Helena Arachidonic Acid Reagent Procedure

Cat. No. 5364

Helena Arachidonic Acid Reagent is intended for use in platelet aggregometry studies.

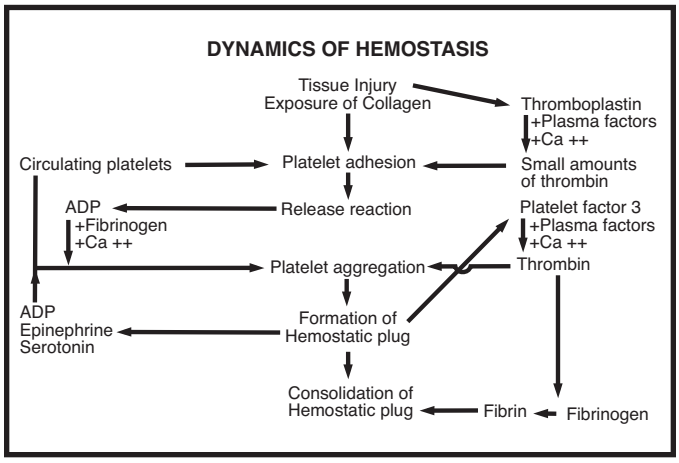
SUMMARY

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]

This procedure is performed on a turbidimetric aggregometer, as first described by Born.[3] The change in absorbance is recorded as platelet rich plasma is stirred in a cuvette with an aggregating reagent added.



PRINCIPLE

In-vivo, platelets participate in primary hemostasis by first adhering and then aggregating at the site of an injured blood vessel. Platelet aggregation can be followed in-vitro by adding inducers such as collagen, ADP, epinephrine and Arachidonic acid to stirred platelet rich plasma. The increase in light transmittance is recorded as the platelets aggregate. The absorbance (OD) change is measured and recorded as the platelets aggregate.[4]

It has been suggested[5] that Arachidonic Acid testing be performed before other tests to screen for drug effects that might affect platelet responsiveness.

REAGENT

1. Arachidonic Acid Reagent

Ingredients (after reconstitution):

Sodium Arachidonate 5 mg/mL

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.

Preparation for Use: Prepare a stock solution by reconstituting one vial with 1.0 mL of distilled or deionized water. Mix gently until completely dissolved.

Storage and Stability: The Arachidonic Acid Reagent should be stored in dry form at 2 to 8°C and is stable until the expiration date on the vial. Arachidonic Acid should be kept stoppered when not in use to avoid oxidation. Reconstituted reagent is stable for 24 hours at 2-8°C.

Signs of Deterioration: If the reagent is discolored (yellow tint) immediately upon reconstitution and fails to yield at least 70% aggregation with normal platelets, the reagent should not be used. Also, if the reconstituted reagent is left open too long, oxidation of the Arachidonic Acid may occur. If the solution becomes discolored (yellow tint) and fails to induce at least 70% aggregation with normal platelets, the reagent should not be used.

INSTRUMENTS

Helena Arachidonic Acid Reagent is suitable for use with any turbidimetric aggregation monitoring device. Recommended is the Helena AggRAM Analyzer (Cat. No. 1484).

SPECIMEN COLLECTION AND HANDLING

Specimen: Plasma obtained from whole blood collected with 3.2% sodium citrate as an anticoagulant is the specimen of choice. Hemolyzed samples may cause erroneous aggregation results.

Patient Preparation:

1. The patient should be fasting for at least eight hours prior to drawing the specimen.
2. It is preferable that the patient be free of all drugs. However, it is essential for normal platelet function that the patient not have taken aspirin or aspirin containing drugs for 7-10 days prior to testing.

Specimen Collection: Blood may be collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique. Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. For blood specimens with hematocrits

(HCT) of < 55% (normal), 9 parts of freshly collected whole blood should be immediately added to one part anticoagulant.[6] For blood specimens with hematocrits outside the normal range, adjust the amount of whole blood added to the anticoagulant according to the following formula.[7]

$$\text{Parts whole blood to One part anticoagulant} = \frac{0.6}{(1 - .\text{HCT})} \times 9$$

Particular care should be taken when using evacuated test tubes. These tubes are designed to draw 9 parts blood to 1 part anticoagulant. If the hematocrit is determined abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant. During all phases of collection and post collection of a specimen, only plastic or siliconized glass labware should be used.[8]

Specimen Preparation:

1. Prepare platelet rich plasma (PRP) by centrifuging both of the anticoagulated samples at 100 x g 10-15 minutes at room temperature. DO NOT BRAKE THE CENTRIFUGE. Remove the PRP from the cells with a plastic pipette and place in a plastic tube labeled PRP. Cap the tube and maintain at room temperature. Wait 30 minutes after PRP is removed before testing.
2. Prepare platelet poor plasma (PPP) by recentrifuging the remaining blood samples at 1600-2000 x g (or an equivalent centrifugation force) for 10-15 minutes at room temperature. DO NOT BRAKE THE CENTRIFUGE. Remove PPP, place in a plastic tube labeled PPP and cover. Maintain at room temperature.
3. A platelet count should be performed on the patient PRP and a normal control PRP. The platelet count should be standardized (usually 250,000/mm^3) by adjusting the PRP with autologous platelet poor plasma. The control should be run at the same platelet count as the patient samples. If the patient platelet count is low, the control PRP should be adjusted to the same platelet count. Platelet counts below 100,000/mm^3 may give variable results.

Storage and Stability: Plasma as well as whole blood should always be stored at room temperature (15-30°C). Cover samples to maintain the pH. Tests should be performed within three hours after sample collection.

