AMERICAN BIOCHEMICAL & PHARMACEUTICALS LTD.

REF ABP-COL-T1
3 X 0.5mL, Lyophilized
Collagen, TYPE 1

Symbol Guide

IVD — In-vitro Diagnostics Product
Consult Operations Manual
Distributor
Do Not Reuse
Contains Sufficient for < N < Tests
Consult Instructions For Use
NON STERILE — Non-Sterile
Caution see instructions
Temperature Limitations
Biohazardous
Manufacturer

EC REP
Global House
5 Ashley Avenue
Epsom, Surrey KT18 5AD
United Kingdom

PRODUCT DESCRIPTION
Collagen Reagent is a lyophilized preparation of soluble calf skin (Type1) fibrillar collagen. The working concentration of the reconstituted collagen reagent is 1.9mg/mL. The final concentration is 0.19mg/mL.

INTENDED USE
Type 1(fibrillar) collagen is intended for use in inducing platelet aggregation shape change and platelet activation. Platelet response to collagen inducer aggregation or activation. Partial responses (%Aggregation), extended LAG PHASE (seconds) and other parameters have been described for heritable and acquired dysfunctions. Collagen is sensitive to the presence of Aspirin at low concentrations.[11]

TEST PRINCIPAL
Collagen reagent is a potent agonist. When collagen reagent is added to PRP or other appropriate samples Normal platelets will change their shapes, demonstrate adhesive properties, release endogenous ADP and induce an aggregation response.[1,2,3,4,5]

When collagen reagent is introduced to Platelet Rich Plasma or other appropriate normal test samples, expected results should be attained. When Collagen Reagent is introduced into abnormal test samples, one or more parameters will not match expected results and requires further study.

NON STERILE — Collagen Reagent is intended for In-Vitro DIAGNOSTIC USE ONLY.

Collagen Reagent is an animal derived product that has met or passed all required test for infectious diseases. Appropriate Personnel Protective Equipment and Standard Precautions MUST be practiced when working with this product.[6,9]

MATERIALS PROVIDED
3 x 0.5mL vials Collagen Reagent

RECONSTITUTION
Collagen Reagent must be brought to room temperature (15°-20° C) prior to reconstitution. Reconstitute each vial with 0.5mL purified water.
Do Not Dilute

REAGENT STORAGE
The reconstituted Collagen Reagent may be stored for up to 30 days at 2-8° C when in its original, tightly sealed container.

REAGENT DISPOSAL
Unused or expired Collagen Reagent must be disposed of as a hazardous waste in accordance with local regulations and laboratory policy.[6,9]

PROFESSIONAL LABORATORY USE ONLY

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

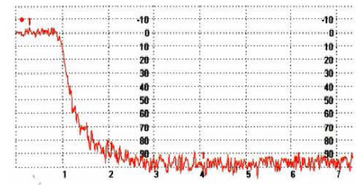
CE

EXPECTED VALUES[5,10,12,13]
Expected values vary by concentration, sample type, disease state and other factors. Reference ranges must be:

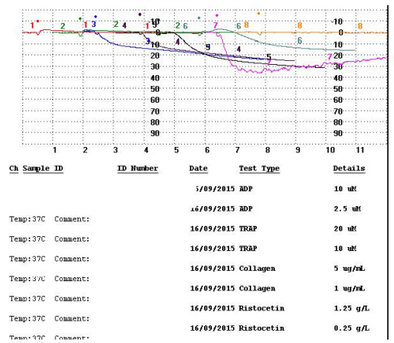
COLLAGEN REAGENT	WORKING CONCENTRATION	FINAL CONCENTRATION	FINAL AGGREGATION (%)
	1.9mg/mL	0.19mg/mL	61-99

LAG PHASE	PRIMARY SLOPE	AUC@6 MINUTES	OTHER
≥ 60 SECONDS	35-67	365	DO NOT DILUTE

NORMAL COLLAGEN AGGREGATION RESPONSE

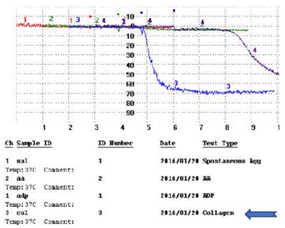


INHERITED PLATELET DISORDER: GLANZMANN'S THROMBASTENIA
(Confirmed by genetic test results)



Ch	Sample ID	ID Number	Date	Test Type	Details
			16/09/2015	ADP	10 uM
			16/09/2015	ADP	2.5 uM
			16/09/2015	TRAP	20 uM
			16/09/2015	TRAP	10 uM
			16/09/2015	Collagen	5 ug/mL
			16/09/2015	Collagen	1 ug/mL
			16/09/2015	Ristocetin	1.25 g/L
			16/09/2015	Ristocetin	0.25 g/L

REAGENT RESPONSES TO VARIOUS REAGENTS



#	Ch	Sample ID	ID Number	Date	Test Type
1	1	saline	1	2016/01/28	Spontaneous Agg
2	2	AA	2	2016/01/28	AA
3	1	adp	1	2016/01/28	ADP
4	3	collagen	3	2016/01/28	Collagen

LIMITATIONS
Detailed clinical, dedication and social histories are required for accurate interpretation of test results. In addition, spices, supplements, herbal extracts, caffeine, tobacco, alcohol as well as prescription and over the counter drugs may interfere with test results. [1,5,10,11]

TEST PREPARATIONS

MATERIALS PROVIDED
Collagen Reagent, Type 1 3x0.5mL

Collagen 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
Collagen Reagent will perform as described when used on most Light Transmission Aggregometers.

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

PATIENT PREPARATION[5,7,8]
1. Clinical, medication, family and social histories required prior to testing.
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 know to affect platelet function.
4. Patients should avoid fatty meals and food products prior to specimen collections.

SPECIMEN COLLECTION

SPECIMEN COLLECTION
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) [6]
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

PREPARATION OF PLATELET RICH PLASMA (PRP) & PLATELET POOR PLASMA (PPP) TEST SAMPLES[6]

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 (37.5°C)
6. Set the 100% baseline by placing the blank into the test well.
   a. Press the Blank Button
   b. Remove the Blank for 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.

COLLAGEN RESPONSES[5,13,14,15]

CONDITION	COLLAGEN RESPONSE
Thrombasthenia	Absent
Bernard Soulier Syndrome	Normal
Storage Pool Defect	Reduced
Cyclo-oxygenase Deficiency	Reduced
Thromboxane Synthetase Deficiency	Reduced
Aspirin Ingestion	
Ehlers Danos Syndrome	Normal
von Willebrands Disease	Normal

FURTHER TESTING:
If the test results, when properly interpreted, are abnormal:
1. Repeat the test on a different day.
   a. If those results are abnormal, review the patient's histories for possible interferences.
2. Specialty review or consult may be required.

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

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