

# **INSTRUCTION MANUAL FOR THE CHRONO-LOG® MODEL 700 WHOLE BLOOD LUMI- AGGREGOMETER**

## **FOR IN-VITRO DIAGNOSTIC USE**

Measuring platelet aggregation and ATP secretion in  
whole blood or platelet rich plasma

## **CHRONO-LOG CORP.**

2 West Park Road Havertown, PA 19083

Tel: 800-247-6665 or 610-853-1130

[www.chronolog.com](http://www.chronolog.com)





<b>Table of Contents</b>		<i>Preparation</i>	43
<b>Manufacturer</b>	4	<i>Calibration Procedure</i>	43
<b>EC Rep</b>	4	<b>Troubleshooting Guide</b>	45
<b>Power requirements</b>	4	<b>AGGRO/LINK®8 FOR WINDOWS</b>	45
<b>Fuse</b>	4	<i>INTRODUCTION</i>	45
<b>Warnings:</b>	4	<i>STARTING AGGRO/LINK®8</i>	46
<b>VENTILATION</b>	4	<i>FILE</i>	46
<b>HAZARDS</b>	4	<i>EDIT</i>	50
<b>MISUSE</b>	4	<i>AGGREGOMETER</i>	55
<b>POSITIONING INSTRUMENT FOR DISCONNECT</b>	4	<i>VIEW</i>	57
<b>SUMMARY AND EXPLANATION</b>	4	<i>WINDOW</i>	57
<b>OVERVIEW OF HEMOSTASIS</b>	5	<i>HELP</i>	58
<b>PRINCIPLES OF PLATELET AGGREGATION TESTS</b>	6	<i>Introduction</i>	59
<b>INSTRUMENT SPECIFICATIONS</b>	8	<i>General Description</i>	59
<i>Operating Specifications</i>	8	<i>Starting vW CoFactor Assay</i>	59
<i>Environmental Specifications</i>	8	<i>File</i>	59
<i>General</i>	8	<i>Edit</i>	62
<i>Instrument controls</i>	9	<i>Standards</i>	63
<b>INSTALLATION PROCEDURE</b>	9	<i>Aggregometer</i>	65
<b>Instrument Function Verification</b>	10	<i>Window</i>	67
<b>Platelet Aggregation Procedure</b>	10	<i>Help</i>	67
<i>PRINCIPLE</i>	10	<i>View</i>	67
<i>SPECIMEN</i>	10	<i>Results</i>	68
<i>PREPARATION</i>	12	<i>DATA BACKUP</i>	69
<i>CALIBRATION</i>	14	<b>BIBLIOGRAPHY</b>	69
<i>QUALITY CONTROL</i>	14	<b>Assistance and Distributor Information</b>	77
<i>PROCEDURE – STEPWISE (Whole</i>			
<i>Blood/Luminescence)</i>	15		
<i>CALCULATIONS</i>	21		
<i>REPORTING RESULTS</i>	21		
<i>INTERPRETATION</i>	25		
<i>PROCEDURE NOTES</i>	25		
<i>LIMITATIONS OF THE PROCEDURE</i>	26		
<i>PROCEDURE – STEPWISE (PRP/Luminescence</i>			
<i>Aggregation):</i>	26		
<i>CALCULATIONS</i>	31		
<i>REPORTING RESULTS</i>	31		
<i>Reference Ranges</i>	32		
<i>Platelet Abnormalities</i>	33		
<i>Reporting Format</i>	34		
<i>INTERPRETATION</i>	35		
<i>PROCEDURE NOTES:</i>	35		
<i>LIMITATIONS OF PROCEDURES:</i>	35		
<b>vW CoFactor Assay</b>	36		
<i>PRINCIPLE</i>	36		
<i>SPECIMEN</i>	36		
<i>EQUIPMENT AND MATERIALS</i>	36		
<i>Performance Parameters</i>	37		
<i>PROCEDURE</i>	37		
<b>SAMPLE DISPOSAL</b>	42		
<b>SHUT DOWN PROCEDURE</b>	42		
<b>MAINTENANCE AND SERVICE</b>	42		
<b>SELF-CALIBRATION OF OPTICAL CIRCUITS</b>	43		

**Manufacturer:** Chrono-log Corp.



2 West Park Road  
Havertown, PA 19083  
USA  
Phone: 1-610-853-1130  
Email: chronolog@chronolog.com

**EC Rep:**



BioTop Medical  
Poortgebouw Noord,  
Rijnsburgerweg 10  
2333 AA Leiden  
Netherlands  
Phone 31 71 528 01 12  
Fax 31 71 528 01 15

**Power requirements:** 115 or 230VAC; 50 or 60 Hz 150 watts max. The main supply voltage fluctuations are not to exceed 10% of the nominal supply voltage.

**Fuse:** For 115 VAC, IEC T1.25AL250V;  
For 230 VAC, IEC T0.63AL250V.



#### Warnings:

**WARNING- FIRE HAZARD** For continued protection replace only with the same type and rating of fuse.



**Caution** – This Instrument is intended for indoor use only. Failure to operate in a protected environment in accordance with instructions may present a shock hazard.

**Warning:** Patient specimens should be handled as if they contain infectious materials, in accordance with national guidelines for Biosafety/Hazard Group 2. In the United States, the universal precautions recommended by the Department of Labor, Occupational Safety and Health Administration apply. (Occupational Exposure to Blood Borne Pathogens; final rule (29 CFR 1910, 1030) FEDERAL REGISTER, pp 64004-64182.

#### VENTILATION

To ensure that the system has adequate ventilation, please adhere to the following instructions carefully:

- Do not place Aggregometer within a closed in wall or on top of cloth material which can act as insulation.
- Do not place Aggregometer where it will receive direct sunlight.
- Do not place Aggregometer next to a heat source of any kind including heating vents.
- Make sure that all openings on the Aggregometer remain unobstructed, especially the fan guard on the back of the unit.

#### HAZARDS

- There are no hazards associated specifically with use of the Whole Blood Lumi Aggregometer.

- Normal precautions, which apply to the handling of blood, should be observed in handling the samples.
- If it is felt necessary to disinfect the Electrode Assembly, it may be dipped in a 1:10 dilution of household bleach for about 10 seconds, removed and the bleach washed off with distilled water.
- Non-factory authorized service personnel should not remove the instrument cover due to the presence of high voltages inside the instrument.

#### MISUSE

- To prevent electrical shock, your Aggregometer power cable must be plugged into a properly grounded power source. Do not use adaptor plugs or remove grounding prong from cable. If an extension cable must be used, use a three wire cable with properly grounding plugs.
- Do not spill liquids or food on your Aggregometer.
- Do not put objects or materials other than cuvettes in the heater block wells.
- Do not push any object through the fan guard.
- Do not use detachable mains supply cord sets with insufficient capacity. Hospital grade three connector power cords are available from the factory or your distributor.

#### POSITIONING INSTRUMENT FOR DISCONNECT

1. Turn off instrument at power switch.
2. Disconnect DIN and USB cables.
3. Rotate instrument to access C13 power cord connector and remove it from the power inlet module.

#### SUMMARY AND EXPLANATION

When a blood vessel is damaged, platelets adhere to the wound edges, aggregate, synthesize prostaglandins and release serotonin, ADP and ATP. Prostaglandin synthesis and release products cause further aggregation. The coagulation cascade is initiated, thrombin generated, fibrin formed and the platelet plug anchored to the damaged vessel.

Defects in platelet function due to lack of a cell membrane glycoprotein, cytoplasmic storage granules, platelet enzymes or a plasma factor often result in excessive bleeding after trauma, frequent bruising or nosebleeds, or excessive menstrual blood loss. Yet patients who present with one or more of these clinical signs are much more likely to be tested initially for coagulation abnormalities than for platelet dysfunction. Further, surgical patients are not routinely screened for platelet defects despite the fact that many cases of platelet dysfunction are discovered only after excessive or recurrent post-surgical bleeding.

In 1962, Born described the aggregation of platelets by ADP and modified a colorimeter to monitor continuously this aggregation in platelet rich plasma. These modifications included incubation at 37°C, stirring and recording the change in light transmission over time on a pen recorder.

In 1977, Feinman et al designed an instrument to measure simultaneously platelet aggregation and ATP secretion. This instrument utilized infra-red light to measure aggregation and a sensitive photomultiplier at right angles to the aggregation light path to measure ATP secretion by firefly luminescence. This was the first Lumi-Aggregometer.

In 1980, Cardinal and Flower described the impedance method for measuring aggregation in whole blood. A very small electric current is passed between two electrodes. During initial contact with the blood the electrodes become coated with a monolayer of platelets. When an aggregating agent is added, platelets aggregate on the monolayer increasing the impedance. This increase in impedance is recorded on a pen recorder. The addition of impedance measurement to the Lumi-Aggregometer created the Whole Blood Lumi-Aggregometer.

In 1984, Wojenski and Silver published a rapid protocol using the Whole Blood Lumi-Aggregometer for the routine laboratory. This protocol is based on the simultaneous measurement of platelet aggregation and ATP secretion in blood. The protocol takes less than 5 mL of blood and can be completed in 30 minutes. This is less time than it previously took just to prepare and standardize platelet rich plasma.

