English

Plateletworks®
Arachidonic Acid

REF Cat. No. 5864, 5854

CE

INTENDED USE
Plateletworks Arachidonic Acid (AA) is an in-vitro diagnostic screening test performed on whole blood for the qualitative determination of 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-base 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 thrombotic events.[1]
Platelet aggregation is performed to identify abnormal platelet function, to quantitate platelet response, and monitor platelet inhibition by drug therapy.[2]
Several platelet aggregating agents include thrombin, collagen, ADP, arachidonic acid, antigen-antibody complexes, serotonin and vasopressin. 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 as stimulating substances to induce ADP release and thromboxane A2 release.[2]
It has been suggested that arachidonic acid[3] testing be performed before other tests to screen for drug effects that might affect platelet responsiveness.

PRINCIPLE
Traditional platelet aggregometry, the reference method for testing platelet function, is based on the addition of the platelet agonist to a blood sample (usually platelet rich plasma). Platelet aggregation may be assessed using various agonists such as ADP, collagen and others. Arachidonic acid is a fatty acid which is liberated from the human platelets on activation and is converted by the enzyme cyclooxygenase into a potent inducer of platelet aggregation. Ingestion of aspirin or other similar drugs inhibits cyclooxygenase-1 (COX-1) thus inhibiting platelet aggregation. It has been reported that 5-60% of individuals taking aspirin are resistant to the effects of aspirin on the platelets.[4,5]
The Plateletworks methodology is an adaptation of platelet aggregometry that is extremely simple, inexpensive, and quick to perform (results are available in about five minutes). This two-step method involves using a cell counter to measure total platelet count in a whole blood sample and then to redetermine the platelet count on a second sample that has been exposed to a known platelet agonist. The agonist will stimulate those platelets which are functional to aggregate into clumps, and they will not be counted as platelets in the second sample. The difference in the platelet count between samples one and two provides a direct measurement of platelet aggregation and is reported as percent aggregation.

Platelets rendered inactive or non-functional by aspirin or other inhibitors of cyclooxygenase-1 do not aggregate.
Because of the differences in light transmission aggregometry in platelet rich plasma and Plateletworks whole blood aggregation with arachidonic acid, cutoff values were used to evaluate test results. An aggregation of equal to or greater than 60% is considered normal aggregation and an aggregation of less than 60% is considered consistent with aspirin effect.

REAGENTS
For In Vitro Diagnostic Use
Each Plateletworks kit contains baseline tubes and agonist tubes. The tube contents are as follows:
- EDTA (baseline) tube – 0.024 mL of 7.5% K_3EDTA solution (1.80 mg)
- AA tube (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.

Note: All reagents are of non-human origin. The arachidonic acid is isolated from porcine liver and purified.

Storage and Stability
All Plateletworks tubes should be stored at 2-8°C prior to use. When stored at 2-8°C, the tubes are stable until the marked expiration date.

INSTRUMENT
The Plateletworks tubes can be run on any hematology analyzer utilizing impedance methodology for determining platelet counts from whole blood. For detailed instructions, refer to the appropriate Operator's Manual.

SPECIMEN COLLECTION AND PREPARATION
Blood samples should be collected via routine method (i.e., indwelling catheter line, venipuncture, etc.). No anticoagulation of the blood is required. Samples should be drawn in a manner to prevent contamination with tissue thromboplastin, indwelling IV solutions, and other interfering substances. Blood collection guidelines are described in the NCCLS document H3-A3, Vol. 11, No. 10, July 1991: "Procedure for the Collection of Diagnostic Blood Specimens by Venipuncture".

Venipuncture
When using a venipuncture technique, needles of 22 to 19 gauge should be used to reduce the potential for platelet activation during specimen collection. For pediatric patients, a 21 to 23 gauge needle may be used. Withdraw 2.0 cc of blood and discard it. Then collect a 2.5 cc sample of fresh whole blood for testing (1.0 cc for baseline tube and 1.0 cc for agonist tube).

