CONDUCTING TESTS

P/N	Description	Unit Volume	Stock Conc. WB/PRP	Final Conc. WB/PRP	Volume Per Test WB*	Volume Per Test PRP**	Tests Per Unit WB	Tests Per Unit PRP
386	Thrombin	1.0 mL	10 Unit/mL	1 Unit	100µL	50µL	10	20
385	Collagen	1.0 mL	1 mg/mL	2µg/mL	2µL	1µL	500	1000
390	Arachidonic Acid	0.7 mL	50 mM	0.5 mM	10µL	5µL	70	140
396	Ristocetin	0.5 mL	125 mg/mL	1.0 mg/mL/ 1.25 mg/mL	8µL	5µL	62	100
395	Chrono-lume	4x1.25 mL	N/A	N/A	100µL	50µL	50	100
384	ADP	5.0 mL	1 mM	10µM	10µL	5µL	500	1000
393	Epinephrine	5.0 mL/50 mL	10 mM/1 mM	50µM/5µM	5µL	2.5µL	1000	10000
387	ATP Standard	5.0 mL	2µmole	2 nmole	5µL	5µL	1000	1000

* In a 1.0 mL sample – typically 450 µL blood diluted with 450µL of physiological saline plus 100 µL of CHRONO-LUME Reagent or 1.0 mL blood sample without CHRONO-LUME when testing with Ristocetin.

** In a 500µL sample – typically 450 µL platelet rich plasma plus 50 µL of CHRONO-LUME Reagent or 500 µL platelet rich plasma without CHRONO-LUME when testing with Ristocetin. (Reduce volumes by HALF with P/N 365 rubber spacers for a 250 µL microvolume sample.)

NOTE: Multiple Stock solutions are not required. To change Final Concentration, adjust pipette volume.

Since each test requires only micro-volumes of reagent, it is essential that introduction of excess reagent be avoided. Therefore, remove the excess reagent adhering to the outside of the tip by wiping the outside of the micropipette tip after drawing the reagent.

It is important that the tip of the micropipette is immersed in the sample and the reagent forcefully injected. DO NOT introduce the reagent above the sample in the cuvette or run down the side of the cuvette since the reagent will cling to the side of the cuvette and will not mix with the sample.

EC REP **BioTop Medical**
Poortgebouw Noord, Rijnsburgerweg 10
2333 AA LEIDEN, The Netherlands

UK RP: OBELIS UK LTD.,
Sandford Gate, Oxford, OX4 6LB, UK

NOTE: For best results, reconstituted working reagents should be kept on ice. Reconstituted CHRONO-LUME®, EPINEPHRINE, ARACHIDONIC ACID and RISTOCETIN should also be kept in the dark.

PROCEDURES

PROCEDURES utilizing AGGRO/LINK® with Windows-compatible software and following NCCLS GP2-A3 format are available on disk or via Email (proced@chronolog.com) for:

- Whole Blood Aggregation
- Whole Blood Aggregation with ATP Release
- Optical Aggregation with PRP
- Optical Aggregation with ATP Release

CONTROL AND PATIENT SAMPLES – collect normal control free of drugs known to affect platelets and patient samples into sterile evacuated tube with a non-wettable lining and 1/10 volume of 3.2% buffered sodium citrate. Draw 20-30 mL blood for Optical PRP testing or 5-10 mL blood for Whole Blood testing.

Normal Ranges

NOTE: The following Normal Ranges were obtained from various laboratories and publications. They should be used as a guideline only. Normal ranges should be established for aggregation and ATP release in each and every laboratory.