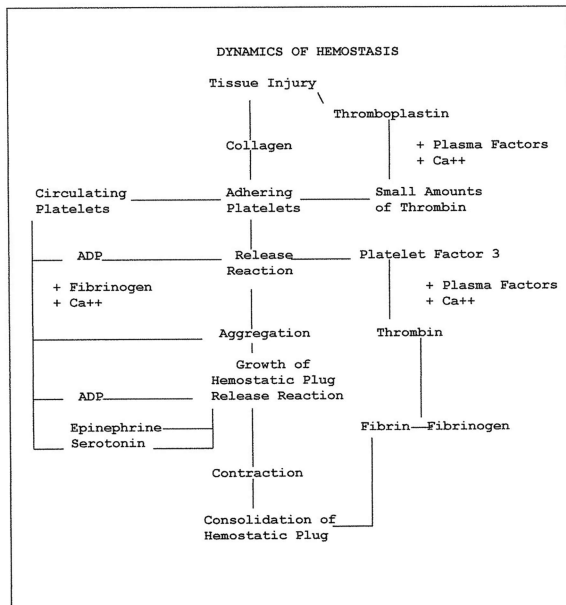
## OVERVIEW OF HEMOSTASIS

Hemostasis - from the Greek word for blood; and, the Greek word meaning standing - is a complex, delicately balanced system of interactions that keeps blood circulating as a fluid through the blood vessels. A simplified representation of the process is shown below.

There are three elements of the hemostatic mechanism:

- blood vessels
- plasma proteins known as coagulation factors;
- platelets

A defect or abnormality in the interactive process of hemostasis may lead to abnormal bleeding or to inappropriate clotting.



## Abnormalities of the hemostatic mechanism

**Blood vessels** - abnormalities of the endothelial cell lining of the blood vessels can arise from injury, inflammation, infection or atherosclerosis. The endothelium may lose its normal anti-thrombotic properties and begin to synthesize and release compounds that promote thrombosis. Vascular disorders that result in abnormal blood vessel support structures may contribute to disordered hemostasis.

**Coagulation proteins** - abnormal levels of coagulation factor levels or defective function of the coagulation factors can disturb hemostatic balance. These defects can be hereditary or they can be acquired, through a pathological process, for example.

**Platelets** - disorders of the platelet component of hemostasis can arise from abnormal numbers of platelets (quantitative defects) or functional impairment (qualitative defects). Defects in the platelet component of the hemostatic system can also be acquired or inherited.

## Platelet function

The platelets (or thrombocytes) are small, discoid cells that circulate in the blood along with red cells and leukocytes. The cell's nucleus is lost during the maturation process. Platelets have cytoplasmic granules known as dense granules and alpha granules. These contain compounds that amplify the platelet response if they are exocytosed (released, secreted).

The main function of platelets is the maintenance of blood vessel integrity by prevention of red cell migration through the vessel wall. Platelets also prevent vascular leakage by plugging any sites of damage or injury. In the case of damage or injury that exposes the subendothelium and/or basement membrane, circulating platelets are recruited to the site to form a platelet aggregate. This physiologic reaction is known as formation of the primary hemostatic plug.

## Formation of the primary hemostatic plug

Platelets adhere and aggregate at any site of subendothelial exposure. Exposure of the subendothelium results in the unmasking of the structural protein Collagen. Collagen is a platelet stimulus.

Platelets adhere to the now exposed Collagen fibrils. The platelets change shape and pseudopods are formed. The shape change and pseudopods result in closer contact with other individual platelets. Granule contents are exocytosed. More platelets are recruited and stimulated to undergo shape change, pseudopod formation and granule release. This aggregated mass physically prevents leakage at the site.

Von Willebrand Factor (vWF) (found in the alpha granules of the platelet and circulating in the blood in association with Factor VIII) is classified as an adhesive protein. It interacts with a binding site on the platelet membrane and acts to strengthen the platelets' adherence to the endothelium.

## Qualitative platelet function disorders

### Defective adhesion

- von Willebrand Disease -quantitative or qualitative defect in plasma vWF (vWF)
- Bernard-Soulier Syndrome (BSS) -lack of platelet membrane glycoprotein Ib (GPIb)

### Defective aggregation

- Afibrinogenemia - deficiency of plasma fibrinogen
- Glanzmann's Thrombasthenia -defective or deficient platelet membrane glycoprotein IIb-IIIa (GP IIb-IIIa)

### Defective platelet granule secretion

- Storage pool deficiency (SPD) -deficiency in dense granule contents (ADP, ATP and/or serotonin)
- Gray platelet syndrome -deficiency in alpha granule contents [platelet factor 4 (PF4), platelet vWF, thrombospondin, and platelet derived growth factor (PDGF)]
- Arachidonic Acid metabolic pathway abnormalities
- Defective liberation of Arachidonic Acid from the platelet membrane
  - Deficiency of the enzyme cyclo-oxygenase
  - Deficiency of the enzyme thromboxane synthetase
- Secretion defects with normal granule contents and normal Arachidonic Acid metabolic pathway
  - Defective cytosolic calcium mobilization
  - Defective early responses -myosin light chain phosphorylation; phosphatidylinositol metabolism

### Defective or Blocked Receptors to Specific Agonists (in addition to BSS and thrombasthenia)

- Defective response to Epinephrine - myeloproliferative disorders (MPD)
- Defective response to Collagen
- Defective response to U46619 drug (the stable analog of thromboxane A<sub>2</sub>)

Defective Platelet Coagulant Activities - the platelet contribution to and interaction with the coagulation scheme.

### Miscellaneous defects

- Congenital
  - May-Heggelin Anomaly
  - Down's Syndrome
  - Thrombocytopenia with absent radii (TAR syndrome)
- Acquired
  - Uremia
  - Extracorporeal circulation

## PRINCIPLES OF PLATELET AGGREGATION TESTS

Platelets are known to aggregate under a variety of conditions and in the presence of a number of different reagents. "Platelet aggregation" is a term used to denote the adherence of one platelet to another. The phenomenon can be induced by adding aggregating agents to platelet-rich plasma or whole blood. Platelet aggregation depends on the presence of calcium, fibrinogen and one or more plasmatic factors, and an aggregating agent. Platelet aggregation will vary with different aggregating agents and with their concentration. For optical aggregometry, ADP, Epinephrine, Collagen and Ristocetin are used extensively for screening

purposes and provide the most immediate information for basic diagnostic considerations.

The selection of these reagents has some basis in theory. Both ADP and Epinephrine (adrenaline) are contained within the platelet in storage organelles and are released from the platelet during formation of the primary hemostatic plug and may thereby induce further platelet aggregation. Consequently, in-vitro platelet response to these reagents has proven to be of help in determining the nature of a patient's bleeding disorder.

Collagen, on the other hand, is not contained in the platelet but is found in the supporting connective tissue of the blood vasculature and is considered to be the first aggregating or pro-coagulant factor that the platelet encounters following vascular trauma. Hence, in-vitro study of the platelet response to Collagen has assumed considerable importance.

Other reagents such as Thrombin, the calcium ionophore A23187, Arachidonic Acid, Ristocetin, Bovine Factor VIII, and serotonin have also been used to study platelet response for more specific purposes.

Platelet aggregation is the most useful in-vitro test of platelet function presently available. It is a diagnostic tool, which can provide insight that is difficult or impossible to obtain by other techniques, thus aiding in patient diagnosis and proper selection of treatment or therapy. Experience with this technique has delineated a spectrum of inherited and acquired platelet dysfunctional states.

Platelet aggregation is clinically significant in the detection and diagnosis of acquired or congenital qualitative platelet defects. The platelet's ability or inability to respond to particular aggregating reagents is the basis for differentiating platelet dysfunctions as shown in the table below.

AGGREGATION STUDIES ON SELECTED PLATELET FUNCTION DEFECTS			
DEFECT	Platelet Aggregation by ADP	Platelet Aggregation by Collagen	Platelet Aggregation by Ristocetin
Thrombasthenia	Decreased	Decreased	Normal
Thrombopathia or Thrombocytopathy	Normal (1st phase)	Decreased	Normal
von Willebrand's Disease	Normal	Normal	Abnormal
Non-steroidal, Anti-inflammatory drugs	Normal (1st phase)	Decreased	Abnormal

Three types of aggregation tests will be described:

- Optical aggregation in PRP
- Impedance aggregation in whole-blood
- Measurement of ATP release (luminescence)

### Optical Aggregation Tests

In-vitro platelet aggregation is an effort to characterize the in-vivo ability of the platelets to form the primary hemostatic plug.

Platelets in a suspension of plasma are isolated from an anticoagulated blood sample by a relatively low centrifugal force centrifugation. This material, known as platelet rich plasma (PRP), is removed from the blood sample following centrifugation. The tubes are then returned to the centrifuge and spun at a higher force to prepare platelet poor plasma (PPP).

The Born type aggregometer or optical aggregometer is a fixed wavelength spectrophotometer with a sample chamber (or chambers) heated to 37°C. Provision is made for stirring of the sample because platelet to platelet contact is necessary to the determination of in vitro platelet aggregation.

The CHRONO-LOG® sample chambers are designed so that a beam of infra-red light shines through two cuvettes, one containing PRP (the sample) and one containing PPP (the reference). Silicon photodiodes detect the light able to pass through the samples: PRP is arbitrarily considered to be 0% light transmission or 0% aggregation; PPP is considered to be 100% light transmission or 100% aggregation. The difference in light transmission outputs from the photodiodes is transferred to recording devices.

The twin well, dual infra-red beam design ensures reproducibility and, as will be seen later, allows simultaneous measurement of aggregation and luminescence. Each channel has a separate well for the test (PRP or washed platelets; 0% light transmission) and corresponding reference (PPP or buffer; 100% light transmission) samples. The optical aggregation output is proportional to the continuously measured difference in light transmission between the test and reference samples. Pressing a single pushbutton sets the 0% and 100% light transmission baselines. With the sensitivity of the twin-well dual-beam detection system, a count difference of only  $50 \times 10^3/L$  between the test and reference sample is needed for testing. If the light transmission difference between the test and reference samples is insufficient for accurate testing, the aggregation output cycles continuously between the baselines to warn the operator.

