Helena Platelet Aggregation Reagents

Cat. No. 5366, 5367, 5368, 5369

Helena Platelet Aggregation Reagents are intended for use in platelet aggregometry studies.

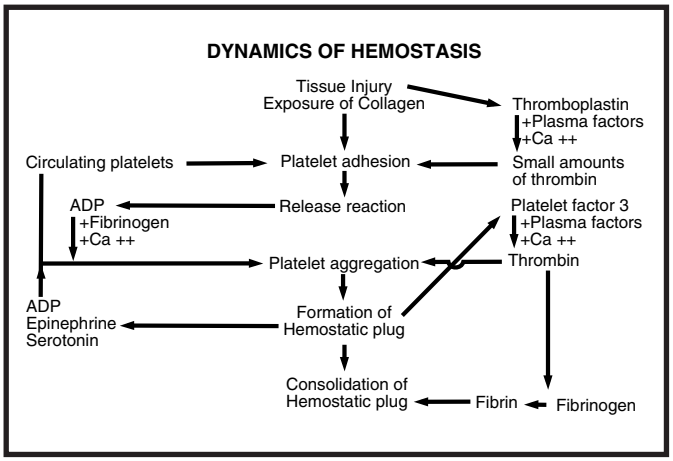
SUMMARY

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 and thrombin 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 aggregating reagents added. ADP, collagen and epinephrine are reagents commonly used to induce platelet aggregation. Helena Platelet Aggregation Reagents are stable, sensitive reagents which are conveniently packaged.



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 and epinephrine 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]

REAGENTS

1. **ADP (Adenosine diphosphate) Reagent**
   Ingredients (after reconstitution):
   ADP 200 μM
   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 ADP Reagent should be stored in the dry form at 2 to 8°C and is stable until the expiration date on the vial. The reconstituted reagent is stable for 1 week at 2 to 8°C or three months at -20°C. The working solutions should be used within three hours of preparation.
   Signs of Deterioration: If the dry, unreconstituted reagent is not uniformly white in appearance, it should not be used.
2. **Epinephrine Reagent**
   Ingredients (after reconstitution):
   Epinephrine bitartrate 3 mM
   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 Epinephrine Reagent should be stored in dry form at 2 to 8°C and is stable until the expiration date on the vial. The reconstituted reagent is stable for 1 week at 2 to 8°C.
   Signs of Deterioration: If the dry, unreconstituted reagent is not uniformly white in appearance, it should not be used.
3. **Collagen Reagent**
   Ingredients:
   Collagen (equine tendon) 100 μg/mL
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.
   Preparation for Use: The reagent is ready for use. Mix gently in swirling motion before use.
   Storage and Stability: The Collagen Reagent is stable in its liquid form in an unopened vial at 2 to 8°C until the expiration date on the vial. The contents of an opened vial are stable for four weeks at 2-8°C.
   Signs of Deterioration: The reagent should appear as a uniform clear suspension once it has been swirled. If discoloration is noted or the suspension is not uniform, the reagent should not be used.

INSTRUMENTS

Helena Platelet Aggregation Reagents are suitable for use with any turbidimetric aggregation monitoring device. Recommended is the Helena AggRAM (Cat. No. 1484) or the PACKS-4 (Platelet Aggregation Chromogenic Kinetic System) (Cat. No. 1471).

SPECIMEN COLLECTION AND HANDLING

Specimen: Plasma obtained from whole blood collected with 3.2% sodium citrate as an anticoagulant is the specimen of choice.

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 hematocritis (HCT) of <55% (normal), 9 parts of freshly collected whole blood should be immediately added to one part anticoagulant.[5] For blood specimens with hematocrits outside the normal range, adjust the amount of whole blood added to the anticoagulant according to the following formula.[6]

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

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.

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:

Platelet Aggregation Kit **Cat. No. 5369**

ADP Reagent (2 x 1.0 mL)
Collagen Reagent (2 x 1.0 mL)
Epinephrine Reagent (2 x 1.0 mL)

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Materials Required But Not Provided:

Platelet Aggregometer (AggRAM Analyzer or PACKS-4 Analyzer recommended)
Plastic pipette tips
Plastic or siliconized test tubes
Pipettes to deliver 50 µL or 100 µL
Pipettes to deliver 450 µL or 225 µL
0.85% Saline (preservative free)

STEP BY STEP METHOD

NOTE: The AggRAM and the PACKS-4 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 and prepare blood specimen according to directions in SPECIMEN COLLECTION AND HANDLING section.
2. Reconstitute the aggregation reagents according to the directions in the REAGENT section.
3. ADP, Epinephrine and Collagen reagents should be used undiluted and will have the final concentrations given below when mixed with the PRP during testing.

