ARACHIDONIC ACID 25 mM
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 oxygenase 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 addition 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 turbidity 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 2-8°C 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.
Whole Blood can be used for impedance aggregation within 3 hours from collection.

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. After 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 publications. They should be used as a guideline only.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Conc.</th>
<th>Aggregation (%)</th>
<th>ATP (nmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic Acid</td>
<td>0.5 mM</td>
<td>74 – 99 (± 2 σ)</td>
<td>0.56 – 1.40</td>
</tr>
</tbody>
</table>

Normal ranges in Whole Blood (WB) ± 2 σ

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Conc.</th>
<th>Aggregation (ohms)</th>
<th>ATP (nmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic Acid</td>
<td>0.5 mM</td>
<td>5 – 17</td>
<td>0.6 – 1.40</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Arachidonic Acid Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombasthenia</td>
<td>Absent</td>
</tr>
<tr>
<td>Bernard-Soulier Syndrome</td>
<td>Normal</td>
</tr>
<tr>
<td>Storage Pool defect</td>
<td>Absent or Primary wave only</td>
</tr>
<tr>
<td>Cyclo-oxygenase deficiency</td>
<td>Reduced</td>
</tr>
<tr>
<td>Thromboxane synthetase deficiency</td>
<td>Reduced or Absent</td>
</tr>
<tr>
<td>Aspirin ingestion</td>
<td>Reduced or Absent</td>
</tr>
<tr>
<td>Ehlers-Danlos syndrome</td>
<td>Normal</td>
</tr>
<tr>
<td>Von Willebrand disease</td>
<td>Normal</td>
</tr>
</tbody>
</table>

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


CONTENT

<table>
<thead>
<tr>
<th>Arachidonic Acid</th>
<th>3 x 5 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instruction for use</td>
<td>1 item</td>
</tr>
</tbody>
</table>

REF. 311501WB

In Vitro Diagnostic Medical Device

Temperature limitation

Batch code (LXXX)

Manufacturer

Keep dry

Non-sterile

Use by (year/month)

Catalogue number

Do not reuse

Fragile, handle with care

Keep away from heat

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