PRODUCT DESCRIPTION
Arachidonic Acid is a lyophilized preparation of sodium arachidonate. The working concentration of the reagent is 5mg/mL.

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.[8,11,12]

PRINCIPLE
Arachidonic Acid is a fatty acid present in the granules and membranes of human platelets.[16] It is liberated from phospholipids and, in the presence of the enzyme cyclo-oxygenase, incorporates oxygen to form the endoperoxide prostaglandin G_2 (PGG_2). PGG_2 is then quickly transformed to prostaglandin H_2 (PGH_2) which in turn is converted to thromboxane A_2 a potent inducer of platelet aggregation. Ingestion of aspirin or aspirin-containing compounds inhibits cyclo-oxygenase mediated oxygen consumption, thus precluding all subsequent events leading to platelet aggregation.[8,11,13]

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.[12]

ARACHIDONIC ACID

Aspirin

Cyclo-oxygenase

$PGG_2 \rightarrow PGH_2$

Thromboxane A_2

Inhibition

Platelet Aggregation   Thromboxane B_2 (Inactive)
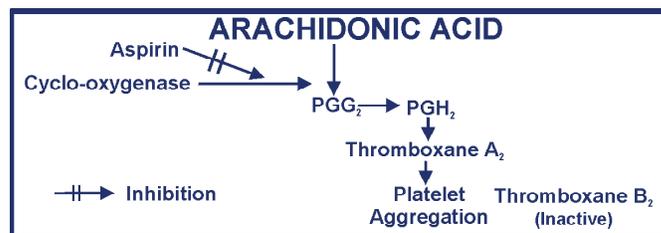
PRECAUTIONS
Arachidonic Acid is for IN-VITRO DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION.

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

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.

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, chylous, icterus, thrombocytopenia (<75,000/mm³) clots in specimen, and hypofibrinogenemia. Reuse of disposable items may result in inaccurate test results.

Observe standard precautions throughout the specimen collection, sample preparation and analytical processes.[2,3] Dispose of sharps and biological waste in accordance with laboratory policy.

Syringe Technique (recommended)[8]
a.   Use a butterfly needle for the venipuncture.

b.   Draw 9.0mL of blood into a plastic syringe. Avoid excess suction.
c.   Remove the needle from the syringe and immediately and gently dispense the blood into a plastic [polypropylene]4 tube containing 1.0mL of 0.11M Sodium Citrate anti-coagulant. The ratio of blood to anti-coagulant must be 9 parts of blood to 1 part anti-coagulant.[5]
d.   Cover and invert 4-5 times gently to mix.
e.   Maintain at room temperature (15° to 28°C).

NOTE: When the patient's hematocrit is < 30% or > 55%, the blood to anti-coagulant volumes must be adjusted.[4]

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°to28°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/mm³. 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
NOTE: Reagents must be at room temperature (15° to 28°C) prior to reconstitution. Stored reagent must be brought to room temperature prior to use.

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.2) A prolonged response (time from addition of reagent to onset of aggregation) will be observed for up to 8 days following aspirin ingestion.[2] (Fig.3)
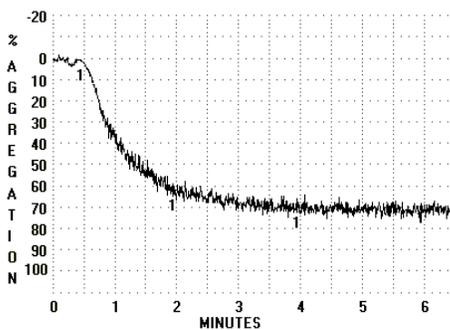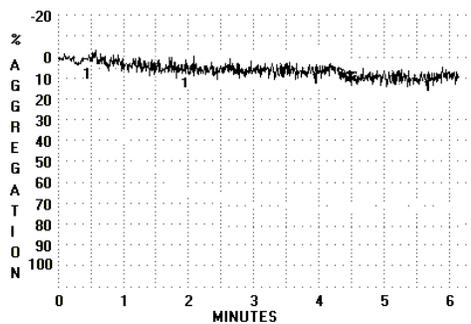
Figure 1 Normal Aggregation
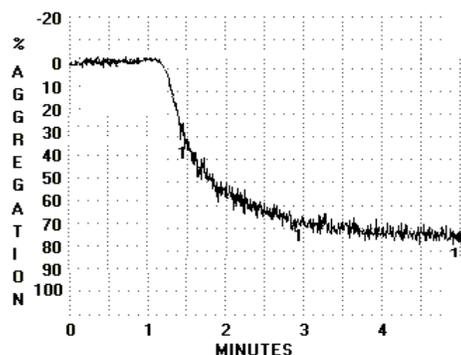

Figure 2 Abnormal Response (Aspirin Effect)

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 5.0mg/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.[4,8,9,10]

Table 2

TYPICAL PLATELET AGGREGATION RESPONSES FOR NORMAL DONORS @ 250,000 PLATELETS/mm³   [total aggregation at 5 minutes]

	ADP	Arachidonic Acid	Collagen [Type I]	Epinephrine
Final Conc.	2.0×10^{-5} M	500µg/mL	0.19mg/mL	1.0×10^{-4} M
Lag Phase [sec]	<10	<=20	<60	0
Primary Slope	38-67	>20	35-67	7-34
Total Aggregation (%@5min)	63-89	65-90	61-99	54-101
Biphasic Aggregation	concentration dependent	NO	NO	YES
Other	May show Shape changes	All normal Donors may not Conform PLT CT~175k-300k	Do not Dilute	All normal Donors may not Conform

LIMITATIONS

Arachidonic Acid will oxidize if the vial is left unstoppered. 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.[15] 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.[7,8]

PERFORMANCE CHARACTERISTICS

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

Linearity:

Platelet aggregation induced by common agonists (ADP, Arachidonic Acid, Collagen and Epinephrine) is a nonlinear test system for the following parameters: Lag Phase, Primary Slope, Secondary Slope, biphasic response and disaggregation. The non-linearity is caused by many factors such as the reaction chemistry and instrumentation. Platelet aggregation measures a response rate or activity that is not a quantitative measure of the reactants or their concentration.