Extracorporeal Line
Using a two-syringe technique, flush the extracorporeal blood access line by withdrawing 2.0 cc of blood into a syringe and discarding it. Then use a second syringe to obtain a 2.5 cc sample of fresh whole blood for testing (1.0 cc for baseline tube and 1.0 cc for agonist tube).

Indwelling Catheter Line
Discontinue fluids drip, if necessary, and flush the line with 5 mL saline. Using a two-syringe technique, withdraw 2.0 cc of blood into a syringe and discard it. Then use a second syringe to obtain a 2.5 cc sample of fresh whole blood for testing (1.0 cc for baseline tube and 1.0 cc for agonist tube).

NOTE: The appropriate amount of blood from the hypodermic needle/syringe must be added to the EDTA and agonist tubes within one minute after completion of the draw.

PROCEDURE
Materials Provided
The Plateletworks Kit includes agonist tubes, baseline tubes and a % Aggregation/Inhibition chart. A Plateletworks Aggregation/Inhibition Calculation Wheel (584) is also available upon request.

Cat. No. Contents
5864 Plateletworks Arachidonic Acid Kit
25 AA Tubes
25 EDTA Tubes

5854 Plateletworks Combo - 10 Kit
10 AA Tubes
10 ADP Tubes
10 Collagen Tubes
10 EDTA Tubes

Materials Required but Not Provided
Impedance cell counter
Blood collection materials (syringes, blood collection set, etc.)

Step-by-Step Method
Note: Allow Plateletworks tubes to equilibrate to room temperature (20-24°C) before adding sample.
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 dispense 1.0 cc of blood 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.
4. The baseline tube is then run on the cell counter, recording the platelet count.
5. 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.
6. 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 last the Arachidonic Acid tube. All counts can be completed in 10 minutes.
7. The percent platelet aggregation is then calculated
   a) from the % Aggregation/Inhibition Chart supplied in the packaged tubes, or
   b) using the Plateletworks Calculation Wheel.
   c) calculated by the appropriate formula.

$$\frac{\text{Baseline Platelet Count} - \text{Agonist Platelet Count}}{\text{Baseline Platelet Count}} \times 100 = \%\ \text{Aggregation}$$

Quality Control
Quality control 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. These 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 that fall within the normal range established by the laboratory. If the first normal control value is outside the normal reference interval, a second normal control should be run. If the second normal control value is also outside the normal reference interval, the assay should be considered out of control and no testing should be performed. In this case, contact Helena's Technical Support for assistance.

REFERENCE VALUES
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 patient population.[6] The data are as follows:

Agonist	Reference Range
AA	60-100% aggregation

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.

	Aspirin	Non-Aspirin	Total
Normal PW-AA	33	130	163
Abnormal PW-AA	232	4	236
Total	265	134	399

Normal (positive) = ≥60% Aggregation
Abnormal (negative) = <60% Aggregation

Overall Agreement	90.7%
Positive Agreement	87.6%
Negative Agreement	97.0%

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 addition to the Plateletworks tubes.
- It is recognized that many drugs and compounds (prescription and non-prescription) may affect platelet aggregation. The most common of these is aspirin. Therefore, a complete medical history 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 when performed on an automated cell counter. Therefore, platelet counts obtained should be scrutinized in light of the patient's clinical circumstance and previous platelet count results. Plateletworks results should always be interpreted in light of the clinical history and condition of the patient.
- It may be beneficial for any abnormal baseline results to be further investigated using additional platelet testing methodology, such as platelet count, bleeding time, assessment of platelet morphology, and others.
- Do not use Plateletworks tubes past their expiration date or those which have been improperly stored.
- Plateletworks results may be affected by poor technique (e.g., improper blood sample volume, delayed test performance beyond recommended procedure, etc.).