When a stimulus is added to the cuvette containing PRP and the platelets respond, changes in light transmission occur and are recorded over time by the recording device.

PRP, which is turbid, is stirred in a test cuvette maintained at 37° C. The light transmittance through this turbid sample is measured relative to the PPP blank. When the agonist is added, the platelets will form increasingly larger aggregates and the PRP will begin to clear, allowing more light to pass through. This increase in light transmittance is directly proportional to the amount of aggregation and is amplified and digitized into a computer using AGGRO/LINK®8 Software.

When the platelets undergo shape change in response to a stimulus (agonist; aggregating agent), their larger size allows less light to pass through the PRP: this is recorded as less light transmission through the sample relative to the PPP. If the dose of aggregating agent is strong enough to cause the platelets to adhere to each other and form aggregates, more light is able to pass through the PRP sample. The change in light transmission recorded, over time, shows a trend towards the platelet poor plasma, or 100% light transmission.

In-vitro aggregation recordings are characterized by their appearances:

- shape change
- a first wave of aggregation (primary aggregation) that may reverse and return towards the PRP baseline
- irreversible second wave aggregation that occurs when the platelets' secreted granule contents become the stimulus and cause additional aggregation.

Aggregation curves are also characterized by:

- the maximum amount of change in light transmission caused by the agonist (per cent aggregation)
- the slope -or rate- of the aggregation, in % change of aggregation per minute.

Multiple aggregating agents and dosages are usually used to stimulate the platelets. Different aggregating agents stimulate different pathways of activation in the platelets: either binding sites or metabolic pathways. Different concentrations of agonists are used to elicit a family of curves (dose response curves).

The pattern of responses to these test panels is compared to established normal response patterns and established abnormal response patterns. This information is considered to relate to the platelet function component of hemostasis.

#### **Impedance Aggregation in Whole Blood**

In vitro platelet aggregation is an effort to characterize the in vivo ability of the platelets to form the primary hemostatic plug.

Platelets are tested in anti-coagulated blood, without the need to isolate them from other components of blood. Because there is no need to centrifuge the specimen to produce an optically transparent suspension of cells, the entire platelet population is tested. The process of testing consumes less technical time; and labile factors in the blood that may influence platelet function are preserved.

The CHRONO-LOG® Whole Blood Aggregometers consist of sample receptacles heated to 37°C. There is provision made for stirring of the samples utilizing magnetic stir bars or non-magnetic disposable stir bars. Cuvettes containing the test sample and a stir bar are placed in the receptacles.

The impedance (or electrical resistance) method of aggregation is non-optical. An electrode assembly is inserted into a cuvette containing a whole blood test sample. The electrode assembly consists basically of two precious metal wires that are immersed in the sample. An AC voltage in the millivolt range is applied to the probe circuit. The instrument measures the electrical resistance or impedance between the two immersed wires. During a brief period of equilibration, a monolayer of platelets forms on the exposed portions of the wires, resulting in a stable impedance value. This stable baseline of impedance is assigned a value of zero ohms of resistance. An aggregating agent is added to the cuvette and the stimulated platelets aggregate to the platelet monolayer on the immersed wires. This accumulation of platelets adds electrical resistance to the circuit, which is measured and quantified in ohms (the measurement of electrical resistance). The change in impedance is displayed as a function of time on a computer with the CHRONO-LOG® AGGRO/LINK®8 Software. The changes in resistance are measured and quantified in ohms (the measurement of electrical resistance). Tests are generally run for

four to six minutes after the addition of an agonist.

Results of impedance aggregation tests are quantified by:

- ohms of aggregation at a given time in the test
- slope, or rate of the reaction, in ohms change per minute
- by maximum extent of aggregation, in ohms.

The increase in impedance is directly proportional to the mass of the platelet aggregate. Impedance aggregation in blood is more sensitive to the aggregating effects of Ristocetin so it may be more sensitive to von Willebrand disease than the bleeding time or vWF (Ristocetin co-factor) assay. Sensitivity is also increased to hyperaggregation and drugs such as aspirin and dipyridamole when compared to light transmission in platelet rich plasma. Impedance aggregation in blood is not dependent on the optical characteristics of the sample, so tests can be performed on lipemic and thrombocytopenic samples. As centrifugation is not required, impedance aggregation is especially useful in conditions where megathrombocyte count is increased.

The impedance method for measuring platelet aggregation allows the study of platelets in the more physiologic whole blood environment. Sample preparation is greatly reduced, preserving labile modulators such as prostacyclin and thromboxane A<sub>2</sub>, resulting in a testing environment proven to be more sensitive to the effects of many anti-platelet drugs (e.g., Aspirin, Dipyridamole, ReoPro™).

#### Measurement of ATP release (luminescence)

ATP release is measured by a visible light range luminescence technique in PRP and whole blood. The basic principle of the Lumi-aggregometer is to measure secretion by a sensitive luminescent (luciferin-luciferase) assay for extra-cellular ATP in combination with the simultaneous measurement of aggregation.

The ATP released by the platelet dense granules binds with CHRONO-LUME® (luciferin-luciferase) and generates a light. The measurement of luminescence is in principle simple, but the amount of visible light is small and a stable, high-gain photomultiplier tube is required to measure luminescence simultaneously with aggregation. Since the optical aggregation measurement light source is in the infra-red range, interference with the photo-multiplier measurement in the visible range is avoided.

The photo-multiplier is sensitive to luminescence created by CHRONO-LUME® Reagent (luciferin-luciferase) reacting with ATP secreted by dense granules in whole blood, diluted blood, platelet rich plasma or washed platelets. Luminescent measurement of ATP secretion provides unequivocal evidence of normal or impaired dense granule release. This increases the sensitivity of the test to secretion defects and storage pool deficiency.

CHRONO-LOG® Whole Blood Lumi-Aggregometers can measure both aggregation and ATP release; aggregation can be measured in PRP by the optical method that records changes in light transmission or by the impedance method

## INSTRUMENT SPECIFICATIONS

### Operating Specifications

- Display - Liquid Crystal Display (LCD) unit with a 24 character by 2-line capability. The LCD displays the actual temperature in degrees Celsius, the luminescence gain, the set stirrer RPM, operating mode and warning messages.
- Heater Block - Electronically controlled heater block can be set between 35.0°C and 39.0°C in 0.1°C steps. Both the temperature setting and actual temperature of heater block are displayed on front panel. Operation is prevented while temperature is outside  $\pm 0.2^\circ\text{C}$  of the temperature setting.
- Stirrer - Nine stirring speeds from 400 RPM to 1200 RPM in 100 RPM steps and a stirrer stopped position. The selected stirring speed is displayed on front panel. Normally, stirrer speed accuracy is better than 0.01%. There is a feature that prevents operation if stirrer is not within  $\pm 10$  RPM of the selected setting.
- Luminescence - Nine gain setting selected and displayed on front panel. Gain settings are: 2X, 1X, 0.5X, 0.2X, 0.1X, 0.05X, 0.02X, 0.01X AND 0.005X
- Impedance Aggregation - Automatic baseline setting from a front panel push button. Gain fixed at 50% of scale equals 20 $\Omega$  change. Calibration resistor is automatically inserted for a few seconds when baseline button is released. An over range detection prevents operation if baseline does not set.
- Optical/Aggregation/Turbidometric - Automatic baseline setting from a front panel push button with an over-range detection to prevent operation if baseline does not set.
- Output resistance for analog output - Less than 10,000 ohms.
- Computer Interfaces - Serial RS-232 and USB
- Power requirements - 115 or 230VAC; 50 or 60 Hz 150 watts max. The main supply voltage fluctuations are not to exceed 10% of the nominal supply voltage.
- Fuse - For 115 VAC, IEC T1.25AL250V; for 230 VAC, IEC T0.63AL250V.
- DIN input connector for 4 channel configuration.

### Environmental Specifications:

**Caution – This Instrument is intended for indoor use only. Failure to operate in a protected environment in accordance with instructions may present a shock hazard. Use of the instrument in a manner contrary to manufacturer guidelines may result in protection impairment.**

- Operating Temperature: 15° to 30° C (60° to 86° F)
- Storage Temperature: -20° to 60° C (-4° to 140° F)
- Relative Humidity: 5% to 85% (Noncondensing)
- Operating Altitude: -16 to 2000 M (-50 to 6,560 ft.)
- Storage Altitude: -16 to 10,600 M (-50 to 35,000 ft.)

### General

- Stirring Speed - crystal controlled front panel selectable for each channel, from 400 to 1200 RPM in 100 RPM steps with Precision  $\pm 0.01\%$ .
- Temperature - heater block  $37^\circ \pm 0.2^\circ\text{C}$ , electronically controlled, displayed on front panel meter.
- Incubation Wells - six (6) wells for 1 mL cuvettes at  $36.5^\circ\text{C} \pm 1.0^\circ\text{C}$  for each channel



- Dimensions – 14" (36cm) wide, 8.5" (22 cm) high, 18" (46cm) deep.
- Weight - 40 lbs (18kg).
- Recommended Recorder(s) - CHRONO-LOG® Model 709 Dual Pen. Others must have two pens, 1 megohm minimum input impedance and 100mV range.
- Pollution Degree 2
- Installation Category II

#### Instrument controls

- Power ON/OFF switch - powers instrument on and off.
- Set Baselines Pushbutton(s) - sets instrument baseline when held down, allowing the recorder baseline to be set using the Pen Recorder Zero Control. The Pen Recorder is set on the right of the chart. Pressing and releasing, sets optical aggregation gain when in the Optical mode. In the Impedance mode, the baseline adjusts the impedance reading of the sample to the impedance zero baseline then adds 20 ohms  $\pm$  0.2 ohms of impedance to the impedance baseline then adjusts the Gain so that 20 ohms equals 50% of scale.

