are listed below:

Condition	**Arachidonic Acid Aggregation**
Thrombasthenia	Absent
Bernard-Soulier Sydrome	Normal
Storage Pool defect	Absent or Primary wave only
Cyclo-oxygenase deficiency	Reduced
Thromboxane synthetase deficiency	Reduced or Absent
Aspirin ingestion	Reduced or Absent
Ehlers-Danlos syndrome	Normal
Von Willebrand disease	Normal

XII. PERFORMANCES

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.

XIII. PRECAUTIONS

1. For whole blood test application it is necessary to use only the reagent in the form of acid because other preparations like Arachidonic Acid Sodium Salt lyse the red cells releasing ATP which hide the platelet proper activity.
2. Carry out the test in fasting subjects, not taking drugs containing Acetylsalicylic Acid or other drugs that interfere with platelet aggregation for one week.
3. Thrombocytopenic patients may show PRP aggregation curves with heights lower than the normal values. Consequently when such patients are under analysis particular attention must be observed in the preparation of PPP, that must be rigorously without platelets.
4. the presence of red blood cells in the PRP will cause the total observed aggregation to be reduced.

XIV. BIBLIOGRAPHY

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CONTENT	***REF. 311501WB***
Arachidonic Acid	3 x 5 mg
Instruction for use	1 item

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (LXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CE IVD