INTENDED USE
Arachidonic Acid is for use in routine platelet aggregation studies for the differential diagnosis of aspirin-like release defects and Storage Pool Disease. It is also used to evaluate the inhibitory effect of aspirin on platelet aggregation.6,9,12

PRINCIPLE
Arachidonic Acid is a fatty acid present in the granules and membranes of human platelets.14 It is liberated from phospholipids and, in the presence of the enzyme cyclo-oxygenase, incorporates oxygen to form the endoperoxide prostaglandin 


In vitro addition of Arachidonic Acid to normal platelet rich plasma results in a burst of oxygen consumption, thromboxane formation and platelet aggregation.13 However, in the presence of aspirin or aspirin-containing compounds, these reactions are absent.15

PRECAUTIONS
Arachidonic Acid is for IN-VITRO DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Platelet Aggregometer
2. Purified water (distilled, deionized or reagent grade), pH 5.3 - 7.2
3. Pipettors (0.4mL and 0.05mL volumes)
4. Disposable Stir bars
5. Aggregometer cuvettes

INSTRUMENTATION
Arachidonic Acid will perform as described when used on most optical platelet aggregometers.1 Follow the manufacturer’s instructions for operating the aggregometer in use.

MATERIALS PROVIDED
Arachidonic Acid, 3 x 0.5mL. Store at 2° to 8° C prior to reconstitution.

SPECIMEN COLLECTION AND PREPARATION OF TEST SAMPLE
Refer to the current NCCLS Approved Guideline H21 A2 for detailed specimen collection and sample preparation instructions.6

1. PATIENT PREPARATION:
   Patients should refrain from taking aspirin or medications containing aspirin, other medications and dietary supplements known to affect platelet function for 7 - 10 days prior to specimen collection. Patients should fast and avoid fatty foods and dairy products for 12 hours prior to specimen collection.6

2. SPECIMEN COLLECTION:
   Blood collection should be performed with care to avoid stasis, hemolysis, contamination by tissue fluids, or exposure to glass. Keep specimens at room temperature.8

   Each of the following can cause test results to be inaccurate; and affected specimens should be rejected: hemolysis, RBC contamination, lipemia, chylosus, icterus, thrombocytopenia (<75,000/mm3) clots in specimen, and hypoalbuminemia. Reuse of disposable items may result in inaccurate test results.

   Observe standard precautions throughout the specimen collection, sample preparation and analytical processes.12 Dispose of sharps and biological waste in accordance with laboratory policy.

   Syringe Technique (recommended)6
   a. Use a butterfly needle for the venipuncture.

   Evacuated Collection Tube Technique
   1. Use a butterfly needle for the venipuncture.
   2. Draw blood using (plastic) tubes containing 0.11M Sodium Citrate anti-coagulant.
   3. Gently invert 4-5 times to mix.

   NOTE: When using plastic vacuum collection tubes, make sure the citrate anti-coagulant is 0.11M by checking the label. Colored tops do not vary with differing citrate concentrations. Follow the manufacturer’s instructions for specimen collection.

   PREPARATION OF PLATELET RICH PLASMA (PRP) AND PLATELET POOR PLASMA (PPP)
   1. Prepare platelet rich plasma by centrifuging the anti-coagulated blood at 150 X g for 10 minutes at room temperature (15° to 28°C).
   2. Examine the plasma layer for red cells. If red cells are present, re-centrifuge at 150 X g for an additional 5 minutes.
   3. Using a plastic transfer pipette, observe and carefully remove the platelet layer without disturbing the buffy coat or red cells, and transfer to a container labeled (PRP). Cap the container and allow it to stand at room temperature.
   4. Prepare the platelet poor plasma by centrifuging the remaining blood specimen at 2500 X g for 20 minutes. Examine the platelet poor plasma for hemolysis, then transfer it to a plastic tube labeled PPP.
   5. The platelet count of the PRP should be 250,000 ± 50,000/mm². The platelet count may be reduced by using PPP prepared from the sample.

   NOTE: If using Arachidonic Acid as an agonist, do not adjust the platelet count.

   RECONSTITUTION
   Reconstitute a vial of Arachidonic Acid with 0.5mL purified water. The reagent may appear cloudy, but will become clear and colorless within a few minutes.

   REAGENT STORAGE
   ARACHIDONIC ACID MUST BE KEPT STOPPERED AT ALL TIMES WHEN NOT IN USE.

