AMERICAN BIOCHEMICAL & PHARMACEUTICALS LTD.

REF ABP-ARA-1
3 X 0.5mL, Lyophilized
Arachidonic Acid

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PRODUCT DESCRIPTION
Arachidonic Acid is a lyophilized preparation of sodium arachidonate. The working concentration of the reconstituted Arachidonic Acid reagent is 5.0 mg/mL.

Sodium salts of Arachidonic Acid are very sensitive to oxidation and may deteriorate rapidly once opened and exposed to air. Recap the vial immediately after use.

A yellow tinge is an indication oxidation has occurred and the reagent may no longer perform as expected.

INTENDED USE
Arachidonic Acid is intended for use in routine platelet aggregation studies.

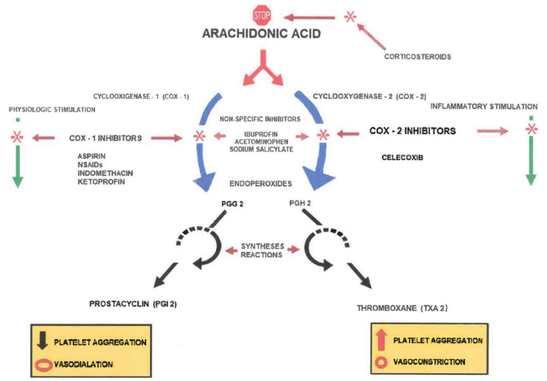
Arachidonic Acid can confirm the presence of Aspirin in a test sample and indicate its effect on platelet function.

Arachidonic Acid is used in the differential diagnosis of Storage Pool Disease and Aspirin-like release defects. [1,2,3,4,11,12]

TEST PRINCIPLES
Arachidonic Acid is a strong agonist. It is an omega-6 fatty acid that is located in the membrane of the granules of platelets.[5,6,7] When Arachidonic Acid is added to Platelet Rich Plasma (PRP), it is converted, in a multi-step process, including the enzymatic incorporation of oxygen bi cyclo-oxygenase 1 (COX1), in to thromboxane A2 (TXA2). TXA2 activates platelets and causes a granule discharge from the platelet and then the platelet shape changes. In Light Transmission Aggregometry (LTA), this causes a decrease in light transmission which is represented by a single wave of platelet aggregation.[8,9]

Aspirin, Aspirin-like defects and Storage Pool Disease inhibit platelet aggregation by interrupting the COX 1 pathway.[1,2,9,12,13,14]

Sodium arachidonate is 10 times more potent in inducing aggregation of human platelets suspended in buffer than in plasma (PRP).



IVD NON STERILE Arachidonic Acid reagent is intended for *IN VITRO DIAGNOSTIC USE ONLY.*

In accordance with laboratory policy appropriate Personal Protective Equipment, including lab coat, gloves and eye protection should be used when working with Arachidonic Acid. [15]

MATERIALS PROVIDED
Arachidonic Acid Reagent, Type 1 3x0.5mL
Arachidonic Acid Reagent Storage prior to reconstitution 2 - 8°C.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Platelet Aggregometer
2. Purified Water (distilled, deionized or reagent grade) (pH 5.3 - 7.2)
3. Pipettes and tips
4. Sample tubes and caps
5. Siliconized Aggregometer cuvettes
6. Plastic coated micro stir bars

INSTRUMENTATION
Arachidonic Acid Reagent will perform as described when used on most Light Transmission Aggregometers.

Follow the aggregometer manufacturer's instructions for USE and sample size requirements.

RECONSTITUTION
Allow the Arachidonic Acid reagent to come to room temperature prior to reconstitution.

To reconstitute a vial of Arachidonic Acid, add 0.5mL of purified water to the vial and recap the vial.

Initially, the reagent may appear to be cloudy. It will become clear and colorless in a few minutes.

Refrigerated reagent must come to toom temperature prior to use.

Recap the Arachidonic Acid Reagent IMMEDIATELY after use.

REAGENT STORAGE
Reconstituted Arachidonic Acid Reagent is stable for 24 hours when stored at 2° to 8°C in a tightly stoppered vial. It is stable for up to eight weeks when stored at -20°C.

ARACHIDONIC ACID MUST BE KEPT IN A TIGHTLY STOPPERED VIAL AT ALL TIMES WHEN NOT IN USE.

REAGENT DISPOSAL
Unused or expired Arachidonic Acid reagent must be disposed of as a hazardous waste in accordance with local regulations and laboratory policy.

PROFESSIONAL LABORATORY USE ONLY

PERFORMANCE CHARACTERISTICS
Studies have confirmed that Arachidonic Acid will perform as described, prior to its expiration date, when storage, usage and procedural instructions are followed.

