English

PLATELETWORKS®
ADP and Collagen

REF **Cat. No. 5860, 5862, 5850, 5852, 5854**

CE

INTENDED USE

Plateletworks is an in vitro diagnostic screening assay for the determination of % platelet aggregation or % platelet inhibition in fresh whole blood. The samples may be taken during cardiac interventional procedures or other surgical or treatment settings facing similar risks from thrombosis. Plateletworks may also be used when there are general concerns about the effects of drug therapies on platelet function. The change in platelet count due to activation and aggregation of functional platelets is measured using an electronic impedance-based cell counter.

SUMMARY

The anucleate, multifunctional platelet circulates as a cellular component of the blood and provides the first line of defense against bleeding. Subsequent to vasculature injury, platelets adhere to collagen that becomes exposed as a result of endothelial disruption. They then undergo a radical shape change and release adenosine diphosphate (ADP), a physiological agonist which activates additional platelets. Ultimately, a primary hemostatic plug is formed due to the cyclic and self-perpetual stimulation of platelets as they aggregate, release other agonists, and interact with the proteins of the coagulation cascade to form a fibrin clot.

To determine the functionality of primary hemostasis in a given patient, platelets must be assessed in two ways: 1) by measuring the platelet count, and 2) by testing platelet function. If the platelet count is low (generally < 50,000/μL), the ability of the platelets to aggregate properly may be compromised. However, even with a normal platelet count, the platelets may be dysfunctional due to congenital or acquired conditions. If platelets are not aggregating normally, the outcome may be either hemorrhage or unwanted clotting. More specifically, inadequate platelet aggregation may lead to bleeding complications, and platelets that are excessively activated can potentially initiate a thrombotic event such as myocardial infarction, ischemia, stroke, reocclusion following coronary angioplasty, and others.

As a result, it may be desirable to assess platelet count and platelet aggregation or platelet inhibition in certain patient populations. In particular, these include patients undergoing cardiopulmonary bypass, coronary angioplasty, and individuals receiving drug therapies known to affect platelet aggregation. Test results obtained may be useful for pre-and post-surgical determinations for more definitive therapy. Further, platelet aggregation studies performed using multiple agonists may also be helpful in this regard.

PRINCIPLE

Traditional platelet aggregometry, the reference method and “gold standard” for testing platelet function, is based on the addition of platelet agonists such as collagen, ADP and others to a blood sample (usually platelet rich plasma). Platelet aggregation may be assessed using these agonists, individually and/or as a panel, as part of the diagnostic intervention into various conditions of congenital and acquired platelet dysfunction. The chart below represents what the different agonist results suggest at baseline.[1] It may be beneficial for any abnormal baseline results to be further investigated using additional platelet testing methodologies such as platelet count, bleeding time, assessment of platelet morphology by electron microscopy, measurement of platelet-specific proteins indicative of platelet activation, and others. Such follow-up testing should be conducted in light of patient history, the clinical presentation, and physician discretion.

Condition	Collagen	ADP
Afibrinogenemia	N	D
Bernard Soulier Syndrome	N	N
Glanzman’s Thrombasthenia	D	D
Aspirin Ingestion	D	D
von Willebrand’s Syndrome	N	N
Uremia	D	D
Storage Pool Disease	D	D

N = Normal, D = Decreased

These agonists serve to stimulate the platelet, and the pattern of aggregation is recorded on a strip chart. The curve is then interpreted by the operator. Unfortunately, the reproducibility of results is dependent on many factors, and the test is highly labor intensive and expensive. For these reasons, platelet aggregometry is not routinely performed in most laboratories. As a result, platelet aggregation testing capabilities have been largely unavailable despite the strong clinical need for this data.

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 as per the following equation:

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

Platelets rendered inactive or non-functional by pharmaceutical intervention are considered inhibited. The Plateletworks results may be expressed as percent inhibition instead of percent aggregation.

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

Users may refer to the % Aggregation/Inhibition Chart in each box of Plateletworks tubes.