ACCURACY, PRECISION AND REPRODUCIBILITY
Accuracy

In platelet aggregation, accuracy is a relative parameter and is dependent on the test system.

Precision and Reproducibility

The limitations of platelet aggregation makes it difficult to provide typical precision or reproducibility ranges. However, there is an experienced based consensus for these parameters (see below). Each laboratory must establish its own limits for test acceptability.

Test to Test Reproducibility:	better than ± 7.5%
Instrument to Instrument Reproducibility:	better than ± 15%
Reagent Lot to Lot Variation:	better than ± 10.5%
Laboratory to Laboratory (same test system):	better than ± 12.5%

REFERENCES

1. Born, GVR and Cross, MJ. The Aggregation of Blood Platelets. J. Physiol [London] 168:178, 1963.
2. Centers for Disease Control and Prevention. Guidelines for Isolation Precautions in Hospitals. Centers for Disease Control and Prevention. 1996; Vol 17; 1:53 - 80.
3. National Committee for Clinical Laboratory Standards. NCCLS: Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline. NCCLS document M29. Wayne, PA.
4. McCabe-White, M and Jennings, LK. Platelet protocols: Research and Clinical laboratory Procedure. Academic Press. London. 1999, p 35.
5. Newhouse, P and Clark, C. The Variability of Platelet Aggregation., in Triplet, DA,ed. Platelet Function: Laboratory Evaluation and Clinical Application. ASCP. Chicago. 1978. p 69.
6. National Committee for Clinical Laboratory Standards. NCCLS Collection, Transport and Processing of Blood Specimens Approved Guideline- Second Edition. NCCLS Document H 18-A2. Wayne, PA.
7. Weiss, HJ: Aspirin and platelets in drugs and hematologic reactions. Dimittov and Nodine (eds.). Grune and Stratton, New York, 1974.
8. Triplett, DA, Harms, CS, Newhouse, P, Clark, C: Platelet Function.Laboratory Evaluation and Clinical Application. ASCP, 1978.
9. Day, HJ, Holmsen, H: Laboratory tests of platelet function. Annal Clin Lab Sci, 2:63, 1972.
10. Owen, CA, Bowie, EJW, Thompson, JH: The diagnosis of bleeding disorder. Little, Brown and Co., 1975.
11. Bye, A., Lewis, Y, O'Grady, J: Effect of a single oral dose of aspirin on the platelet aggregation response to arachidonic acid. Br J Clin Pharmac 7a:293, 1979.
12. Ingerman, CM, Smith, JB, Shipiro, S, Sedar, A, Silver, A, Silver, MJ: Hereditary abnormality of platelet aggregation attributable to nucleotide storage pool deficiency, Blood 52:332, 1978.
13. Moncada, S, Vane, JR: Arachidonic Acid metabolites and the interactions between platelets and blood vessel walls. N Eng J Med 300:1142, 1979.
14. William, WJ, Beutler, E, Erslev, A, Rundies, RW: Hematology, Mc-Graw Hill, New York, 1977.
15. Marcus, AJ: Platelet Aggregation. In Coleman RW, Hirsh J, Marder VJ, Salzman EQ: Hemostasis and thrombosis: Basic principles and clinical practice. pg 380. JB Lippencott Company, 1982.
16. Marcus, AJ: Platelet Aggregation. In Coleman RW, Hirsh J, Marder VJ, Salzman EQ: Hemostasis and thrombosis: Basic principles and clinical practice. pg 472. JB Lippencott Company, 1982.

PRODUCT AVAILABILITY

PRODUCT	NET CONTENTS	CATALOG NUMBER
Arachidonic Acid	3 x 0.5mL	101297
ADP	3 x 0.5mL	101312
BETA/Pak®		
(ADP, Collagen, Ristocetin)	1 x 0.5mL each	101580
Collagen	3 x 0.5mL	101562
Epinephrine	3 x 0.5mL	101311
Lyophilized Platelets	3 x 4 mL	101595
Lyophilized Platelets	1 x 10 mL	101258
PAR/Pak® II		
(ADP, Collagen, Epinephrine)	2 x 0.5mL each	101310
Ristocetin		
AggRecetin® 1.5mg/mL	15mg	100968
AggRecetin 1.0-1.5mg/mL	15mg	100970
AggRecetin Bulk	100mg	101241
vW Factor Assay®	10 Determinations	101246
vW Factor Assay	20 Determinations	103025
vW Abnormal Control Plasma	3 x 0.5mL	101270
vW Normal Reference Plasma	3 x 0.5mL	101269
vW Normal Control Plasma	3 x 0.5mL	106426

BIO/DATA CORPORATION

155 Gibraltar Road, PO Box 347, Horsham, PA 19044-0347 U.S.A.
(800) 257-3282 U.S.A.   (215) 441-4000 Worldwide
(215) 443-8820 Fax Worldwide
E-mail: bdc@biodatacorp.com
Internet: www.biodatacorp.com

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EC REP   EMERGO EUROPE, Molenstraat 15, 2513 BH The Hague, The Netherlands