When the Impedance output is connected to the Chart Recorder, the Pen baseline should be set at 10% from the LEFT side of the chart. This will be the baseline at the start of the aggregation test.

When the Optical output is connected to the Chart Recorder, the Pen baseline should be set at 10% from the RIGHT side of the chart. This will be the baseline corresponding to PPP (total aggregation). Releasing the pushbutton sets the optical aggregation gain to 80 small chart divisions between the PRP baseline (zero aggregation) and the PPP (total aggregation) baseline.

The Luminescence output baseline is always set 10% from the right side of the chart to set the baseline corresponding to select zero luminescence.

- Select – The select switch allows for setting of the Gain, Temperature and the Stirring Speed with the use of the SET switch.
  - Gain Range: 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2.
  - Stirring Range: 400 to 1200 RPM in 100RPM increments.
  - Temperature Range: 35.0°C to 39.0°C in 0.1°C increments.
- Set – Sets either the Gain, Temperature or Stirring Speed, depending on which feature is selected. Each time the SET button is pushed, the selected function is incremented until it reaches its maximum setting.
- Mode – Sets the assay to either Impedance or Optical mode.
- Heater Block Cover Interlock Switch - photomultiplier high voltage power supply OFF when open, ON when closed. Protects the photomultiplier tube used to detect luminescence from ambient light when the heater block cover is open.

## INSTALLATION PROCEDURE

**NOTE:** For a two channel instrument, Model 700, approximately 3.5 sq. ft. (0.325 sq. m.) of bench space is required. For a four channel instrument, Model 700 and 700-X approximately 5 sq. ft. (0.465 sq. m.) of bench space is required.

1. Unpack the CHRONO-LOG® Aggregometer and supplies from their shipping containers.
2. Check that the items received match the Packing List.
3. Check that the Instruments were not damaged in shipping.
4. Store the reagents as specified on each label.
5. The system will interface to a computer with the following minimum requirements:
  - Windows®-based PC with an i3 Core processor or higher.
  - 8GB of system memory.
  - Windows® 7 or later operating system.
  - Super VGA or Higher Monitor (17 inch or larger).
  - Screen resolution of 800x600 or better.
  - Minimum Hard Disk Space required: 500GB.
  - Mouse or compatible pointing device.
6. Place the Aggregometer and Computer in a clean dry area on a bench top at a height of 28-35".
7. Check that the voltage select switch on the Aggregometer is set to the proper voltage. If the voltage select switch is changed, then the fuse also needs to be changed to maintain protection. Plug the power cords, as appropriate, into a 115 or 220 Volts AC filtered power strip and then plug the power strip into a grounded power outlet.
8. Computer Connections: (See Connection Charts in Appendix A).
  - a. Turn ON the computer then install USB Driver located on the installation CD in the USB folder. Double-clicking the CDM\*.exe file will initiate drive installation.
  - b. Turn ON the Model 700 then plug the USB cable into the computer and the Model 700 unit. The first time this is done the computer will display the message "NEW HARDWARE FOUND". The computer should complete the USB installation.
  - c. If this is a dual module system, connect the DIN/DIN cable between modules.
9. Software installation. If computer was purchased through Chrono-log, the software is pre-installed, proceed to step 10.
  - a. Install the AGGRO/LINK®8 and the vW software by following the on screen instructions. The software packages are in separate folders on the CD. Right click each Setup.exe file (in turn) and run as Administrator to start installation of each software and follow on-screen instructions to complete the installation.
  - b. If you are installing an upgrade from a previous version, save the software into a new folder such as C:\Program Files\AGGROLINK8v13x to allow older software to remain in place until any desired validation or training is completed.
  - c. It is recommended that the software be run as Administrator for all Windows user accounts. Insufficient privileges may result in run time errors. Please consult your IT staff if technical support is required.
  - d. For Windows 7 or later operating system, after the software packages are installed, right click the icons or programs. Select the compatibility tab and select "Disable desktop Composition" for all users. This can also be disabled at CONTROL PANEL, ALL CONTROL PANEL ITEMS, PERFORMANCE INFORMATION AND TOOLS, ADVANCED TOOLS, ADJUST APPEARANCE AND PERFORMANCE OF WINDOWS, PERFORMANCE OPTIONS, deselect ENABLE DESKTOP COMPOSITION.
10. Start the AGGRO/LINK®8 Software on the Computer. During the software start-up, the computer will test the communication. The Program will respond with

AGGREGOMETER READY on the lower right side of the status bar when communication is established.

- a. If communication is not established, the Program will continue searching and respond with AGGREGOMETER NOT CONNECTED on the lower right side of the status bar if connection cannot be established.
  - b. Check the cable and make sure the Aggregometer has power.
  - c. To retry, select "Aggregometer" from the ribbon, then "Connect" from the dropdown menu.
11. Close the AGGRO/LINK®8 Software.
  12. Start the vW Software on the Computer. During the software start-up, the computer will test the communication. The Program will respond with AGGREGOMETER READY on the lower right side of the status bar when communication is established.
    - a. If communication is not established, then the Program will continue searching and respond with A/L NOT CONNECTED on the lower right side of the status bar if connection cannot be established.
    - b. Check the cable and make sure the Aggregometer has power.
    - c. To retry, select "Aggregometer" from the ribbon, then "Connect" from the dropdown menu.
  13. When the Aggregometer is first turned on, the LCD displays will indicate a temperature less than 35°C. After about 15 minutes the LCD display on each channel will show a stable temperature reading corresponding to your desired setting.
  14. The System is now installed and ready for use.

**NOTE: Software packages cannot be run simultaneously on one Aggregation system. Be sure to close the AGGRO/LINK®8 Software prior to opening the vW Software and vice versa.**

### Instrument Function Verification

1. Temperature Check – set to 37°C all channels. Model 700 will prevent running of tests until temperature is reached and will monitor temperature throughout testing.
2. Stirring - take a cuvette with stir bar and place in each reaction well to observe it spinning. Model 700 will monitor stirring throughout testing.
3. Impedance Circuit Function - when the unit has reached operating temperature, plug in each of the electrode assemblies. Place in a cuvette with 1 mL of saline to test the ability of the electrode to respond to the baseline and gain setting. Push the Baseline line button to confirm the gain is set at 50% of the graph and the baseline at 0% of the graph. This verifies the circuit is complete.
4. Luminescence circuit function - with the test well doors closed, increase each LUMINESCENCE GAIN from X 0.005 to X2. Observe the changes (increased oscillations and/or a position change) in the luminescence baseline. Decrease to 0.1 setting and open and close each door. Observe the fact the circuit shuts off, then on. These maneuvers will verify the luminescence circuitry is operational.

**NOTE: Remember to decrease each Luminescence Gain to X 0.005 setting prior to the start of PRP-Lumi Aggregation testing or to X0.02 prior to the start of Whole Blood-Lumi Aggregation testing.**

5. Optical Circuit Function - insert a cuvette containing saline or distilled water into the reaction well for PRP. LEAVE THE PPP WELL VACANT. Press and HOLD the SET BASELINE button and observe the tracing at the 100% light

transmission baseline. Release the SET BASELINE button and observe a return to the PRP 0% baseline.

### Platelet Aggregation Procedure

#### PRINCIPLE:

The Whole Blood/Luminescence clinical protocol is based on the work of Sweeney et al, which is a variation of the Ingberman-Wojenski and Silver method contained in "A Quick Method for Screening Platelet Dysfunctions Using the Whole Blood Lumi-Aggregometer". This Protocol is written for running Luminescence aggregation in whole blood with simultaneous measurement of ATP release using a CHRONO-LOG® 700 Series Whole Blood/Optical Lumi-Aggregometer with AGGRO/LINK®8 software and Reusable or Disposable Electrodes.

The PRP/Luminescence Protocol is written for running Born method of Turbidimetric aggregation with simultaneous measurement of ATP release using a CHRONO-LOG® 700 Series Whole Blood/Optical Lumi-Aggregometer with AGGRO/LINK®8 Software.

#### SPECIMEN:

##### Preparation and Handling of Blood Specimens:

It is extremely important that care be taken in the collection, handling, and preparation of the patient's blood specimen.

**Warning:** Patient specimens should be handled as if they contain infectious materials, in accordance with national guidelines for Biosafety/Hazard Group 2. In the United States, the universal precautions recommended by the Department of Labor, Occupational Safety and Health Administration apply. (Occupational Exposure to Blood Borne Pathogens; final rule (29 CFR 1910, 1030).

Essential precautions can be summarized as follows:

- Do not pipette by mouth.
- Wear disposable gloves during all specimen and assay manipulations.
- Avoid use of sharp-pointed or glass liquid handling devices, which may puncture skin.
- Do not smoke, eat, or drink in the laboratory area.
- Avoid splashing any liquid specimens and reagents and the formation of aerosols.
- Wash hands thoroughly on completion of a manipulation.

#### Patient Preparation:

Subjects for platelet aggregation tests should be resting, fasting and non-smoking. Subjects should avoid taking any prescription or over the counter medications known to affect platelet function for ten (10) days to two (2) weeks prior to the test. A partial list of medications with known antiplatelet effects can be found below.