REAGENTS

For In Vitro Diagnostic Use

Each Plateletworks kit contains baseline tubes and/or agonist tubes depending on the configuration of the kit. The tube contents are as follows:

- EDTA (baseline) tube: 24 μL containing 1.8 mg of K_3EDTA (liquid)
- ADP tube (gray top): 10μg ADP (bacterial), 3.2 mg sodium citrate, buffer salts (lyophilized)
- Collagen tube (white top): 10 μg collagen (equin tendon), 3.2 mg sodium citrate (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. Each Plateletworks tube requires a 1.0 cc fresh, human blood sample.

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. When stored at room temperature (20-24°C), ADP tubes are stable for one month. However, it is not recommended that Collagen tubes be stored at room temperature for longer than one week.

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 19 to 22 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 Kits include agonist tubes, baseline tubes and a % Aggregation/Inhibition chart. A Plateletworks Aggregation/Inhibition Calculation Wheel (584) is also available upon request.

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

Materials Required but Not Provided

Impedance cell counter

Blood collection materials (syringes, blood collection set, etc.)

Step-by-Step Method

Note: If kept refrigerated at 2-8°C, 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 an appropriate cell counter, recording the platelet count.
5. Mix the ADP tube an additional 3 to 5 times and run on the cell counter. Record the platelet count.
6. The Collagen tube is mixed 4-5 times each minute for 4 minutes.
7. The Collagen tube is then run on a cell counter, recording the platelet count.
8. The percent platelet aggregation is then calculated
   a) from the % Aggregation/Inhibition Chart supplied in the package of tubes.
   b) using the Plateletworks Calculation Wheel.
   c) calculated by the appropriate formula.

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 5 days and should have prior platelet aggregation tests that fall within the normal range established by the laboratory. 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 (1-800-231-5663, ext. 600).

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

REFERENCE VALUES

The reference value for each Plateletworks agonist tube was determined on samples collected from healthy volunteers. Each laboratory should establish their own reference range with their normal patient population.[13] The data are as follows:

Agonist	Reference Range
Collagen	70-100%
ADP	86-100%

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, between the ages of 18 and 85, at three clinical sites. This includes normal, healthy volunteers, patients undergoing cardiopulmonary bypass surgery, and patients undergoing cardiac catherization.

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.

Regression analysis (correlation coefficients) was performed to assess the agreement between the two methods. A positive correlation was demonstrated for each agonist. See Figures 1 and 2.

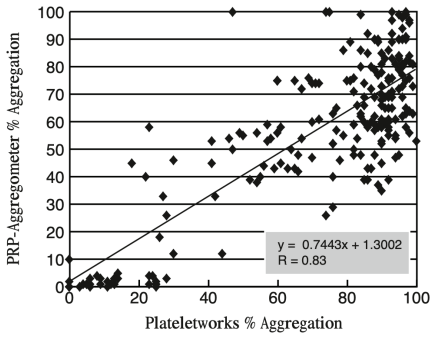

Figure 1: ADP

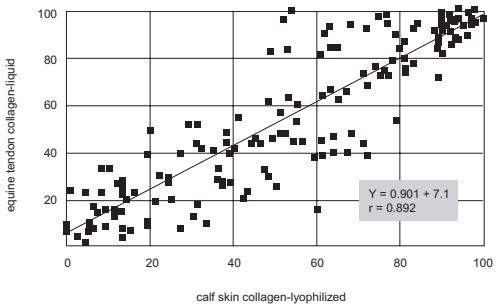

Figure 2: Collagen

However, it is recognized that correlation coefficients measure the strength of the relationship between the methods and not the agreement between them.[2] Further, since the aggregation system is bounded by 100% as the upper limit of aggregation regression analysis is not expected to describe a predictive relationship. Therefore, the data from the clinical sites where substantial equivalence testing was performed were also subjected to the non-parametric analysis of Spearman Rho which "tests for a positive correlation without specifying linearity". The results from these analyses are shown (with the regression analysis).

