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**HELENA LABORATORIES**

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HELENA LABORATORIES LABELING – Style/Format Outline

1. PRODUCT {Test} NAME
2. INTENDED USE and TEST TYPE (qualitative or qualitative)
3. SUMMARY AND EXPLANATION
4. PRINCIPLES OF THE PROCEDURE

{*NCCLS lists SAMPLE COLLECTION/HANDLING next}*

1. REAGENTS (name/concentration; warnings/precautions; preparation; storage; environment; Purification/treatment; indications of instability)
2. INSTRUMENTS required – Refer to Operator Manual (... for equipment for; use or function; Installation; Principles of operation; performance; Operating Instructions; Calibration\* {\*is next in

order for NCCLS – also listed in “PROCEDURE”}’ precautions/limitations/hazards; Service and maintenance information

1. SAMPLE COLLECTION/HANDLING
2. PROCEDURE

{*NCCLS lists QUALITY CONTROL (QC) next}*

9) RESULTS (calculations, as applicable; etc.)

10) LIMITATIONS/NOTES/INTERFERENCES

11) EXPECTED VALUES

12) PERFORMANCE CHARACTERISTCS

13) BIBLIOGRAPHY (of pertinent references)

14) NAME AND PLACE OF BUSINESS OF MANUFACTURER

15) DATE OF ISSUANCE OF LABELING (instructions)

For Sales, Technical and Order Information, and Service Assistance,   
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Form 364

Helena Laboratories

1/2006 (Rev 3)

English

**Plateletworks**®

**Arachidonic Acid**

Cat. No. 5864, 5854



**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 acid3 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: 24 µL containing 1.8 mg of K3EDTA (liquid)

• Arachidonic Acid tube (yellow top): 125 µg arachidonic acid, 3.2 mg sodium citrate, buffer salts, bovine serum albumin (lyophilized)

**Note:** All reagents are of non-human origin.

**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 CLSI Document GP41-Ed7, April 2017: "Collection of Diagnostic Venous Blood Specimens”.

**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. The agonist tube should be tested within ten minutes after sample addition to the tube.

**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.

**Baseline Platelet - Agonist Platelet**

**Count Count**

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **X 100 = % Aggregation**

**Baseline Platelet Count**

**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.

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. The agonist tube should be tested within ten minutes after sample addition to the tube.

- 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 103/µL can be accurately obtained. Agonist platelet counts can be measured in samples meeting the limits of aggregation detection (>27 x 103/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 EP28-A3c, Vol. 28, No. 30, October 2010.

**Plateletworks®**

**Cat. No.**

Plateletworks Arachidonic Acid Kit 5864

25 Baseline (EDTA) Tubes

25 AA Tubes

Plateletworks Combo-10 Kit 5854

10 Baseline (EDTA) Tubes

10 Collagen Tubes

10 ADP Tubes

10 AA Tubes

Plateletworks ADP Kit 5860

25 Baseline (EDTA) Tubes

25 ADP Tubes

Plateletworks Collagen Kit 5862

25 Baseline (EDTA) Tubes

25 Collagen Tubes

Plateletworks Combo-15 Kit 5850

15 Baseline (EDTA) Tubes

15 Collagen Tubes

15 ADP Tubes

Plateletworks Combo-25 Kit 5852

25 Baseline (EDTA) Tubes

25 Collagen Tubes

25 ADP Tubes

**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

Diluent Solution for ICHOR II & BC-3600 (2 x 5.5.L) 5881

Ichor II E-Z Cleanser (100 mL) 5882

Diluent Solution for ICHOR II & BC-3600 (20 L) 5884

Rinse Solution for ICHOR II & BC-3600 (2 x 5.5.L) 5885

Lyse Reagent for ICHOR II & BC-3600 (500 mL) 5886

Tri-Level Control for ICHOR II & BC-3600 5887

Calibrator for ICHOR II & BC-3600 5888

Thermal paper for ICHOR II & BC-3600 (5 rolls) 5889

Mindray BC-3600 Analyzer 5890

Probe Cleanser for Mindray BC-3600 (1 x 17 mL) 5893

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Test System: Plateletworks Arachidonic Acid

Analyte: Platelet Aggregation Percentage

Complexity: Moderate.



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Pro 119

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