Normal Ranges in Platelet Rich Plasma (Mean +/- 1 SD)

Reagent	Conc.	Agg. (%)[2]	ATP (nmole)
Thrombin	1 Unit	N/A	>0.5[3]
Collagen	2µg/mL	70 - 94	0.74 - 1.92[3]
Arach. Acid	0.5 mM	74 – 99[2,*]	0.56 - 1.40[3]
ADP	5µM	69 - 88	0.41 - 0.63[2]
	10µM	71 - 88	0.5 - 1.06[2]
Epinephrine	5µM	78 - 88	0.40 - 0.52[2]
Ristocetin	1.25 mg/mL	87 - 102[2,*]	N/A

(* +/- 2 SD)

Normal Ranges in Whole Blood (Mean +/- 2 SD)

Reagent	Conc.	Agg. (ohms)[16]	ATP (nmole)[16]
Thrombin	1 Unit	N/A	>0.5[4]
Collagen	1ug/mL	12 - 33	0.43 - 2.2
	2µg/mL	15 - 27[1]	0.5 - 1.7[1]
	5µg/mL	11 - 42	0.54 - 2.9
Arach. Acid	0.5 mM	7 - 29	0.45 - 2.7
ADP	5µM	5 - 26	0.11 - 2.0
	10µM[3]	6 - 33	0.21 - 1.9
Ristocetin	1.0 mg/mL	> 5Ω; < 70 sec. lag time[8]	N/A

CALCULATION OF ATP RELEASE – the AGGRO/LINK Software calculates ATP release. If using a chart recorder, the following formula is used for calculation:

$$\frac{\text{Luminescence of test}}{\text{Gain of test}} \times \frac{\text{Gain of standard}}{\text{Luminescence of standard}} \times 2 \text{ nmoles}$$

REFERENCES

(1) Ingerman-Wojenski CM and Silver MJ: A Quick Method for Screening Platelet Dysfunctions Using the Whole Blood Lumi-Aggregometer. Thromb Hemostas 51-2 (154-156) 1984.

(2) White M MC, Jennings LK: Platelet Protocols: Research and Clinical Laboratory Procedures. Academic Press, 1999.

(3) Podczasy JJ, Lee J, Vucenik I: Evaluation of Whole-Blood Lumiaggregation.Clin Appl. Thrombosis/Hemostasis, 3(3):190-195, 1997.

(4) Sweeney JD, Hoernig LA, Michnik A and Fitzpatrick JE: Whole Blood Aggregometry. Influence of Sample Collection and Delay in Study Performance on Test Results. Am J Clin Pathol 1989; 92:676-679.

(5) Feinman RD, Detwiler TC and Ingerman-Wojenski C: The Lumi-Aggregometer as a Research and Clinical Tool. The Plts.: Physiology and Pharmacology. Copyright 1985 by Academic Press, Inc.

(6) Sirridge, M: Laboratory Evaluation of the Bleeding Patient. Clinics in Laboratory Medicine, Vol. 4, No. 2, June 1984.

(7) Nieuwenhuis HK, Akkerman J-W N and Sixma JJ: Patients With a Prolonged Bleeding Time and Normal Aggregation Tests May Have Storage Pool Deficiency: Studies on One Hundred Six Patients. Blood 70-3 (620-623) 1987.

(8) Sweeney JD, Hoernig LA and Fitzpatrick JE: Whole Blood Aggregation in von Willebrand Disease. Am J Hematol 32: 190-193 (1989).

(9) Philipp CS, Dilley A, Miller CH, Evatt B, Baranwal A, Scheartz R, Bachmann G and Saidi P: Platelet functional defects in women with unexplained menorrhagia. JTH, 1:477-484.

(10) Sweeney JD, Hoernig LA, Behrens AN, Novak E, and Swank RT: Von Willebrand's Variant (type II Buffalo). American Journal of Clinical Pathology Vol. 93, No. 4, (522-525) 1990.

(11) Miller JL: Platelet-Type von Willebrand's Disease. Thrombosis and Hemostasis Vol. 7, No. 4, 1985.

(12) Taylor ML, Misso NLA, Stewart GA, Thompson PJ: The effects of varying doses of aspirin on human platelet activation induced by PAF, Collagen and arachidonic acid. Br J Clin Pharmac., (1992), 33, 25-31.

(13) Ingham Medical Center, Lansing, MI.

(14) Riess H, Braun G, Brehm G and Hiller E.: Critical Evaluation of Platelet Aggregation in Human Whole Blood. Am J Clin Pathol. Vol. 85, No. 1, (50-56) 1986.