EXPECTED VALUES
Expected values vary by concentration, sample type, disease state and other factors. Reference ranges must be determined locally:

ARACHIDONIC ACID	WORKING CONCENTRATION	FINAL CONCENTRATION	FINAL AGGREGATION (%)
	5.0 mg/mL	500 µG/mL	60-90%

LAG PHASE	PRIMARY SLOPE	FINAL AGGREGATION @ 6 MINUTES (%)	BIPHASIC AGGREGATION	AUC@6 MINUTES
≤ 20	<20	65 - 90	NO	414

NOTE: NOT ALL NORMAL DONORS CONFORM TO THE EXPECTED RESULTS.

Reference ranges must be established by each laboratory.

Figure 1: Normal Response

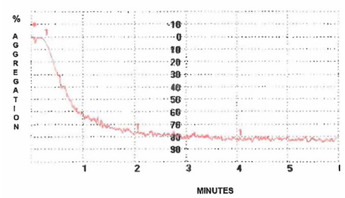


Figure 2: Abnormal Response: Aspirin Effect

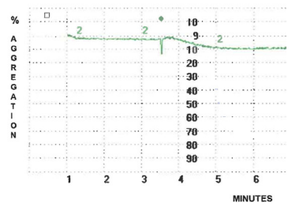
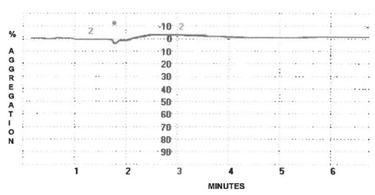


Figure 3: ABNORMAL RESPONSE: GLANZMANN'S THROMBASTHENIA



RESULTS
A typical response to Arachidonic Acid will appear as a single wave of aggregation. (Fig 1) A single, 600 mg dose of Aspirin will result in the absence of a response to the Arachidonic Acid for up to five days (Fig 2). A heritable, Aspirin-like defect will appear as an inhibited response to Arachidonic Acid. (Fig 3)

LINEARITY
Platelet Aggregation is a non-linear test system. Platelet Aggregation can be induced by a variety of agents. It is a biological reaction. The underlying reaction, test conditions/instrumentation type, agonist and agonist concentration, among other factors contribute to reaction response. Platelet Aggregation is not a quantitative test. It measures the rate and extent of a response to the agonist in a concentration dependent manner.
The following parameters are reported for agonist induced platelet aggregation: Primary Aggregation, Primary Slope, Secondary Aggregation, Secondary Slope (biphasic response), Lag Phase, Disaggregation, AUC @ 6minutes, Maximum aggregation, and Final aggregation.

ACCURACY, PRECISION AND REPRODUCIBILITY
Accuracy
Accuracy is a relative parameter in Light Transmission Aggregometry (LTA). It depends on the test system.

Precision and Reproducibility
The nature and limitations of LTA make it difficult to provide the usual precision or reproducibility ranges for the test. Consensus reports refer to the following ranges and experts recommend that each laboratory establish its own limits for test acceptability.[14,16,17]

Test to Test Reproducibility:	better than ± 7.5%
Instrument to Instrument Reproducibility:	better than ± 15%
Reagent Lot to Lot Variation:	better than ± 10.5%

QUALITY CONTROL
Laboratories should follow the Westgard Rules for low volume tests.

A known and drug free donor should be tested at a frequency that is in accordance with laboratory policy. Limited Proficiency tests are available from accredited and professional organizations.[21]

LIMITATIONS
Arachidonic Acid will oxidize if the vial is left uncapped. The oxidized reagent will appear yellow in color. Do not use the reagent once oxidized.

Do not adjust the platelet count when using Arachidonic Acid as an agonist. Diluting PRP, particularly with PPP, will generate sub-optimal results.

Because of the loss of albumin in washed platelets preparations, the Arachidonic Acid should be diluted with preservative free, physiologic saline to an appropriate concentration for the platelet preparation to be tested.[18,19,20]

TEST PREPARATIONS

PATIENT PREPARATION[2,11,14,17]
1. Prior to being tested, Clinical, medication, family and social histories are required.
2. Patients should refrain from taking Aspirin or other anti-platelet medications for 7-10 days, or as directed by their physician.
3. Patients should avoid supplements, herbal preparations, energy drinks or other products known to affect platelet function.
4. Patients should avoid fatty meals and food products prior to specimen collections.

SPECIMEN COLLECTION[14,15,16,21]
Refer to the current CLSI Approved Guidelines H 58 -A: Platelet Function Testing by Aggregometry for detailed specimen collection and sample preparation instructions and related references.

EVACUATED SPECIMEN COLLECTION TUBE TECHNIQUE (PREFERRED)
1. Use a 21 or 23 gage winged needle set for specimen collection
2. Remove the tourniquet as soon as blood starts to flow
3. Collect the blood specimen in 2.7µL plastic evacuated specimen collection tubes containing 0.105/0.11 M (2.3%) buffered sodium citrate anticoagulant.
4. Gently invert each tube 4 -5 times to assure complete mixing.
5. Maintain specimens at room temperature without removing the caps.
6. Observe Standard Precautions through out the specimen collection process and follow appropriate laboratory policies for post phlebotomy patient care and disposal of sharps and supplies.

