BIO/DATA CORPORATION

English

Epinephrine

IVD

(Adrenaline)

REF 101311

PRODUCT DESCRIPTION
Epinephrine is a lyophilized preparation of adrenalin. The working concentration of the reconstituted reagent is 1 x 10^{-3} M.

INTENDED USE
Epinephrine (adrenalin) reagent is for routine use in platelet studies for the evaluation of hypersensitivity of platelets in Platelet Rich Plasma and evaluation of platelet responses to a weak agonist.

PRINCIPLE
When added to platelet rich plasma, Epinephrine stimulates platelets to aggregate. Aggregation induced by Epinephrine is referred to as primary aggregation. Normal platelets will further respond by releasing endogenous ADP from their granules. Release of endogenous ADP results in a secondary wave of aggregation.[8,10,11]

PRECAUTIONS
Epinephrine is for *PROFESSIONAL LABORATORY USE AND IN-VITRO* DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION.

NOTE TO USER: Any serious incident that occurs in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

MATERIALS PROVIDED
Epinephrine, 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 (0.5mL, 0.45mL, 0.05mL volumes)
4. Disposable Stir bars
5. Aggregometer cuvettes

INSTRUMENTATION
Epinephrine 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/mm^3) 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 anticoagulant. The ratio of blood to anti-coagulant must be 9 parts of blood to 1 part anticoagulant.[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 anticoagulant 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 anticoagulant.
   3. Gently invert 4-5 times to mix.

   NOTE: When using plastic vacuum collection tubes, make sure the citrate anticoagulant 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/mm^3. 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 Epinephrine with 0.5mL purified water.

REAGENT STORAGE
The reconstituted Epinephrine is stable for 30 days when stored at 2°-8°C in its original tightly sealed container.

TEST PROCEDURE
The platelet rich plasma must be held at room temperature for at least 30 minutes prior to testing.
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 Epinephrine directly into the platelet rich plasma. Do not allow reagent to run down the wall of the cuvette. The final concentration of Epinephrine in the platelet rich plasma is 1 x 10^{-4}M.
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 Epinephrine aggregation patterns are illustrated in Figures 1 and 2. Epinephrine will induce two distinct waves of aggregation in normal platelet rich plasma.[8,10,11]

EXPECTED VALUES
Expected ranges for each reagent at various concentrations used to induce platelet aggregation should be established by each laboratory, see Table 1.[4,8,9,10]

Table 1

TYPICAL PLATELET AGGREGATION RESPONSES FOR NORMAL DONORS @ 250,000 PLATELETS/mm^3 [total aggregation at 5 minutes]

	ADP	Arachidonic Acid	Collagen [Type I]	Epinephrine
Final Conc.	2.0x10^{-5} M	500µg/mL	0.19mg/mL	1.0x10^{-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

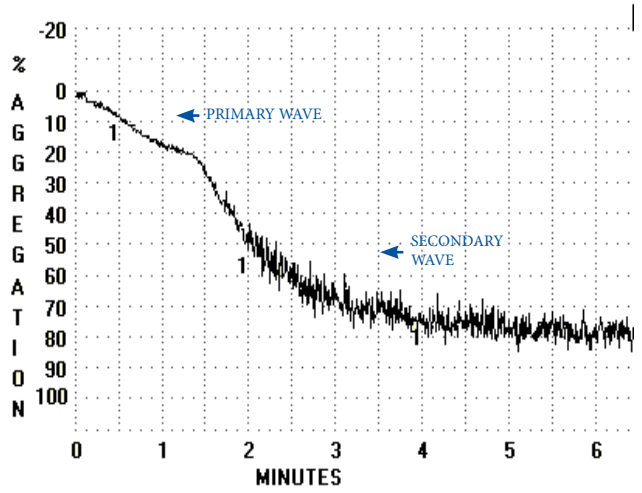

Fig. 1 Normal Aggregation

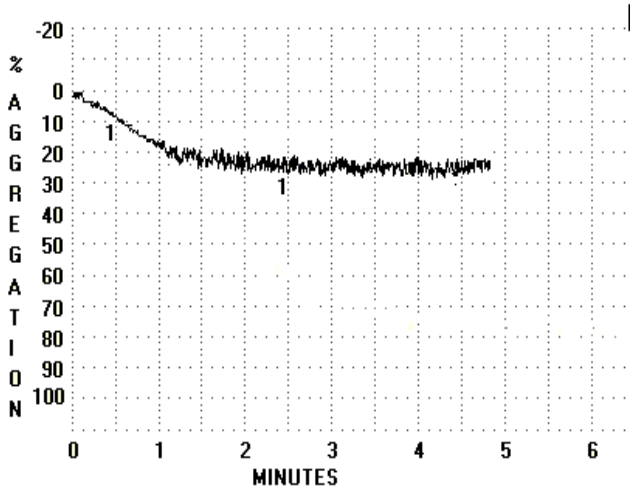

Fig. 2 Abnormal Aggregation

LEGEND: Results of Epinephrine induced platelet aggregation on normal and abnormal plasmas. The final concentration of Epinephrine in PRP is 1 x 10^{-4}M.
Spike mark indicates the addition of reagent.

LIMITATIONS[3]
Spurious results will be observed when the platelet count of the platelet rich plasma is less than 75,000 platelets/uL.

Platelet rich plasma which has not been held at room temperature for at least 30 minutes prior to testing may yield abnormal results.

Several reports indicate that platelet rich plasma from 20-50% of the normal population will exhibit only a primary wave of aggregation in response to Epinephrine.

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

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