(15) Gengo FM, Rainka M, Robson M, Gengo MF, Forrest A, Hourihane M and Bates V: Prevalence of Platelet Nonresponsiveness to Aspirin in Patients Treated for Secondary Stroke Prophylaxis and in Patients with Recurrent Ischemic Events. Jrnl of Clin Pharmacology, Vol 48 No 3, 335-343, 2008.

(16) Pyle-Eilola, Amy L.; Carter, Chris M.; Cucci, Jodi; Chandler, Wayne L: Establishment of Reference Internals for Whole Blood Luminescent Platelet Aggregometry. AACC 2017 Poster.

CHRONO-LOG Corporation
CE UK CA
2 WEST PARK ROAD
HAVERTOWN, PA 19083 USA
1-800-CHRONOLOG
IN PA 610-853-1130
chronolog@chronolog.com
www.chronolog.com

EC REP **BioTop Medical** RgtPl1 01/2024
UK RP: OBELIS UK LTD.

CHRONO-LOG CORPORATION

CHRONO-PAR® AND CHRONO-LUME® REAGENTS FOR PLATELET FUNCTION TESTING & SECRETION STUDIES IN WHOLE BLOOD AND PLATELET RICH PLASMA

(For In-Vitro Diagnostic Use — Measuring platelet aggregation and ATP secretion in whole blood or platelet rich plasma)

(For Laboratory Professional Use)

INTRODUCTION

CHRONO-PAR® and CHRONO-LUME® reagents are used to confirm normal platelet function and to diagnose platelet dysfunctions.

The following CHRONO-PAR and CHRONO-LUME reagents are suitable for use in both Whole Blood and Platelet Rich Plasma:

***ADP* (P/N 384)** – In PRP, with low concentrations, (<1µM), shape change is followed by primary aggregation and disaggregation. At higher concentrations of 1-5 µM a biphasic response is often visible. Second wave aggregation requires the synthesis of thromboxane A2 and is affected by cyclooxygenase inhibitors such as aspirin. Aggregation with ADP in Whole Blood requires higher concentrations of ADP (typically 5 to 20 µM).

***Arachidonic Acid* (P/N 390)** – A direct test for prostanoid synthesis, as aggregation requires conversion to thromboxane A2 by cyclooxygenase, a process which is inhibited by aspirin. Responses from no aggregation to below the normal range frequently indicate drug ingestion some time during the previous days.

***ATP Standard* (P/N 387)** – For the quantitation of ATP Release. Supplied as 2 µmole of lyophilized adenosine 5' triphosphate. 5 µL added to any size test sample provides a 2 nmole standard.

***CHRONO-LUME* (P/N 395)** – For the quantitation of ATP Release in the detection of aspirin use and the diagnosis of Storage Pool and Secretion Disorders. Luciferin-Luciferase binds with ATP, generating light, which is proportional to the amount of ATP released by the platelets in the test sample.

NOTE: CHRONO-LUME reaction is time and temperature dependent. Be sure to incubate with each sample for two minutes only before starting test.

***Collagen* (P/N 385)** – A lag phase follows addition of reagent to test sample, during which collagen polymerizes into fibrils for platelet activation. Low concentration collagen (1-2 µg/mL) is inhibited by cyclooxygenase inhibitors such as aspirin; normally, higher concentrations (5µg/mL) are not affected.

***Epinephrine* (P/N 393)** – Shape change is not seen with this agonist. Higher concentrations (≥5 µM) produce a biphasic curve with second-wave aggregation dependent on thromboxane A2 synthesis. Epinephrine is not recommended as a standard agonist for Whole Blood testing clinically, as fewer than 50% respond to this very weak agonist. The recommended anti-coagulant for Whole Blood testing with epinephrine is 1.5% trisodium citrate with 2 U Heparin per mL of citrate.

***Ristocetin* (P/N 396)** – For the detection of von Willebrand Disease (a quantitative or qualitative defect in plasma von Willebrand Factor) and Bernard Soulier Syndrome (a lack of platelet membrane glycoprotein GPIb) and Glanzmann's Thrombasthenia with aggregation-disaggregation pattern. Ristocetin results can also be affected by aspirin.

