BIO/DATA CORPORATION

Collagen

(Soluble Calf Skin)

PRODUCT DESCRIPTION
Collagen is a lyophilized preparation of soluble calf skin collagen (Type 1). The working concentration of the reconstituted reagent is 1.9mg/mL.

INTENDED USE
Collagen reagent is intended for routine use in inducing Platelet Rich Plasma responses to activation, aggregation and inhibition.

PRINCIPLE
When collagen is added to platelet rich plasma, the platelets adhere to the collagen. Following this adhesion, normal platelets will change their shape, release endogenous ADP, and aggregate.[8,10,11]

PRECAUTIONS
Collagen (Type 1) is for *PROFESSIONAL LABORATORY USE ONLY AND IN-VITRO* DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION.

MATERIALS PROVIDED
Collagen, 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 volumes)
4. Disposable Stir bars
5. Aggregometer cuvettes

INSTRUMENTATION
Collagen will perform as described when used on most optical platelet aggregometers. 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 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° 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/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 Collagen with 0.5mL purified water.

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

TEST PROCEDURE
Testing must be completed within 4 hours of specimen collection.[8]
1. Place a stir bar in each cuvette
2. Prepare an aggregometer blank by pipetting 0.5mL platelet poor plasma into a cuvette.
3. Pipette 0.45mL platelet rich plasma into a second cuvette. Incubate at 37°C for 2 minutes.
4. Set, if required, the 0% and 100% baselines according to the manufacturer's instructions for the aggregometer in use.
5. Add 0.05mL Collagen directly into the platelet rich plasma. Do not allow reagent to run down the wall of the cuvette.
6. 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 Collagen aggregation patterns are illustrated in Figs. 1 and 2. Following addition of Collagen to platelet rich plasma, a lag phase occurs during which no aggregation is observed. Normal platelets will then exhibit a shape change followed by a large, single wave of aggregation.

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/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)	62-101	65-90	63-109	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

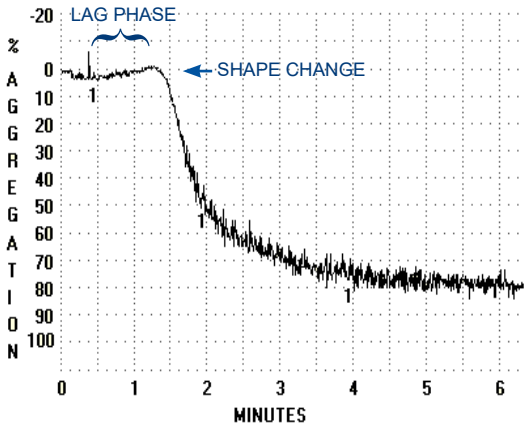

Fig. 1 Normal Aggregation

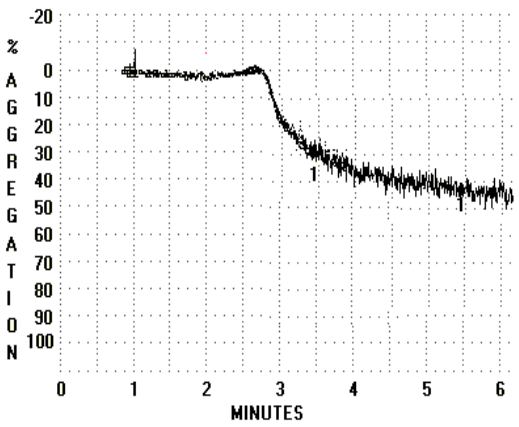

Fig. 2 Abnormal Aggregation

LIMITATIONS

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 make 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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Printed in U.S.A.

Instructions for Use No. 101563 Rev. L