   Restopper the vial immediately after removing reagent. Reconstituted Arachidonic Acid is stable for 24 hours at 2° - 8° C. For long term storage, freeze at -20°C for up to 8 weeks.

   TEST PROCEDURE
   Testing must be completed within 3 hours of specimen collection.8
   1. Prepare an aggregometer blank by pipetting 0.5mL platelet poor plasma into a cuvette.
   2. Pipette 0.45mL platelet rich plasma into a second cuvette. Incubate at 37°C for 3 minutes and add a stir bar.
   3. Set, if required, the 0% and 100% baselines according to the manufacturer’s instructions for the aggregometer in use.
   4. Add 0.05mL Arachidonic Acid directly into the platelet rich plasma. Do not allow reagent to run down the wall of the cuvette. The final concentration of Arachidonic Acid in the platelet rich plasma test mixture is 500 µg/mL.
   5. Allow the aggregation pattern to generate for 5 minutes.

   QUALITY CONTROL
   Laboratories should follow generally accepted quality control practices when proficiency testing is not available.

   To assure proper instrument operation and reagent performance, a control specimen should be evaluated each day that tests are performed. The control specimen should be prepared in the same manner as the test specimen. For qualitative platelet aggregation studies, the control should consist of fresh platelet rich plasma collected from a (specified and qualified) normal donor who has not ingested aspirin containing compounds within 10 days of testing and has a history of normal platelet function.

   RESULTS
   Typical Arachidonic Acid aggregation patterns are illustrated in Figs. 1-3. Ingestion of a single dose (600mg) of aspirin will result in absence of Arachidonic Acid aggregation for as long as 5 days (Fig. 3) A prolonged response (time from addition of reagent to onset of aggregation) will be observed for up to 8 days following aspirin ingestion.7 (Fig. 3)
LETS/mm²

**Figure 1 Normal Aggregation**

**Figure 2 Abnormal Response (Aspirin Effect)**

**Figure 3 Abnormal Response (Mild Aspirin Effect 5-8 days post ingestion)**

**LEGEND:** Results of Arachidonic Acid-induced platelet aggregation on normal and platelet-rich plasma. The working concentration of Arachidonic Acid is 5.0 mg/mL. The final concentration of PRP is 500 μg/mL. Spike mark indicates addition of reagent.

**EXPECTED VALUES**

Expected ranges for each reagent at various concentrations used to induce platelet aggregation should be established by each laboratory, see Table 2.

**TYPICAL PLATELET AGGREGATION RESPONSES FOR NORMAL DONORS @ 250,000 PLATELETS/mm²**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>ADP</td>
<td>2.0 x 10⁻⁶ M</td>
</tr>
<tr>
<td>Arachidonic Acid</td>
<td>500 μg/mL</td>
</tr>
<tr>
<td>Collagen (Type I)</td>
<td>0.19 mg/mL</td>
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<tr>
<td>Epinephrine</td>
<td>1.0 x 10⁻⁶ M</td>
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</table>

**LIMITATIONS**

Arachidonic Acid will oxidize if the vial is left unstopped. Oxidized reagent will appear yellow in color and should not be used. Because Arachidonic Acid binds to albumin, the concentration required to induce aggregation in platelet rich plasma is higher than the concentration required in washed platelet suspensions. For testing washed platelets, Arachidonic Acid should be diluted with physiologic saline to an appropriate concentration for the platelet preparation in use.

It has been noted that on occasion, sub-optimal aggregation occurred when Arachidonic Acid was added to platelet rich plasma which had been diluted with platelet poor plasma. However, aggregation appeared normal when the same platelet rich plasma was tested in the undiluted form.

A detailed patient history is required for accurate test interpretation. Patients should be questioned about the recent ingestion of any medication, because a number of prescription and nonprescription drugs may interfere with platelet aggregation. Substances such as caffeine, tobacco, herbal extracts (or supplements) and alcohol may affect results.

**PERFORMANCE CHARACTERISTICS**

Studies have shown that this product will perform as described prior to its expiration date when procedural and storage directions are followed.

**REFERENCES**


**PRODUCT AVAILABILITY**

<table>
<thead>
<tr>
<th>PRODUCT</th>
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<th>CATALOG NUMBER</th>
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<td>Arachidonic Acid</td>
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<tr>
<td>ADP</td>
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<td>101312</td>
</tr>
<tr>
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<td>Epinephrine</td>
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<td>Lyophilized Platelets</td>
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<tr>
<td>Lyophilized Platelets PAR Pak® II</td>
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<td>vW Factor Assay</td>
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