***Thrombin* (P/N 386)** – For the quantitation of maximum ATP Release at 1U/mL, not for aggregation. Secretion in response to Thrombin is independent of thromboxane synthesis. Absent or decreased secretion to Thrombin may be indicative of storage pool deficiency or a secretion defect.

NOTE: CHRONO-PAR Reagents are shipped at ambient temperatures. Upon arrival, store as recommended.

Material Required But Not Provided

1. Aggregometer
2. Cuvettes
3. Stir Bars
4. Micropipettes – Adjustable from 0.5µL to 100µL required for reagents.
5. Pipettes – 100µL to 1 mL required for blood samples.
6. CHRONO-LOG P/N 397 Saline - or Sterile physiological saline for irrigation (0.85% or 0.9% w/v) for CHRONO-PAR® Reagent preparation and for dilution of the Whole Blood specimen

Avoid blood bank saline because it may be an incorrect osmolarity. Cell counter diluents are not suitable because they contain EDTA, which inhibits platelet aggregation. Infusion salines are inappropriate because they contain benzyl alcohol or other preservatives/additives. Such preservatives inhibit platelet function.

7. CHRONO-LOG P/N 398 Purified Water – or sterile bottled distilled water for irrigation are suitable for CHRONO-PAR® Reagent preparation.

Should be pyrogen free (ATP free) for reconstituting reagents and not contain preservatives such as benzyl alcohol which inhibits platelet function. DO Not use water from a Millipore System.

8. Ice for maintaining Reconstituted Working Reagents at appropriate temperatures.
9. Plastic conical tubes
10. KimWipes®
11. Electrode Assembly [Reusable]
12. Electrode Assembly [Disposable] — See Instrument Manual for Reagent Instructions.

NOTE: All CHRONO-PAR Reagents should be thawed at room temperature or hand thawed and should not be placed in a warm water bath.

INTERPRETATION OF RESULTS

Aggregation and luminescent ATP secretion curves in blood and PRP can be interpreted as follows:

- By direct comparison to a normal drug free control which also provides real time quality control.
- Comparison to published normal values that can be verified and reproduced by any laboratory.
- Collagen or Arachidonic Acid releases ATP equal to or greater than 50% of that released in response to Thrombin. ADP and epinephrine induce less ATP release.
- In a study of one hundred and six patients with storage pool deficiency (SPD), 23% had normal optical (PRP) aggregation responses to ADP, epinephrine and collagen; and 44% had miscellaneous aggregation abnormalities. The authors concluded that SPD is common, heterogeneous and not necessarily associated with optical (PRP) aggregation abnormalities.[7]
- Simultaneous measurement of aggregation and ATP release provides unequivocal evidence of dense granule secretion.[5] The threshold value at which storage pool deficiency should be considered has been reported to be less than 0.5 nmole ATP in response to 1U thrombin.[4]

LIMITATIONS

- Tests should be performed within 3 hours of venipuncture.
- Many drugs inhibit platelet function. Unless the aim of testing is to demonstrate drug-induced inhibition, patients should be free of drugs known to affect platelets for two weeks prior to testing.
- For a platelet count of <225,000/µL, run ADP tests UNDILUTED.
- Further Clinical and Laboratory evaluation may be required to confirm diagnosis.

QUALITY CONTROL

It is good laboratory practice to run a run a normal donor, free of drugs known to affect platelets, whenever reagents are reconstituted or thawed.

WARRANTY

CHRONO-PAR® AND CHRONO-LUME® REAGENTS which fail to demonstrate aggregation and release in normal controls free of drugs known to affect platelets, before expiration and when stored and reconstituted as directed, are replaced at no charge. This warranty applies only in the United States.