   If other concentrations are desired, make working solutions with saline.

	Final concentration
ADP	20 µM
Collagen	10 µg/mL
Epinephrine	300 µM

4. Prepare aggregometer for use as recommended in the Operator's Manual.
5. Pipette 450 µL of platelet poor plasma (PPP) into a cuvette. This blank will be used to set the 100% aggregation.
6. Pipette 450 µL of platelet rich plasma (PRP) into cuvettes with a stir bar. Incubate the cuvette at 37°C for one to three minutes.
7. Insert the PPP cuvette into the appropriate channel and set the instrument to 100% aggregation.
8. Iinsert the PRP cuvette into the appropriate channel.
9. Add 50 µL of the aggregating reagent dilutions to the PRP cuvette and record the percent aggregation. (Instrument sets 0% when the aggregating agent is added and the channel activated.)

NOTE: For maximum sensitivity to borderline response, aggregation may be performed with lower concentrations

of reagents. Dilutions of reagents made in 0.85% saline can be used to obtain minimum concentration required to produce normal aggregation. Each laboratory should establish the optimal range of dilution for its own instrument, reagent and normal population.

Quality Control: Known normal PRP 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 is noted by the presence of a primary and secondary wave. Prior to the addition of an aggregation reagent, random oscillations in the trace are seen. After exposure to the aggregation reagent, a delay in response, or oscillations, occurs followed by platelet shape changes. This alteration is the first platelet response to a stimulus and is seen as a decrease in oscillations and an increase in optical density. Small aggregates of platelets start to form and continue to form larger masses. This is noted by the ascent of the curve which is termed the primary wave. As the ascent nears completion, the trace may turn one of two directions. If the "platelet release reaction" occurs, the aggregation process continues to become irreversible (secondary wave), and the trace will continue to ascend. However, if the release reaction does not occur, the trace will reverse itself and continue downward to the baseline. This can be caused by aspirin ingestion 7 to 9 days prior to blood collection.

One of the several methods used to quantitate platelet aggregation is the Weiss formula. It measures the initial and maximum O.D. to give a result in percent aggregation.[8]

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

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 aggregation. For the effects of various drugs on platelet aggregation activity, refer to Young, et al.[9] Prior to testing, patients should refrain from smoking or drinking, and if possible, from taking medication.

REFERENCE VALUES

The table below illustrates the results of aggregation studies conducted at Helena Laboratories with a group of twenty-one normal donors using the Helena PACKS-4 and 50 normal donors with the AggRAM. These 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.

REAGENT	Combined % Aggregation Actual Range	Estimated Range
ADP	77-99	70-100
Collagen	80-98	70-100
Epinephrine	63-97	70-100*

*This range is for individuals who exhibit a secondary wave of aggregation and represents the maximum aggregation observed. A percentage of normal individuals with no known bleeding disorders do not exhibit a secondary aggregation with epinephrine and their % aggregation is about 20-60%.[11]

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	±	±
*Wiskitt 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 COMPARISON

Studies were done on specimens using the AggRAM and PACKS-4 Analyzers. The results were as follows.

ADP – N = 65
Y = 0.957X + 4.8
r = 0.982

Collagen – N = 66
Y = 1.071X – 2.1
r = 0.978

Epinephrine – N = 64
Y = 0.890 + 8.1
r = 0.955

X = PACKS-4
Y = AggRAM

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Other Supplies and Equipment

The following items, needed for performance of the Helena Platelet Aggregation Procedure, must be ordered individually.

	Cat. No.
AggRAM Analyzer	1484
AggRAM Stir Bars	1489
PACKS-4 (Platelet Aggregation Chromogenic Kinetic System)	1471
AggRAM/PACKS-4 Cuvettes (200)	1473
PACKS-4 Magnetic Stir Bars (30/pkg)	1474
PACKS-4 Pipette Tips (1000/box)	
50- 200 μL	1475
100-1000 μL	1476

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