15 - 28°C

1. Evacuated specimen collection tubes with light blue tops may contain 3.2% or 3.8% sodium citrate. Check the label for the proper concentration.[3,6,7,9]
2.Underfilled tubes should be rejected
3. Blood collection should be performed with care to avoid patient anxiety, stasis, hemolysis and contamination by tissue fluid, or any exposure to glass.
4. Make sure the winged needle set is intended for phlebotomy use.
5. Each of the following can cause test results to be inaccurate
   a. Visible RBC contamination
   b. Hemolysis
   c. Icterus
   d. Lipemia
   e. Clots

These are unacceptable specimens and should be rejected.
6. Test results may also be affected if the patient has thrombocytopenia (thresholds are agonist and analyzer dependent) or hypofibrinogenemia. Follow laboratory policies when such specimens have been collected.
7. If the patient's hematocrit is less than 30% or greater than 55%, the blood to anticoagulant ratio must be adjusted. (see H58 -A for instructions)
8. Specimens must be tested within four hours of collection.

SAMPLE (PRP & PPP) PREPARATION[9,11,13,14]

PREPARATION OF PLATELET RICH PLASMA (PRP) & PLATELET POOR PLASMA (PPP) TEST SAMPLES
Check the RCF Nomogram in the centrifuge manual to confirm the proper settings.

1.Prepare Platelet Rich Plasma test samples first.
2.Centrifuge the unopened specimen collection tubes at 150 x g for 10 minutes at room temperature.
3. Do not engage the centrifuge's brake.
4. Carefully remove the tubes from the centrifuge. Examine the plasma layer for the presence of Red Blood Cells (RBCs)
   a. If there are RBCs present, re-centrifuge for an additional five minutes at 150 x g.
5. Using a plastic transfer pipette, carefully remove the PRP layer without disturbing the buffy coat and transfer the PRP to labeled plastic sample tubes and cap the tubes. Maintain the PRP at room temperature.
6. To prepare the PPP, recap the specimen collection tubes and re-insert them into the centrifuge. Centrifuge those specimens at 1500 x g for 20 minutes.
7. Check for hemolysis.
   a. If the PPP is hemolyzed, it is unacceptable for use as a blank.
8. Carefully transfer the PPP to pre-labeled plastic tubes and cap them. Maintain them at room temperature.[6,9]

1. PRP should have nominal platelet count greater than 200,000/cumm
2. PPP must have a platelet count less than 10,000/cumm
3. Platelet counts on PRP and PPP can not be performed using automated hematology analyzers. Those analyzers were neither designed or intended for counting these samples. It is best to count PRP and PPP, if necessary using a hemocytometer.
4. PRP platelet counts should not be adjusted using PPP.
5. PRP has a maximum useful life of four hours from the time of collection.

GENERIC LTA TEST PROCEDURES
1. Place the appropriate number of test cuvettes in to the incubation wells.
2. Add a new, plastic coated stir bar to each cuvette.
3. Prepare the PPP blank by pipetting 0.250 µL of PPP in to a cuvette.
   DO NOT PLACE A STIR BAR IN THE BLANK TUBE
4. Pipette 0.225 µL of PRP (patient sample) into each test cuvette containing a stir bar.
5. Place the PRP sample tubes in the incubation block
   a. Select the timer button for the test channel, and a countdown will begin.
   b. Incubate the PRP test samples for a pre-set incubation period and temperature (37°C)
6. Set the 100% baseline by placing the blank into the test well.
   a. Press the Blank Button
   b. Remove the Blank from the test well
7. Place the PRP sample cuvette into the test well
   a. Press the Start Button.
8. Add 0.25 µL of the agonist/reagent into the PRP using the proper pipette and tip to assure the agonist/reagent is directed into the center of the cuvette and not allowed to run down the side of the cuvette.
9. Select inject
10. The test will run for the pre-set test time.
11. An alarm will sound when testing in all channels is completed.

ARACHIDONIC ACID RESPONSES[1,4,9,11]

CONDITION	AA RESPONSE
Aspirin	Abnormal
Antiplatelet Drugs	Abnormal
Intrinsic Release Defect	Abnormal
Glanzmann's Thrombasthemia	Abnormal

FURTHER TESTING:
If the test results are abnormal when properly interpreted:
1. Review clinical history
2. Review the patient's medication record
3. Recheck the patient's social history for use of aspirin containing compounds, supplement use and herbal/spice use.

WARRANTY
This product is warranted to perform to these specifications when used in accordance with labeling. American Biochemical and Pharmacueticals Ltd. disclaims any implied warranty of merchantability and fitness for any other purpose and in no event shall American Biochemical and Pharmacueticals Ltd. be liable for any consequential damages arising out of the aforesaid warranty.

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