CHRONO-PAR® AND CHRONO-LUME® REAGENTS

REAGENT	SUPPLIED AS	PREPARATION	SHELF LIFE & STORAGE	RECOMMENDED VOLUMES
ADP *Cat. #:* 384 *Stock Conc.:* 1 mM	2.5 mg of a lyophilized preparation of adenosine diphosphate.	Tap vial gently to get contents to the bottom. Reconstitute with **5 mL** of irrigation grade physiological **saline.** Allow to sit for 10 minutes with occasional inversion.	*Lyophilized Reagent:* Until expiration date, store frozen below 0°C. *Reconstituted Reagent:* One year or until the expiration date, store frozen at -70°C in volumes suitable for a day's testing. *Working Stock:* 8 hours at 2 - 8°C	*Diluted/Undiluted Blood:* Add 10µL of reagent to 1 mL sample for a final concentration of 10µM. Normal aggregation and ATP Release are seen in whole blood with final concentrations of 5 - 20 µM. *PRP:* Add 5µL of reagent to 500µL sample for a final concentration of 10µM. Normal aggregation & ATP Release are seen in PRP with final concentration of 5-10 µM.
Arachidonic Acid *Cat. #:* 390 *Stock Conc.:* 50 mM	Minimum of 10 mg of arachidonic acid with a purity of better than 99%. Albumin contains 100 mg of bovine albumin, fraction V powder, 96 to 99% pure.	First tap contents gently to the bottom of the vial of **albumin**. Remove stopper and reconstitute the albumin with **1mL** of irrigation grade physiological **saline**. Allow to sit, then mix with occasional swirling. Allow 15 to 30 minutes for the albumin to go completely into solution (Check visually) and gently invert to take up any albumin in plug. The arachidonic acid in the vial is an oily drop, which must be shaken or tapped to the bottom of the vial. Break vial tip with Cap Cracker™ supplied. Pipette reconstituted albumin into both the tip and body of the vial in 100µL aliquots to a total volume of **700µL**. Mix in arachidonic acid on the insides of the vial tip or body by rotating the vial as the albumin is added. Repeat a few times in each section of the vial then vigorously mix the suspension using a transfer pipette. Combine the suspension from the tip with that in the body of the vial and continue mixing until the suspension reaches maximum turbidity. Transfer reagent to microcentrifuge tube and vortex at highest speed for 5 minutes. The reconstituted reagent should appear very milky with numerous small bubbles. Vortex for 2 minutes just before running the test.	*Lyophilized Reagent:* **Albumin** – Until expiration date, refrigerate at 2 - 8°C. **A/A Oily Drop** – Until expiration date, store frozen below -20°C. *Reconstituted Reagent:* 3 months when stored frozen at **-70°C** in the dark in aliquots of 100µL; 1 month when stored frozen at **-20°C** in the dark. Aliquots can be hand thawed and vigorously re-suspended for 2 minutes with a vortex mixer just before use. *Working Stock:* 8 hours at 2 - 8°C in the dark as the Albumin is light-sensitive.	*Diluted/Undiluted Blood:* Add 10µL of reagent to 1 mL sample for a concentration of 0.5 mM. *PRP:* Add 5 µL of reagent to 500 µL sample for a concentration of 0.5 mM. Normal aggregation and ATP Release are seen with final concentrations of 0.5 mM in Whole Blood and 0.5 to 1.0 mM in PRP.
ATP STANDARD *Cat. #:* 387 *Stock Amount:* 2µmole	Lyophilized adenosine 5' triphosphate.	Tap vial gently to get contents to the bottom. Remove stopper, add **5mL** of irrigation grade physiological **saline** for a 2 µmole Standard. Replace stopper and invert gently. Invert again before use. Allow to sit for 10 minutes with occasional inversion. **NOTE**: The appearance of the lyophilized ATP may range from particulate to a thin film coating. Either morphology is suitable for use after reconstitution.	*Lyophilized Reagent:* Until expiration date, store frozen below 0°C. *Reconstituted Reagent:* **2 weeks,** store frozen in aliquots at -20°C *Working Stock:* 24 hours, at 2 - 8°C	*Diluted/Undiluted Blood:* Add 5 µL to 1 mL sample for a 2 nmole standard. *PRP:* Add 5 µL to **500µL** sample for a 2 nmole standard.

