Plateletworks Arachidonic Acid

INTENDED USE
Plateletworks Arachidonic Acid (AA) is an in-vitro diagnostic screening test performed on whole blood to determine platelet inhibition by aspirin. Aspirin inhibits arachidonic acid induced platelet aggregation. The change in platelet count due to activation and aggregation of functional platelets is measured using an electronic impedance-based cell counter.

SUMMARY
Platelets are disk-shaped cells circulating in the blood that are produced from megakaryocytes in the bone marrow. These cells participate in formation of the hemostatic plug and are implicated in some diseases and in some diseases.

Platelet aggregation is performed to identify abnormal platelet function, to quantify platelet response, and to select patients for platelet therapy.

Several platelet aggregating agents include thrombin, collagen, ADP, arachidonic acid, antigen-antibody complexes, and vasoactive agents. It is useful to study these aggregating agents according to their mode of action. ADP, epinephrine, and vasopressin induce aggregation directly while collagen, thrombin and arachidonic acid act to stimulate substances to induce ADP release and further platelet aggregation.

It has been suggested that arachidonic acid testing be performed before other tests to screen for drug effects that might affect platelet responsiveness.

PRINCIPLE
Platelet arachidonic graphometry, the reference method for testing platelet function, is based on the Plateletworks methodology that includes a list of drugs taken for 7-10 days prior to testing should be obtained.

The validity of the Plateletworks assay is dependent on the accuracy of the platelet counts obtained. Multiple factors may potentially interfere with the accuracy of the platelet count. Therefore, cell counters using electronic impedance cell counting principles may be subject to known interfering substances which can impact platelet count results. These include:

- Microcytes, schizocytes, and WBC fragments, which may interfere with the proper counting of platelets and cause elevated platelet counts.
- Agglutinated erythrocytes, which may trap platelets and cause an erroneously low platelet count.
- Giant platelets, which may cause an erroneously low platelet count since they may exceed the upper limit threshold for the platelet parameter.
- Hemolytic reactions, which may interfere with the electronic impedance cell counting principle.
- Giemsa preparations, which may reduce the accuracy of the platelet count.
- Platelet aggregation, due to poor collection techniques or EDTA activation, which may cause a decreased platelet count.

PERFORMANCE CHARACTERISTICS
Correlation Study
Correlation of the Plateletworks assay to platelet aggregometry on platelet rich plasma (PRP) is supported by data generated by testing male and female adults, greater than 18 years of age, at three clinical sites. This includes normal, healthy volunteers, and patients and volunteers who were taking aspirin.

All blood samples were acquired from in-dwelling lines or venipuncture using established methods. For the Plateletworks assays and PRP aggregometry, the manufacturers’ recommendations were followed as to per intravenous procedure in the package insert.

A positive result was equal or greater than 60% aggregation and a negative result was less than 60% aggregation.

The Plateletworks arachidonic acid aggregation was done on samples collected from healthy volunteers. Each laboratory should establish their own reference range with their normal platelet population. The data are as follows:

<table>
<thead>
<tr>
<th>Plateletworks Arachidonic Acid</th>
<th>40-60% Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (positive)</td>
<td>260% Aggregation</td>
</tr>
<tr>
<td>Abnormal (negative)</td>
<td>60% Aggregation</td>
</tr>
<tr>
<td>Overall Agreement</td>
<td>90.7%</td>
</tr>
<tr>
<td>Positive Agreement</td>
<td>87.1%</td>
</tr>
<tr>
<td>Negative Agreement</td>
<td>97.0%</td>
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</tbody>
</table>

LIMITATIONS
- Only fresh, human whole blood should be added to the Plateletworks tubes. Do not collect samples into blood collection tubes containing anticoagulant (i.e., sodium citrate, EDTA, or heparin) prior to testing. Each tube kit contains a sodium citrate collection vial that is to be used instead.
- It is recognized that many drugs and compounds (prescription and non-prescription) may affect platelet aggregation. Therefore, all individuals must be free from any medication known to affect platelet function for a minimum of 7 to 9 days and should have prior platelet aggregation tests performed on samples that within the normal range of platelet function (i.e., baseline). A second control normal range test should be run. If the second normal control value is not within the normal range the assay should be considered out of control and no testing should be performed. In this case, contact Helena’s Technical Support for assistance.