- COX-1 Inhibitors (Acetylsalicylic acid)  
Aspirin and all proprietary or over-the-counter (OTC) preparations containing acetylsalicylic acid
- COX-1 and COX-2 Inhibitors (Nonsteroidal anti-inflammatory drugs [NSAIDs])  
Ibuprofen, Indomethacin, naproxen, Mefenamic acid
- COX-2 Inhibitors (Coxibs)  
Celecoxib

- Inhibitors of Platelet Receptors  
Abciximab ( $\alpha_{IIb}\beta_3$ ), Clopidogrel (P2Y<sub>12</sub>), Prasugrel (P2Y<sub>12</sub>)
- RGD Peptomimetics  
Eptifibatide, Tirofiban
- Phosphodiesterase Inhibitors  
Dipyridamole, Cilostazole
- Anticoagulants  
Heparin, Warfarin, Direct Thrombin Inhibitors (lepirudin, argatroban, bivalirudin)
- Cardiovascular Agents  
 $\beta$ -adrenergic blockers (propranolol), Vasodilators (nitroprusside, nitroglycerin), Diuretics (furosemide), Calcium channel blockers
- Antimicrobials  
 $\beta$ -lactams (penicillins, cephalosporins), Amphotericin (antifungal), Hydroxychloroquine (antimalarial), Nitrofurantoin
- Chemotherapeutic Agents  
Asparaginase, Plicamycin, Vincristine
- Psychotropics and Anesthetics  
Tricyclic antidepressants (imipramine), Phenothiazines (chlorpromazine), Local and general anesthesia (fluothane)
- Miscellaneous Agents  
Clofibrate, Dextran, Guaifenesin (expectorant), Radiographic contrast
- Foods/Herbals  
Alcohol, Caffeine (methylxanthine), Garlic, Onion, Ginger, Fish Oil, Vitamins C and E

#### Type:

Specimen should be drawn with a minimum of trauma or stasis at the venipuncture site and anticoagulated with 3.2% sodium citrate, in the ratio of one part anticoagulant to 9 parts of blood. Blue top tubes may be used.

Needle size should be between 19 and 21 gauge. *A needle with a diameter greater than 1mm (19 gauge) may be traumatic for a vein and adversely affect hemostasis. Needles with diameters under 0.7mm (22 gauge or higher) prolong blood collection and increase the pressure gradient in the needle, which could lead to hemolysis and platelet activation.*

Plastic or non-contact surfaced (siliconized) materials should be used throughout in order to minimize activation of the platelets during sample preparation.

Specimen volumes required for Whole Blood: One (1) discard tube plus (1 or 2) 4.5 mL blue top evacuated tube from each subject (for testing). Keep at room temperature. (20° to 25°C).

Specimen volumes required for PRP: One (1) discard tube plus (5) 4.5 mL blue top evacuated tube from each subject (for testing). Keep at room temperature. (20° to 25°C).

An EDTA blood specimen must be collected from the patient for hematocrit and platelet count. The blood specimen must be collected using plastic equipment throughout.

#### EQUIPMENT AND MATERIALS:

##### Equipment:

1. CHRONO-LOG® 700 Series Aggregometer with Windows® compatible computer installed on a bench top at a height of 28-35”.
2. CENTRIFUGE (for PRP/PPP testing only) – Should have the following features:
  - a. Capable of running at 100-170 g for obtaining Platelet Rich [PRP] sample and at 1500-2400 g for obtaining Platelet Poor [PPP] sample.
  - b. Capable of running at Room Temperature
  - c. OFF setting for Brake
  - d. Either a Fixed Angle or a Swinging Bucket Rotor

##### Materials:

1. P/N 367 Cuvettes (Whole Blood only)
2. P/N 370 Disposable Stir bars (Whole Blood only)
3. P/N 312 Cuvettes (PRP only)
4. P/N 311 Disposable Stir bars (PRP only)
5. P/N 331 .5-10  $\mu$ L Adjustable Pipette
6. P/N 335 Tips for P/N 331
7. P/N 332 10-100  $\mu$ L Adjustable Pipette
8. P/N 337 Tips for P/N 332
9. P/N 333 100-1000  $\mu$ L Adjustable Pipette
10. P/N 339 Tips for P/N 333
11. P/N 366 Insert in Test Block (1 for each channel) (PRP only)
12. P/N 369R Reusable Electrode Assembly
13. P/N 315 Disposable Electrode in Cuvette
14. P/N 320 Adaptor for Disposable Electrodes
15. P/N 393 CHRONO-PAR® Epinephrine (PRP only)
16. P/N 390 CHRONO-PAR® Arachidonic Acid
17. P/N 396 CHRONO-PAR® Ristocetin
18. P/N 395 CHRONO-LUME® Kit with ATP standard
19. P/N 386 CHRONO-PAR® Thrombin
20. P/N 385 CHRONO-PAR® Collagen
21. P/N 384 CHRONO-PAR® ADP
22. P/N 387 CHRONO-PAR® ATP Standard
23. P/N 397 Saline (0.9%) 15mL

***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. Some infusion salines are inappropriate because they contain benzyl alcohol (or other preservatives). Such preservatives/additives inhibit platelet function.***

24. P/N 398 Purified Water 15mL.

***Bottled, sterile, distilled water for Irrigation is recommended for CHRONO-PAR® Reagent preparation. Should be pyrogen free (ATP free) for reconstituting reagents. Avoid any sterile water for injection that contains benzyl alcohol or other preservatives/additives because they inhibit platelet function.***

25. Ice for maintaining Reconstituted Working Reagents at appropriate temperatures.
26. Lintless wipes, such as KimWipes®.

*Gauze squares are not suitable.*

27. Long-stemmed plastic transfer pipettes to take off PRP and PPP for placing into 15 mL polypropylene centrifuge tubes.
28. Blood cell counter dilution vials; 50 mL beakers or similar: for cleaning the electrode assembly
29. Vortex-type mixer: for mixing the Arachidonic Acid
30. 0.5 mL Freestanding polypropylene tubes and twist caps with O-ring seals or 1.7 mL polypropylene microcentrifuge conical tubes with snap tops...for storage of reagent aliquots.
31. Plastic conical test tube and cap (per test subject) for storage of PRP and PPP and for standardizing PRP.
32. Syringe to extract Collagen from vial.
33. Parafilm for the Collagen vial and aliquots.

#### PREPARATION:

##### 1. Aggregometer

- a. Turn Model 700 ON.
- b. Push in MODE button to set each channel to IMPEDANCE or OPTICAL. (NOTE: MODE push button is disabled until Aggregometer reaches temperature setting and when instrument is in the SELECT mode.)
- c. Using SELECT push button for each channel, select TEMPERATURE and use SET push button to set temperature to 37°C. [NOTE: Tests cannot be performed until each channel reaches set temperature.]

**NOTE: Selections and Settings are visible in each Channel's LCD Screen.**

- d. Using SELECT push button for each channel, select STIRRING and use SET push button to set stirring speed to 1200 RPM.
- e. Using SELECT pushbutton for each channel, select GAIN and use SET push button to pre-set Luminescence Gain at 0.01 or 0.02 (for WHOLE BLOOD) or 0.005 (for PRP). NOTE: Select the pre-set Gain setting based on instrument experience.
- f. Leave the LCD screen in GAIN setting until ATP Standard is completed. This allows the SET button to be used to increase the GAIN, if needed.

**NOTE: Lowest Luminescence Gain Setting is x 0.005; Highest Gain setting is x 2.**

- g. For PRP Assay, place Optical Inserts into each channel to provide proper fitting for P/N 312 Cuvette. Be sure Channel # on back of insert matches the channel.
- h. For Whole Blood Assay, remove Optical Inserts from each channel.

##### 2. Reagents

###### a. Arachidonic Acid

Supplied As: Minimum of 10 mg of Arachidonic Acid with a purity of better than 99%. Albumin contains 100mg of bovine albumin, fraction V powder, 96 to 99% pure.

Stock Conc.: 50 mM

Stock Storage: Frozen below -20°C for Arachidonic Acid, Refrigerate at 2-8°C for albumin.

Stock Shelf Life: Until expiration date.

Working Conc.: 0.5 mM

Working Storage: 2-8°C in the dark

Working Shelf Life: 8 hours

Reconstitute with 0.7 mL of the saline-albumin solution

Prepare the **albumin** for the CHRONO-PAR®

Arachidonic Acid. First tap contents to the bottom.

Remove the stopper and reconstitute the albumin with 1 mL of irrigation grade physiological saline. Allow to sit 15 to 30 minutes for the albumin to go completely into solution. Visually inspect bottom of vial to confirm albumin is fully in suspension. Gently invert vial to take up any albumin in the plug and allow to sit for 10 to 15 minutes. Mix with occasional swirling.

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 combined total volume of **700 µL**. Use each 100 µL aliquot to mix in the Arachidonic Acid on the sides of the vials by rotating the vial as the albumin is added. Vigorously mix the Arachidonic acid into the albumin using a plastic transfer pipette. Combine the suspension from the tip with that in the body of the vial and continue mixing until the Arachidonic acid suspension reaches maximum turbidity. Transfer reagent to microcentrifuge tube and vortex at highest speed for 4-5 minutes. The reconstituted Arachidonic acid suspension should appear very milky (not cloudy) with numerous small bubbles. Also, vortex for 2 minutes immediately prior to running each test.

###### b. ADP,

Supplied As: 2.5 mg of lyophilized preparation of adenosine diphosphate.