CHRONO-PAR® AND CHRONO-LUME® REAGENTS *(continued)*

REAGENT	SUPPLIED AS	PREPARATION	SHELF LIFE & STORAGE	RECOMMENDED VOLUMES
CHRONO-LUME *Cat. #:* 395 *Stock Conc.:* 2 µM/L Luciferin/ Luciferase/ 1.25 mL	0.2 mg Luciferin, 22,000 Units d-luciferase, magnesium sulphate, human serum albumin, stabilizers and buffer.	Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with **1.25 mL** of purified **water**. Prior to use, allow to stand for 20 minutes with occasional inversion.	*Lyophilized Reagent:* Until expiration date, store frozen below 0°C. *Reconstituted Reagent:* 30 days, store frozen at -20°C in aliquots suitable for a day's testing. *Working Stock:* 8 hours at 2 - 8°C in the dark.	*Diluted/Undiluted Blood:* Add 100 µL of reconstituted reagent to 900µL of diluted or undiluted blood to measure ATP release. *PRP:* Add 50 µL of reconstituted reagent to 450 µL of platelet rich plasma to measure ATP release.
Collagen *Cat. #:* 385 *Stock Conc.:* 1 mg/mL	Native collagen fibrils (type I) from equine tendons suspended in isotonic glucose solution of pH 2.7.	Can be used directly as supplied. Invert or swirl vial before use, as collagen fibrils are in suspension. **Do not freeze.** If required, collagen can be further diluted in isotonic glucose pH 2.7. **NOTE: Because of its very low pH, organisms do not grow as readily. If asceptic techniques are used (sterile syringe and needle to place one day's use in conical microcentrifuge tube), remaining reagent, stored at 2 - 8°C, is stable until expiration date. Parafilm both original vial and aliquot.**	*As Supplied:* Until expiration date refrigerate at 2 - 8°C. **The reagent aliquot removed from the vial and stored in a conical microcentrifuge tube is stable for one week at 2 - 8°C when parafilmed.**	*Diluted/Undiluted Blood:* Add 1 µL of reagent to 1 mL sample for a final concentration of 1 µg/mL. *PRP:* Add 1 µL of reagent to 500µL sample for a final concentration of 2 µg/mL. Normal aggregation and ATP release are seen with final concentrations of 1-5 µg/mL.[15]
Epinephrine *Cat. #:* 393 *Stock Conc.:* 10mM for Whole Blood Testing 1mM for PRP testing	Lyophilized preparation of 1-epinephrine bitartarate with stabilizers.	Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with **5.0mL** of purified **water** for Whole Blood testing. Dilute the stock 1:10 with physiological saline for PRP testing. Allow to sit for 10 minutes with occasional inversion.	*Lyophilized Reagent:* Until expiration date refrigerate at 2 - 8°C. *Reconstituted Reagent:* 3 months, store frozen at -70°C in the dark and in 100 µL aliquots. *Working Stock:* 8 hours at 2 - 8°C in a dark container.	*Diluted/Undiluted Blood:* Add 5 µL of Stock Solution to 1 mL sample for a concentration of 50 µM. Aggregation and ATP release **may** be seen in Whole Blood at a concentration of 50 µM. *PRP:* Add 5µL of 1:10 Diluted Solution to 500µL sample for a concentration of 10µM. Normal Aggregation and ATP release is seen with 5 - 10 µM in PRP. **NOTE**: Normal subjects exhibit considerable variability that is not correlated with age, sex, stress, diet, platelet count or hematocrit.
Ristocetin *Cat. #:* 396 *Stock Conc.:* 125 mg/mL	Stabilized freeze dried ristocetin.	Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with **0.5mL** of purified **water.** Restopper and allow to sit for 10-15 minutes. Visually inspect bottom of vial to confirm reagent is fully in suspension. Invert gently to take up any reagent remaining in stopper and allow to sit for another 10-15 minutes until all particulate matter is well dissolved. May have a clear to brownish color suspension after reconstitution. Never shake reagent. Swirl gently just before use.	*Lyophilized Reagent:* Until expiration date refrigerate at 2 - 8°C. *Reconstituted Reagent:* 3 months, store frozen at -20°C in volumes suitable for a day's testing. DO NOT STORE AT -70°C. *Working Stock:* 8 hours at 2 - 8°C in the dark.	*Diluted/Undiluted Blood:* Add 8 µL of reagent to 1 mL sample for a concentration of 1.0 mg/mL. Normal aggregation is seen with final concentrations of 0.5 - 1.0.[8] *PRP:* Add 5 µL of reagent to 500 µL sample for a concentration of 1.25 mg/mL. Normal aggregation is seen with final concentrations of 0.9 - 1.25 mg/mL.[11] **NOTE:** To detect Type 2B or Platelet-Type von Willebrand, test with low concentration Ristocetin (0.25 in Whole Blood and 0.5 mg/mL in PRP).[11]
Thrombin *Cat. #:* 386 *Stock Conc.:* 10 Units/mL	Lyophilized thrombin from human plasma.	Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with **1.0mL** of irrigation grade physiological **saline**. Allow to sit for 10 minutes with occasional inversion.	*Lyophilized Reagent:* Until expiration date, store frozen below 0°C. *Reconstituted Reagent:* 3 months, store frozen at -70°C in aliquots suitable for a day's testing. *Working Stock:* 24 hours at 2 - 8°C.	*Diluted/Undiluted Blood:* Add 100 µL of reagent to 1 mL sample for a concentration of 1 Unit/mL. *PRP:* Add 50 µL of reagent to 500µL sample for a concentration of 1 Unit/mL. *Secretion Only:* Maximum ATP release is seen with a final concentration of 1 Unit/mL.