The Plateletworks Arachidonic Acid test is reported as percent aggregation.

The reference value for Plateletworks AA agonist tube was determined on samples collected from healthy volunteers. Each laboratory should establish their own reference range with their normal platelet population. The data are as follows:

<table>
<thead>
<tr>
<th>Agonist Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-60% Aggregation</td>
</tr>
</tbody>
</table>

RESULTS
Plateletworks arachidonic acid aggregation was done on samples from volunteer donors. 134 samples were from individuals not taking aspirin and 265 were from individuals taking aspirin. The results are shown below:

<table>
<thead>
<tr>
<th>PW-AA 232 4 236</th>
<th>PW-AA 33 130 163</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
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<tr>
<td>20</td>
<td>20</td>
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<tr>
<td>30</td>
<td>30</td>
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<tr>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

The baseline tube is then run on the cell counter, recording the platelet count. Continue to mix the AA tube by holding it in the hand and inverting it gently every 8-10 seconds for 2 minutes. Place the tube in a rack and allow to stand for 5 to 8 minutes.

4. The AA Tube is then inverted gently 2 times to mix. Place the tube in the cell counter and record the platelet count.

Note: If running AA tube as part of the Combo kit, count the baseline tube, then the ADP, the Collagen tube and then the AA tube. The agonist tube counts can be performed in 10 minutes.

The percent platelet aggregation is then calculated:

(a) the % Aggregation/Inhibition Chart supplied in the packaged tubes, or
(b) calculated by the appropriate formula.

Baseplate Platelet - Agonist Platelet

Count X 100 = % Aggregation

Baseline Platelet Count

Quality Control
Platelet aggregation testing of the cell counter used to perform the Plateletworks assay should be completed during each shift the system is used. These results will ensure that the instrument is functioning properly.

It is suggested that each laboratory establish its own normal range. No commercial controls for platelet aggregation testing are available. Blood drawn from healthy adults may be used as normal controls for the Plateletworks assay. Aspirin-sensitive individuals may be tested by mixing 30% to 60% of the medication known to affect platelet function for a minimum of 7 to 9 days and should have prior platelet aggregation tests performed on samples that within the normal range of platelet function (i.e., baseline). A second control normal range test should be run. If the second normal control value is not within the normal range, a second normal control should be run. If the second normal control value is within the normal range, the assay should be considered out of control and no testing should be performed. In this case, contact Helena’s Technical Support for assistance.

STORAGE AND STABILITY
All reagents are of non-human origin. The arachidonic acid is isolated from porcine liver and purified.

It may be beneficial for any abnormal baseline results to be further investigated using additional platelet aggregation testing, such as platelet bleeding time, assessment of platelet morphotype, and others.

Materials Required but Not Provided
- EDTA tubes (baseline) – 0.024 mL of 7.5% K$_3$EDTA solution (1.80 mg)
- AA tubes (yellow top) – Upon reconstitution with 1.0 mL fresh whole blood, it contains approximately 125 µg AA lyophilized in the presence of 3.2% sodium citrate, buffer salts and bovine serum albumin.
- EDTA tubes (baseline) – 0.024 mL of 7.5% K$_3$EDTA solution (1.80 mg)

PROCEDURE
1. Obtain the desired fresh whole blood sample. A 1.0 cc whole blood sample is required for each agonist tube and each baseline tube.

2. Immediately dissolve 1.0 cc into each of the baseline tube and the agonist tube.

3. Mix each tube (baseline and agonist) vigorously 15 to 20 times to ensure adequate mixing.
Precision

Precision of the Plateletworks assay was determined using duplicate samples from a healthy volunteer. The duplicate samples were tested on each of twenty (20) days with the AA agonist. The mean was 67%, coefficients of variation were 7.1% within-run and 13.9% for the total test period.

BIBLIOGRAPHY


Litraturo/Bibliografia/Bibliografie/Bibliografia


Test System: Plateletworks Arachidonic Acid
Analyte: Platelet Aggregation Percentage
Complexity: Moderate.

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