Stock Conc.: 1 mM

Stock Storage: Store frozen below 0°C

Stock Shelf life: Until expiration date.

Working Conc.: 10 µM

Working Storage: 2-8°C

Working Shelf life: 8 hours

Reconstitute with 5.0 mL of irrigation grade physiological saline.

There is a very small amount of reagent in this vial.

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. Gently invert before use.

###### c. ATP Standard

Supplied As: Lyophilized adenosine 5' triphosphate

Stock Conc.: 2µmole

Stock Storage: Store frozen below 0°C

Stock Shelf life: Until expiration date.

Working Conc.: 2 nmoles  
Working Storage: 2-8°C  
Working Shelf life: 24 hours

Reconstitute with 5.0 mL of irrigation grade physiological saline.

The appearance of the lyophilized ATP may range from particulate to a thin film coating. Either morphology is suitable for use after reconstitution. Tap vial gently to get contents to the bottom. Remove stopper, add 5 mL of irrigation grade physiological saline for a 2 µmole Standard. Replace stopper and invert gently. Allow to sit for 10 minutes with occasional inversion. Invert again before use.

**d. CHRONO-LUME®**

Supplied As: 0.2mg Luciferin, 22,000 Units d-luciferase, magnesium sulphate, human serum albumin, stabilizers and buffer.  
Stock Conc.: N/A  
Stock Storage: Store frozen below 0°C  
Stock Shelf life: Until expiration date.

Working Conc.: N/A  
Working Storage: 2-8°C in the dark  
Working Shelf life: 8 hours

Reconstitute with 1.25 mL of sterile distilled water

Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with 1.25 mL of sterile distilled water and **allow to stand for 20 minutes prior to use with occasional swirling**. Gently invert before use.

**e. Collagen**

Supplied as: 1 mg of native Collagen fibrils (type I) from equine tendons suspended in isotonic glucose solution of pH 2.7/vial.

Stock Conc.: 1mg/mL  
Stock Storage: Refrigerate at 2-8°C  
Stock Shelf Life: Until expiration date.

Working Conc.: 1, 2 or 5 µg/mL  
Working Storage: 2-8°C  
Working Shelf life: 1 week

Use as supplied.

Invert or swirl vial before use, as collagen fibrils are in suspension. **Do not freeze**. If required, collagen can be further diluted either in isotonic glucose pH 2.7 (enough for one day's testing) or physiological saline (enough for one hour's testing). **Do not dilute entire vial.**

Because of its very low pH, organisms do not grow as readily. If aseptic technique (sterile syringe and needle) is used to remove enough for a day's testing, remaining reagent, if stored at 2 - 8°C, is stable until expiration date. Place the extracted Collagen into a

conical tube to be sure fibrils stay in suspension. Parafilm reagent vial and reagent aliquot during storage. Reagent removed from the vial is stable for one week at 2 – 8°C when parafilmed. Invert gently before use.

**f. Epinephrine**

Supplied As: Lyophilized preparation of l-Epinephrine bitartrate with stabilizers

Stock Conc.: 10 mM for Whole Blood Testing; 1mM for PRP testing  
Stock Storage: Refrigerate at 2-8°C  
Stock Shelf life: Until expiration date

Working Conc.: 50µM/10µM  
Working Storage: 2-8°C in dark container  
Working Shelf life: 8 hours

Reconstitute with 5.0 mL sterile distilled water (dilute 1:10 with physiological saline). For PRP only.

Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with 5.0 mL of sterile distilled water. Allow to sit for ten minutes with occasional inversion. Combine physiological saline and reconstituted Epinephrine in a micro-centrifuge tube to create a 1:10 working stock and invert gently. Allow to sit for 10 minutes with occasional inversion. Gently invert before use.

**NOTE: Normal subjects exhibit considerable variability that is not correlated with age, sex, stress, diet, platelet count or hematocrit.**

**g. Ristocetin**

Supplied as: Stabilized freeze dried Ristocetin  
Stock Conc.: 125 mg/mL  
Stock Storage: Refrigerate at 2-8°C  
Stock Shelf Life: Until expiration date.

Working Conc.: 1.0 mg/mL  
Working Storage: 2-8°C in the dark  
Working Shelf life: 8 hours.

Reconstitute with 0.5 mL of sterile distilled water.

This reagent is an industry high concentration of 125 mg/ml stock. Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with **0.5 mL** of sterile distilled **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.

**h. Thrombin,**

Supplied as: Lyophilized Thrombin from human plasma  
 Stock Conc.: 10 Units/mL  
 Stock Storage: Frozen below 0°C  
 Stock Shelf Life: Until expiration date.

Working Conc.: 1 Unit  
 Working Storage: 2-8°C  
 Working Shelf life: 24 hours.

Reconstitute with 1.0 mL of irrigation grade physiological saline.

Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with 1.0 mL of irrigation grade physiological saline. Allow to sit for 10 minutes with occasional inversion. Invert before use.

### 3. AGGRO/LINK®8 Software for Computer (Optional)

- Turn on the Aggregometer & Computer and Start AGGRO/LINK®8 program. Be sure "AGGREGOMETER Ready" appears in the bottom right-hand corner of the screen. If not, check cables and connectors.
- To have Area Under Curve and Lag Time Calculated and printed on test data, select this option in the Configuration set-up. When selected, a check mark will appear. To disable, leave this box unchecked.
- When ready to begin testing, click on AGGREGOMETER then TEST PROCEDURE and select or set-up the appropriate test page. Click on OK, then select "Run New Patient" under AGGREGOMETER.

**NOTE** – The following Commands are accessible through Icons at top of page:

<input type="checkbox"/> Open	<input type="checkbox"/> Copy the Data Screen	<input type="checkbox"/> Run New (Patient)
<input type="checkbox"/> Move Documents	<input type="checkbox"/> Copy the Trace Screen	<input type="checkbox"/> Run Next (Same Patient)
<input type="checkbox"/> Save Test	<input type="checkbox"/> Copy the Data and Trace screen	<input type="checkbox"/> Restart Current
<input type="checkbox"/> Print Preview	<input type="checkbox"/> Copy Data Screen for Selected traces	<input type="checkbox"/> Stop test
<input type="checkbox"/> Print Test	<input type="checkbox"/> Copy Trace Screen for Selected Traces	<input type="checkbox"/> Set Start Stop Lines
	<input type="checkbox"/> Copy Data and Trace Screen for selected Traces	<input type="checkbox"/> Calculate Slope Amplitude
	<input type="checkbox"/> Merge Copy All Channels	<input type="checkbox"/> Connect to AGGRO/LINK®
	<input type="checkbox"/> Merge Copy Selected Channels	<input type="checkbox"/> Switch between Grid and Data Page
	<input type="checkbox"/> Merge Paste	

### 4. Micro-Pipettes with 2-Stop Control Button

- Description of 2 Stops**
  - First Stop – The measuring stroke for aspirating and dispensing the selected volume
  - Second Stop – To blow out any liquid remaining in the pipette tip after dispensing.

#### b. Volume Setting

When adjusting the volume setting from a higher value to a lower value, turn the knob past the desired volume and then back to the required setting.

#### c. Filling the Pipette Tip

- Press the control button down to the first stop
- Immerse the pipette tip vertically into the liquid
- Aspirate and dispense the liquid three times by using the first stop only.

*NOTE: When pipetting Whole Blood, PRP or Platelets do NOT aspirate/dispense 3 times. Only take up the sample one time.*

- Remove the tip slowly from the liquid. For large volumes, wait approximately 3 seconds before removing the tip from the liquid.
- Wipe off the outside of the tip with a lint-free tissue to remove any excess liquid... Taking precaution that the tissue does not touch the tip opening.

#### d. Dispensing

- Place the pipette tip into the cuvette, so that the end of the tip is immersed in the sample.
- Slightly angle the pipette so that the tip is angled to touch the front wall of the cuvette.
- Press the control button down to the first stop, then press the control button down to the second stop (blow-out) to empty the pipette tip
- Hold the control button down at the second stop, while keeping the pipette tip at a slight angle pull the pipette tip out of the sample
- Let the control button go, once the pipette tip is completely outside of the cuvette.

### CALIBRATION:

#### 1. Impedance

Set Baseline Button automatically sets the baseline at Zero and the Gain at 20 ohms (using the numbers on the right of the graph). Refer to "Procedure – Stepwise" for details.

#### 2. Luminescence

Baseline is set automatically at 100% on the graph using the numbers on the left of the graph. Calibrate ATP Secretion with a 2 nM ATP Standard. (See ATP Standard Procedure for instructions)

#### 3. Optical

Baseline is set from 0% to 100% when the Set Baseline button is pressed and held. Refer to "PRP Lumi Procedure Stepwise" or "VW Procedure Stepwise" for details.

### QUALITY CONTROL:

It is good laboratory practice to run a normal control, free of drugs known to affect platelets, whenever reagents are reconstituted or thawed and/or for every 8-hours of testing. Test results should fall within Normal Ranges established in each laboratory.

Positive controls can be provided by collecting samples from aspirin volunteers and subjects previously diagnosed with a platelet disorder.

## PROCEDURE – STEPWISE (Whole Blood/Luminescence):

### Handling Conditions for Whole Blood:

- Testing can be performed up to three (3) hours after venipuncture.
- Specimens should be kept at room temperature (20-25°C).
- Wait 30 minutes before testing with ADP.

**NOTE REGARDING PLATELET COUNT** - With the exception of ADP, all Reagents can be used to test platelet function in diluted Whole Blood with a final platelet count of  $>125,000/\mu\text{L}$  before dilution. ADP requires a final platelet count of  $>225,000/\mu\text{L}$  for testing with diluted Whole Blood. (For testing with a platelet count of  $<225,000/\mu\text{L}$ , run ADP tests UNDILUTED.)