EXPECTED RESULTS

AGGREGATION RESPONSE WITH SELECTED ABNORMALITIES

Reagent	Final Concentration	Aspirin Effect****	Von Willebrand & Bernard Soulier	Storage Pool/ Secretion Defect	Glanzmann's Thrombasthenia
ADP	5 – 20 µM	**N, R** *	**N**	**N, R** *	**A**
Arachidonic Acid	0.5 mM	**A**	**N**	**N**	**A**
Collagen	1, 2, 5 µg/mL	1,2µg/mL | 5 µg/mL **R** | **N**	**N**	**N**	**A**
Epinephrine	5 – 50 µM	**R***	**N**	**R** *	**A**
Ristocetin	0.25 – 1.0 mg/mL [WB] 0.5 – 1.25 mg/mL [PRP]	Qualitative[6] Defect	** **A,R,H** *** >70 sec. Lag (vW)	**N**	Qualitative[14] Defect

* Second-wave Inhibited
** Type 2B and Platelet-type von Willebrand increased at low concentration of 0.25 mg/mL in Whole Blood and 0.25 - 0.5 mg/mL in PRP.[10, 11] In addition, when cryoprecipitate is added to test sample from patient with Platelet-Type [pseudo] VWD, enhanced response to low concentration Ristocetin will continue, a Type 2B patient will show no response.
*** To distinguish between von Willebrand & Bernard Soulier, add normal plasma or cryoprecipitate to patient sample, vW patient will respond, Bernard Soulier will not.[11]
**** Typical response for donor taking 250 mg aspirin.[12]

ATP SECRETION WITH SELECTED ABNORMALITIES

Reagent	Final Concentration	Aspirin Effect**	Von Willebrand & Bernard Soulier	Storage Pool/ Secretion Defect*	Glanzmann's Thrombasthenia
ADP	5 – 20 µM	**A, R**	**N**	**A,R**	**A**
Arachidonic Acid	0.5 mM	**A**	**N**	**A,R**	**R**
Collagen	1 – 5 µg/mL	**R**	**N**	**A,R**	**R**
Epinephrine	5 – 50µM	**A**	**N**	**A,R**	**A**
Ristocetin	0.25 – 1.0 mg/mL [WB] 0.5 – 1.25 mg/mL [PRP]	— —	— —	— —	— —
Thrombin	1 Unit	**N**	**N**	**A,R**	**R**[5]

* Higher concentrations of any agonist including Thrombin up to 5 Units will induce ATP secretion with a Secretion disorder but will not with a Storage Pool Defect.[5]
** Typical response for donor taking 250 mg aspirin.[12]

Key: A – Absent **H** – Hyper **N** – Normal **R** – Reduced
(Compared to Normal Ranges)