PROCEDURE

Materials Provided:

	Cat. No.
Arachidonic Acid Reagent (2 x 1.0 mL)	5364
Tris-Buffered Saline (1 x 200 mL) (optional)	5365

Materials Required but not provided in the kit:

Platelet aggregometer: (Helena AggRAM Analyzer recommended)
Cuvettes for aggregation recorder
Plastic Pipettes
Plastic or Siliconized Tubes
Pipettes to deliver 50 µL or 25 µL
Pipettes to deliver 450 µL or 225 µL

STEP BY STEP METHOD

NOTE: The AggRAM can be run with the recommended (standard) volumes or with one-half (micro) volumes. Standard volume tests are done at 1000 rpm and micro volume tests are done at 600 rpm. Patient results should be compared to normal ranges run under the same conditions.

The following steps are for standard volume; for micro volume, use one half of the standard volumes.

1. Collect blood specimen and prepare blood specimen according to directions in SPECIMEN COLLECTION AND HANDLING section.
2. Reconstitute the aggregation reagent according to the directions in the REAGENT section.
3. Unlike many other platelet aggregation reagents, it is recommended that Arachidonic Acid be used undiluted. The final concentration in platelet rich plasma is 500 µg/mL
4. Prepare aggregometer for use as recommended in the Operator's Manual.
5. Pipette 450 µL of platelet poor plasma (PPP) into a cuvette and leave at room temperature. Pipette 450 µL of platelet rich plasma (PRP) into a different cuvette and incubate the specimen at 37°C for one to three minutes.
6. Insert the PPP specimen into the appropriate channel and set the instrument to 100% aggregation.
7. Add a stir bar to the warmed PRP specimen, and insert the tube into the channel.
8. Bring the Aggregation reagents to room temperature (15-30°C) before use.
9. Add 50 µL of full strength Arachidonic Acid to the platelet rich plasma and record the aggregation.

Quality Control: Known normal plasma specimens should be used to establish typical aggregation patterns. Normal values for these patterns are then compared with the results from samples with marked variation from the normal range, thus indicating platelet dysfunction.[4]

RESULTS

The analysis of a platelet aggregation curve using Arachidonic Acid may include quantitations of the maximum slope or the maximum % aggregation. Reduction (compared to normal) of the rate and extent of aggregation induced by Arachidonic Acid can be caused by aspirin ingestion within 7 to 9 days prior to blood collection.

One of the several methods used to quantitated platelet aggregation is the Weiss formula. It measures the initial and maximum O.D. to give a result in percent aggregation.[9]

$$\frac{\text{O.D. Initial} - \text{O.D. Maximum}}{\text{O.D. Initial}} \times 100 = \% \text{ Aggregation}$$

Follow the Operator's Reference Manual for the aggregation monitoring instrument being used.

LIMITATIONS

A patient medical history of all prescriptions and non-prescription drugs should be taken before testing. Medication, especially aspirin, may interfere with aggre-

gation. For the effects of various drugs on platelet aggregation activity, refer to Young, et al.[10]
Prior to testing, patients should refrain from smoking or drinking, and if possible, from taking medication.

EXPECTED VALUES

The table below illustrates the results of a study conducted with a group of 50 samples from normal donors using the PACKS-4 and the AggRAM. These values should serve as guidelines for expected values. Because differences may exist among instruments, laboratories and local populations, it is recommended that each laboratory establish its own range of expected values.

	% Aggregation Actual Range
Arachidonic Acid 500 ug/mL	76-97

INTERPRETATION OF RESULTS

The following table may be used as a guideline as to abnormal findings in various platelet disorders.

Defect	ADP Primary Wave	ADP Secondary Wave	Collagen	Epinephrine
*Thrombasthenia	A	A	A	A
*Essential athrombia	A	A	A	A
Aspirin-like defect	N	A	A	+
Bernard Soulier	N	N	+	+
*Wiskott Aldrich	A	A	A	A
Storage Pool Disease	N	A	A	A
von Willebrand's Disease	N	N	N	N

A = Abnormal N = Normal + Not Diagnostic
*In distinguishing between these disorders, use discretion with additional testing as well as clinical symptoms.

PERFORMANCE CHARACTERISTICS

Correlation Studies: Seventy (70) samples were tested on the PACKS-4 and the AggRAM and the results are as follows.

n = 70
y = 1.078 + 3.7
r = 0.985
y = AggRam
x = PACKS-4

BIBLIOGRAPHY

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6. CLSI. Collection, Transport, and Protecting of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition. CLSI document H21-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
7. Triplett, D.A., ED., Standardization of Coagulation Assays: An Overview, College of Am Path, Skokie, IL., pg 4, 1982.
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10. Young, D.S. et al., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., AACC Press, Washington, D.C. 1990.

HELENA PLATELET AGGREGATION SYSTEM

	Cat. No.
Arachidonic Acid (2 x 1.0 mL)	5364
Platelet Aggregation Kit	5369
ADP Reagent (2 x 1.0 mL)	
Collagen Reagent (2 x 1.0 mL)	
Epinephrine Reagent (2 x 1.0 mL)	
ADP Reagent (2 x 1.0 mL)	5366
Epinephrine Reagent (2 x 1.0 mL)	5367
Collagen Reagent (2 x 1.0 mL)	5368
Tris-Buffered Saline (200 mL)	5365

Other Supplies and Equipment

The following items, needed for performance of Helena Arachidonic Acid Procedures, must be ordered individually.

AggRAM Analyzer	1484
AggRAM Stir Bars	1489
Siliconized Cuvettes for AggRAM	1473

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

Shading indicates that text has been modified, added or deleted.

HELENA LABORATORIES

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