INTERFERENCES
- Pseudothrombocytopenia, though infrequent, can result from EDTA-dependent platelet agglutination. Pseudothrombocytopenia may be suspected with the Plateletworks assay if the platelet count determined using the agonist tube is higher than the platelet count determined using the baseline tube (containing EDTA anticoagulant). If pseudothrombocytopenia is suspected, common laboratory practice is to re-draw the blood sample into a sodium citrate collection tube and perform the blood count; the results should be corrected by a factor of 1.1 to account for the sample dilution that occurs with the use of sodium citrate as an anticoagulant. This procedure should be followed using the sodium citrate tube in lieu of the Plateletworks baseline tube, followed by the Plateletworks agonist tube, to determine percent platelet aggregation.
- Cell counters utilizing 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.
  - Chemotherapy, which may increase the fragility of platelets and cause low platelet counts
  - Hemolysis, which contains red cell stroma and may elevate platelet counts.
  - Acid-citrate-dextrose (ACD) blood, which may contain platelet aggregates that could depress the platelet count.
  - RBC inclusions, which may produce a spuriously increased platelet count.
  - Platelet agglutination, 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 adhered to as per instructions provided in the package insert.
A positive result was equal to or greater than 60% aggregation and a negative result was less than 60% aggregation. A comparison study of 337 specimens gave an overall agreement of 87.5%; positive agreement of 93.2%; and negative agreement of 85.0%.
Note: Thrombocytopenic samples may be tested using the Plateletworks assay. As this system utilizes electrical impedance cell counting principles (i.e., Ichor Hematology Analyzer), instrument platelet counts >10 x 10^3/µL can be accurately obtained. Agonist platelet counts can be measured in samples meeting the limits of aggregation detection (>27 x 10^3/µL). Although EDTA-induced thrombocytopenic samples may be tested using the Plateletworks assay, no actual testing was performed on this sample type.

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

Literatur/Bibliografia/Bibliografie/Bibliografia

1. Zucker, M.B., The Functioning of Blood Platelets, Sci Amer 242(6):86-103, 1980.
2. Marcus, A.J. Platelet Aggregation. Hemostasis and Throm-bosis: Basic Principles and Clinical Practice, Coleman, R.W., Hirsh, J., Marder, V.J., and Salzman, E.W., Ed. J.B. Lippincott Co., Philadelphia, 1982, Pg 380-389.
3. Sirridge, M.S. and Shannon, R., Laboratory Evaluation of Hemostasis and Thrombosis, Lea & Febiger, Philadelphia, p. 95, 1983.
4. Gum, P.A. et al., Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol, 88(3):230-235, 2001.
5. Mueller, M.R. et al., Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. Thromb Haemost, 78(3):1003-1007, 1997.
6. Clinical and Laboratory Standards Institute. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. CLSI Document C28-A3c, Vol. 28, No. 30, 2008.

	Plateletworks® Cat. No.
Plateletworks Arachidonic Acid Kit 25 Baseline (EDTA) Tubes 25 AA Tubes	5864
Plateletworks Combo-10 Kit 10 Baseline (EDTA) Tubes 10 Collagen Tubes 10 ADP Tubes 10 AA Tubes	5854
Plateletworks ADP Kit 25 Baseline (EDTA) Tubes 25 ADP Tubes	5860
Plateletworks Collagen Kit 25 Baseline (EDTA) Tubes 25 Collagen Tubes	5862
Plateletworks Combo-15 Kit 15 Baseline (EDTA) Tubes 15 Collagen Tubes 15 ADP Tubes	5850
Plateletworks Combo-25 Kit 25 Baseline (EDTA) Tubes 25 Collagen Tubes 25 ADP Tubes	5852

Other Supplies and Equipment

The following items, associated with the performance of the Plateletworks Kits, must be ordered individually.

	Cat. No.
Plateletworks Calculation Wheel	584
ICHOR II Analyzer	5880
ICHOR II 5.5L Diluent-Plastic Container	5881
ICHOR II E-Z Cleanser (100 mL)	5882
ICHOR II Probe Cleanser (12 x 17 mL)	5883
ICHOR II Diluent Solution (20L)	5884
ICHOR II Rinse Solution (5L)	5885
ICHOR II Lyse Reagent (500 mL)	5886
ICHOR II Tri-Level Control	5887
ICHOR II Calibrator	5888
ICHOR II Paper	5889

For Sales, Technical and Order Information and Service Assistance, call 800-231-5663 toll free.

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

Shaded areas indicate that the text has been modified, added or deleted.

EC REP European Community Authorized Representative
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