**NOTE:** For platelet counts  $<125,000/\mu\text{L}$ , many laboratories run these samples undiluted. Their experience has been that longer incubation may be needed. These samples may take longer to establish a stable baseline and may generate tracings with larger oscillations.

- A. For testing with reusable electrodes, add 1 mL of saline to one cuvette per channel. Add the reusable electrode and place in the incubation well to prewarm.

**NOTE:** This procedure is for using P/N 369R reusable electrode assembly or P/N 315 Disposable Electrodes.

- B. For testing with reusable electrode, prewarm cuvettes and stir bars in the incubation wells. Prewarm enough cuvettes for the entire aggregation study. For testing with Disposable Electrodes, prewarm cuvettes, stir bars and Disposable Electrodes with saline as shown below.
- C. Add 450  $\mu\text{L}$  of physiological saline to all but 2 cuvettes (these will be used for Ristocetin testing). For the remaining 2 cuvettes, add 500  $\mu\text{L}$  physiological saline. Incubate in the incubation wells.

### 1. The ATP standard

**NOTE A:** To run patient and control simultaneously, run patient sample in one channel... or two channels if using 4-channel system and Control in one channel...or two channels if using a 4-channel system.

**NOTE B:** An ATP standard must be run in each channel that will be used for each subject.

- Gently mix the Whole Blood by end over end inversion 3-4 times before pipetting 450 $\mu\text{L}$  of the Whole Blood into prewarmed cuvettes with stir bars and saline (Prepare two (2) cuvettes for each Lumi-channel). Dispense directly into the saline. Do not allow the sample to flow down the side of the cuvette.
- Incubate for five (5) minutes in Incubation Wells.**

**DO NOT** incubate in the test wells. Only incubate in the incubation wells.

- Start AGGRO/LINK®8 program and select **Test Procedure** under **Aggregometer** window. Select number of channels from 1 to 4. Under **Procedure Name**, load **ATP Standard** page. Slope

length can be set from 16 to 99 seconds; Chronolog standard setting is 16 seconds. Check all parameters and click OK.

- Select **"Run New Patient"** under **Aggregometer** window. Test Identification Field at top of RUN TEST Screen will read ATP Standard (DATE). Beginning with Channel 1, type in patient name, ID # and other data, or you can type in the information while test is running by clicking on Test Information under EDIT.

**NOTE** – Patient data can be entered for each Channel or, once entered, can be copied to additional Channels by selecting the appropriate Channel(s) shown in "Copy Data To Selected Channels" and clicking on COPY.

**NOTE** – Test Identification field at the top of the page is used to identify and select test in the Test Directory. For Clinical tests, use patient name.

- Click on OK to start the test. When test screen appears, be sure the Graph Range (time noted at bottom of test graph) is set for a minimum of seven (7) minutes. The Graph Range time can be adjusted under **VIEW** and then **Set Graph Range**.
- Place one of the test cuvettes containing 900  $\mu\text{L}$  pre-warmed diluted blood and stir bar in Channel 1 reaction well.
- Add 100  $\mu\text{L}$  CHRONO-LUME® Reagent to test cuvette. Dispense directly into the sample and do not allow the reagent to flow down the side of the cuvette. (Wipe pipette tip with KimWipe® before adding.)

**Pipetting Reminder For All Volumes:** When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.

- Close the chamber door and select GAIN with the SELECT push button.
- Using the SET push button preset the Luminescence GAIN to X0.01 or X0.02.
- Repeat Steps "f" through "i" for Channels 2, 3 and 4
- Incubate with CHRONO-LUME® in the test well for 2 minutes** during which time stable luminescence baselines should be achieved at the 100% mark using the numbers on the left side of the graph.
- Following the 2 minute incubation and observing stable luminescence baselines at the "100" mark [using the numbers on the left side of the graph], Click on **Stop Test** and **Restart Current Test**, under **Aggregometer [or use Icons]**, to obtain a clean test graph.

**WARNING:** Only use **RESTART CURRENT TEST** to obtain a clean graph. If used to start a new test, the previous test will be erased.

**NOTE:** CHRONO-LUME® is Time and Temperature dependent. To obtain accurate results, be sure to incubate each sample for 2 minutes prior to adding reagent.

- m. Draw up 5 µL of ATP Standard into the micropipette. Wipe tip with a clean KimWipe®.
- n. Click start for the channel 1.

**NOTE:** Before a test is started, the word START is visible in each color-coded bar at the top of the Test Grid. Just before addition of the ATP Standard, Left click on the Color-coded Bar under the appropriate tracing # at the top of the Test Grid. A color-coded Start Line will appear momentarily at that time point on the Test Grid and calculation of the test data will start for that Channel and will be shown on the right side of the grid. After clicking, the word START will then disappear from the color-coded bar.

- o. Open the test well door on Channel 1 and add 5 µL of ATP Standard. Dispense directly into the sample and do not allow the reagent to flow down the side of the cuvette. **Close the test well door.**
- p. Observe the luminescence reaction closely. Typically, the reaction peaks and then begins to deteriorate. The aim is to bring the signal peak to between 60% and 20% using the numbers on the left of the graph. If the peak is less than 60% and before the reaction starts to deteriorate, press the SET push button to increase the Luminescence Gain by one setting. **[Using the SET push button, increase the Luminescence Gain by one step at a time until the tracing peaks between 60% and 20%.]** If the reaction proceeds off scale or goes beyond 20%, do not attempt to decrease the gain to rectify. Instead repeat the test using the appropriate gain setting.

**NOTE A:** Choose to increase the gain at a point when the reaction peak pauses, but before it begins to deteriorate. Increasing the gain too late will result in an improper ATP Standard.

**NOTE B –** If the ATP Standard falls just under 60% (~61, 62, 63), it is not absolutely necessary to increase the gain.

- q. Press SELECT push button to return to Main “TEMP, GAIN, SPEED” LCD Screen and to lock in Luminescence Gain setting.
- r. Repeat steps “m” through “q” for Channels 2, 3 and 4, if required.
- s. Once all ATP signals start to deteriorate, select **Aggregometer and Stop Test.**

**NOTE:** When test is stopped, the screen will change to the Patient Data View and the ATP Standard will be calculated automatically in percent. In addition, the following Text Box will appear: “Test must be saved to use these results for Luminescence gain.” Save?

YES	NO
-----	----

Clicking on YES will save the test and automatically plug the ATP Standard calculation into the Luminescence Gain. Select RUN NEXT icon to run additional tests against this gain. To abandon all ATP standards in this run, click on NO and repeat Standard for

all channels. To preserve one (or several of the ATP standards and rerun in other channels, Click YES to save, then see instructions after “x”.

- t. If it is necessary to change Start and Stop times, click on EDIT then select SET START & STOP TIMES.

**NOTE –** SET START AND STOP TIMES may not be required for Luminescence tracings as the START Line is set automatically at the beginning of the Test Graph (0 seconds) and the STOP Line is set automatically at the end of the test. If adjustments are not needed due to artifacts, proceed to CALCULATE and Click on OK.

- 1) If only calculating Slope and Amplitude:
  - a) A small box will appear with Trace 2 selected. If the Automatic Start Time Feature was used a dotted line will appear on the screen at that point (Start Line) with another dotted line (Stop Line) placed “X” number of minutes after (Test Time set up on procedure page).
  - b) If required, Start & Stop times can be moved:
    1. Individually – Left Click, Hold and Drag to new position where Start Line is on stable Baseline before door opening and Stop Line is after the peak.
    2. Simultaneously – Place cursor near Start Line. Right click, Hold & Drag Start Line to new position on Stable Baseline before door opening. Stop Line will move in tandem.
    3. Click on traces 4, 6 and 8 and repeat above for each.
- 2) If calculating Slope, Amplitude, Lag Time and Area Under the Curve at the same settings:
  - a) Click on Trace 2
  - b) Click on “Set All” to calculate all parameters with one setting.
  - c) Follow Steps 1) “b” and “c” above.
- 3) To calculate Individual Parameters:
  - a) Click on Trace 2
  - b) Click on parameter (i.e. lag time)
  - c) Follow Steps 1) “b” and “c” above.

**NOTE –** When calculating Lag Time individually, only a start time is displayed.

- u. After setting the Start and Stop Times for all Luminescence signals, click on DONE, then select CALCULATE RESULTS under the EDIT window. This command will calculate the ATP peak as a percentage. Check Duration times to be sure Start & Stop Lines were set correctly. Click on OK and calculations will appear in Data Box.
- v. After the calculations have been completed, SAVE the test, using the SAVE command under FILE or the SAVE icon. Tests can be printed using the PRINT command in the FILE WINDOW. PRINT is for printing one test run per page. Use



- CASCADE or TILE under window to view and select tests before printing.
- w. Remove the samples from the reaction wells and discard in their entirety.
- x. Keep Luminescence Gain at Final setting for all tests run with that subject's blood.

**TO PRESERVE VALID ATP STANDARDS IF REPEAT IS NEEDED IN ONE OR MORE CHANNELS:**

- SAVE the first ATP Standard and CLOSE the test.
- Click on RUN NEW Icon and select ATP Standard Test Page
- UNCHECK the channels that had a good Standard in the first run [Ex: UNCHECK Channels 2 and 4]
- RUN the new ATP Standards in remaining channels [Ex: Channels 1 and 3]
- When completed, SAVE the test.

Click on RUN NEXT to begin testing with Luminescence. ATP Standard values in all four channels will be carried over.