Comparative Data:	Plateletworks	vs	PRP Aggregometry
	N		(Pearson)r		Spearman Rho
ADP	225		0.83*		0.68*

*P < 0.001 two-sided test of positive association significant

Comparative Data:	Liquid Collagen	vs	Lyophilized Collagen
	N		(Pearson)r		Spearman Rho
Collagen	154		0.89*		0.88*

*P < 0.001 two-sided test of positive association significant

Note: Thrombocytopenic samples may be tested using the Plateletworks assay. As this system utilizes electrical impedance cell counting principles (i.e., Ichor II Analyzer), instrument platelet counts > 10 x $10^3/\mu L$ can be accurately obtained. Agonist platelet counts can be measured in samples meeting the limits of aggregation detection (> 27 x $10^3/\mu L$). 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 in two ways. First, a sample from a single healthy volunteer was tested on each of ten days with the agonist. (Although the samples were drawn from the same individual, some differences are to be expected due to diurnal physiological variation.) The coefficients of variation was 5.1% for ADP and 5.9% for Collagen.

Additionally, 10 sample aliquots from a single healthy volunteer were tested during a 20 minute interval with the agonist. The coefficients of variation was 4.1% for ADP and 4.9% for Collagen.

This data supports the reproducibility of the Plateletworks assay. Thus, duplicate testing on a routine basis is not required. Nonetheless, any questionable test result should be repeated, and performed in duplicate.

BIBLIOGRAPHY

Bibliografia/Bibliographie/Bibliografia/Literatur

1. Triplett DA et al: Platelet function, laboratory evaluation and clinical application. ASCP, Chicago, 1978.
2. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; Feb: 307-310.

REFERENCES

References/Verveise/Riferimenti/Referencias

1. Badimon L, Badimon JJ, Fuster V: Pathogenesis of thrombosis. In Fuster V, Verstraete M, eds. Thrombosis in cardiovascular disorders. Philadelphia, PA: WB Saunders 1992; 17-39.
2. Born, GVR: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; 194:927.
3. Califf RA, Willerson JT: Percutaneous transluminal coronary angioplasty: prevention of occlusion and restenosis. In: Fuster V, Verstraete M, eds. Thrombosis in cardiovascular disorders. Philadelphia, PA: WB Saunders 1992; 389-408.
4. Cheeseman JE, Mills SP, Hardisty RM: Platelet aggregometry on whole blood: the use of the ELT8/ds blood cell counter in the investigation of bleeding disorders. Clin Lab Haemat 1984; 6:265-272.
5. Despotis GJ, Levine VL, Goodnough LT. Relationship between leucocyte count and patient risk for excessive blood loss after cardiac surgery. Crit Care Med. 1997; 25:1338-1346.
6. Gasperti CM, Gonias ST, Gimple LW, et al: Platelet activation during coronary angioplasty in humans. Circulation 1993; 88:2728-2734.
7. Harker LA, Mann KG: Thrombosis and Fibrinolysis. In: Fuster V, Verstraete M, eds. Thrombosis in cardiovascular disorders. Philadelphia, PA: WB Saunders 1992; 1-16.
8. Ingerman-Wojenski CM, Silver MJ: A quick method for screening platelet dysfunctions using the whole blood lumi-aggregometer. Thromb Hemo 1984; 51(2):154-156.
9. Marcus AJ, Safier LB: Thromboregulation: multicellular modulation of platelet reactivity in hemostasis and thrombosis. FASEB 1993;7:516-522.
10. Sughayer M, Arkin CF: Monitoring coagulation during and after cardiopulmonary bypass surgery. Thromb Hemost. 1990; 12(4):1-7.
11. Schror K: Aspirin and platelets: the antiplatelet action of aspirin and its role in thrombosis treatment and prophylaxis. Sem Throm Hemost 1997; 23(4):349-356.
12. Weiss HJ, Rogers J: Thrombocytopenia due to abnormalities in platelet release reaction. Blood 1972;39:2.

13. 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.

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

Helena Laboratories, Corp.
1530 Lindbergh Drive
Beaumont, TX 77707 USA

Pro 190
12/22(11)

HELENA LABORATORIES

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