**2. Maximum ATP release induced by Thrombin.**

Aggregation is not measured with this Thrombin concentration of 1U/mL. (To measure Aggregation with Thrombin, follow Aggregation testing instructions in Section 3 below and reduce Thrombin concentration to 0.1-0.15 Unit/mL. Sample will Aggregate and then will Clot with Thrombin.)

- a. Select TEST PROCEDURE under the AGGREGOMETER menu. Set-up or select Thrombin procedure page. The Luminescence Gains obtained with the ATP Standard tests will be entered automatically into the Luminescence Gain for each Channel.

**NOTE** – If testing only one subject at a time with a 4-channel system, set up the test page for Thrombin Luminescence Only in one channel and Lumi-Aggregation with agonists in remaining channels and follow the directions for Impedance Lumi-Aggregation below for those channels. If testing patient and control, set up test page for Thrombin Luminescence in two of the channels and Collagen Lumi-Aggregation in two channels. With a 2-channel system, run patient in one channel and control in the other.

- b. Select number of channels from 1 to 4 and check all parameters. Slope length can be set from 16 to 99 seconds; Chrono-log's standard setting is 16 seconds. Click OK.
- c. Now select **Run Same Patient** under **Aggregometer** window. Enter Patient Name and/or ID # in the Test Identification Field at the top of the RUN TEST Screen as tests will be listed in Directory under this information for later extraction. Click OK to start the test.

**NOTE:** If needed, patient data from Trace 1 can be copied to other tracings. Click on appropriate Trace no. and click on COPY.

**NOTE:** The **Test Identification** field at the top of the page is used to identify and select tests in the Test Directory. For Clinical tests, use patient name.

- d. Place one of the prewarmed aliquots of 900 µL diluted blood in each reaction well.

- e. Add 100 µL CHRONO-LUME® Reagent into each test sample. Dispense directly into the sample and do not allow the reagent to flow down the side of the cuvette. Wipe pipette tip with a clean Kim Wipe® before adding. [NOTE: Electrode not needed for Luminescence with Thrombin].

**Pipetting Reminder For All Volumes:** When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.

- f. Close the chamber doors.
- g. If running Aggregation tests in any other channels, insert electrode only into samples for aggregation and follow directions for Impedance Aggregation below.
- h. **Incubate with CHRONO-LUME® in the test well for 2 minutes** during which time a stable baseline of luminescence should be achieved at the 100% mark using the numbers on the left side of the graph.

**NOTE:** CHRONO-LUME® is Time and Temperature dependent. To obtain accurate results, be sure to incubate each sample for 2 minute prior to adding reagent.

- i. After the two (2) minute incubation and baselines have stabilized, **Stop Test** and **Restart Test** under **Aggregometer**. This will eliminate any artifacts from the test graph.

**WARNING:** Only use **RESTART CURRENT TEST** to obtain a clean screen. If used to start a test, the previous test will be erased.

- j. Draw up 100 µL of Thrombin reagent into the pipette tip. Be sure to wipe the tip with a clean Kim Wipe®.
- k. Click start for the appropriate channel.

**NOTE:** Before a test is started, the word **START** is visible in each color-coded bar at the top of the Test Grid. **Just before addition of the reagent, Left click on the Color-coded Bar under the appropriate tracing # at the top of the Test Grid.** A color-coded Start Line will appear momentarily at that time point on the Test Grid and calculation of the test data will start for that Channel and will be shown on the right side of the grid. After clicking, the word **START** will then disappear from the color-coded bar.

- l. Open the chamber door, add the Thrombin, and close the chamber door. Be sure the pipette tip is down into the sample. Dispense directly into the sample and do not allow the reagent to flow down the side of the cuvette.
- m. Repeat "j" through "l" for the other channels (if running Thrombin in multiple Channels) or follow Impedance Aggregation instructions below for other agonists.
- n. If not done previously, while tests are running, gently mix Whole Blood by end over end inversion 3-4 times. Prepare one cuvette for each channel for incubation by adding 450 µL Whole Blood. (The cuvette, stir bar and saline should already be in the incubation wells) & begin 5

minute incubation. Dispense the Whole Blood directly into the saline and do not allow it to flow down the side.

**DO NOT incubate in the test wells. Only incubate in the incubation wells.**

- o. If only testing Thrombin, Luminescence tests can be stopped once all Luminescence signals begin to deteriorate. If testing Lumi-Aggregation with other agonists, wait for all Aggregation signals to complete a minimum 6-minute test run or to reach full amplitude before stopping test. Once all signals are complete, click on **Stop Test** under **Aggregometer** or use **STOP** icon to stop the tests. Tests cannot be stopped separately.
- p. If necessary to change Start and Stop times, click on EDIT and SET START & STOP TIMES. Place Start Line on Stable Baseline before door opening and place Stop Line after the Luminescence peak or place Stop Line on Aggregation tracing at full amplitude.

**NOTE – SET START AND STOP TIMES** may not be required for Luminescence tracings as the START Line is set automatically at the beginning of the Test Graph (0 seconds) and the STOP Line is set automatically at the end of the test. If adjustments are not needed due to artifacts, click on save.

- 1. If only calculating Slope and Amplitude:
  - a) A small box will appear with Trace 2 selected. If the Automatic Start Time Feature was used a dotted line will appear on the screen marking the Start Time. The second dotted line which appears under Set Start & Stop Times marks the Stop Time, automatically placed “X” number of minutes after the Start Time, based on the Duration entered on the Test Procedure Page, or at the end of a Luminescence tracing if test is stopped before.
  - b) If required, Start & Stop times can be moved:
    - 1. Individually – Left Click, Hold and Drag to new position.
    - 2. Simultaneously – Place cursor near Start Line. Right click, Hold & Drag Start Line to new position. Stop Line will move in tandem.
  - c) If aggregation was tested in other channels, Click on Traces 1, 3, 5 and/or 7 and repeat steps b and c above for each.
  - d) To calculate additional luminescence tracings, click on Traces 4, 6 and 8 and repeat steps b and c above.
- 2. If calculating Slope, Amplitude, Lag Time and Area Under the Curve at the same settings:
  - a) Click on appropriate Trace #
  - b) Click on “Set All” to calculate all parameters with one setting.

- c) Follow Steps 1) b through e above. Place Start Line on Stable Baseline before door opening and place Stop Line after the Luminescence peak.
- 3. To calculate Individual Parameters:
  - a) Click on appropriate Trace #
  - b) Click on parameter (i.e. lag time)
  - c) Follow steps 1) b through e above.
- q. After setting the Start and Stop Times, Click on DONE, then select CALCULATE RESULTS under the EDIT window. This command will calculate aggregation in ohms and ATP Release in nmoles. Check Duration times to be sure Start & Stop Lines were set correctly. Click on OK and final calculations will appear in Data Box.
- r. After the calculations have been completed, SAVE the test, using the Save command under File window or use the SAVE icon.
- s. Tests can be printed using the PRINT command in the FILE WINDOW.
- t. Remove the samples from the reaction well and discard the cuvettes in their entirety.

### 3. Impedance Aggregation tests and simultaneous ATP release with Collagen, ADP and Arachidonic Acid

**NOTE A:** 1) Be sure a test cuvette for each channel (with stir bar and 450  $\mu$ L saline) has been prewarmed in incubation well. Add 450  $\mu$ L blood to each test cuvette and continue to pre-warm in incubation well for **5 minutes** before starting tests. If using Disposable Electrode, **DO NOT** remove the disposable electrode as it must remain in the cuvette to be prewarmed.

2) Be sure **MODE** is set to **IMPEDANCE**. Use **MODE** push button to change setting between **OPTICAL** and **IMPEDANCE**. **NOTE B:** **MODE** push button is disabled until Aggregometer reaches temperature setting and when instrument is in the **SELECT** mode.

- a. Set up or Select TEST PROCEDURE under the AGGREGOMETER menu. [Set up or load the procedure that corresponds to the reagents and Whole Blood-Lumi methods being used.] Select number of Test Channels from 1 to 4 and check all parameters. Traces 1,3,5,7 should be set for Impedance while Traces 2,4,6,8 should be set for Luminescence. The Luminescence Gain value obtained with the ATP Standard tests will be entered automatically into the Luminescence Gain for each Channel. **The Impedance gain is set automatically at 20/5 where 20 ohms of impedance aggregation is represented by 5 large blocks or 50% of scale.** Slope length can be set from 16 to 99 seconds. The Chrono-log default setting is 16 seconds. Click OK.
- b. Select **Run Same Patient** under **Aggregometer** window or use **ICON** at top of screen. Patient Test Information page will appear with Patient Data already entered from previous test.

**NOTE –** If needed, patient data from one trace can be copied to other tracings. Click on appropriate Trace No. and Click on **COPY**.

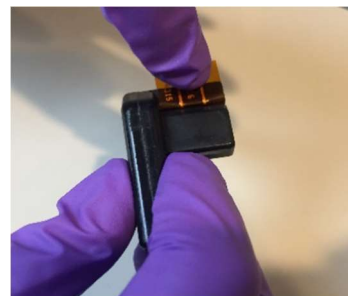
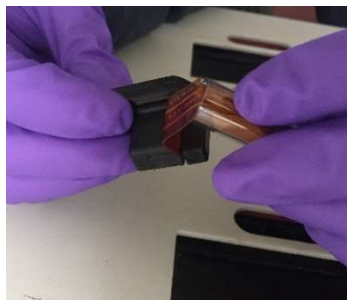
**NOTE - Test Identification** field at the top of the page is used to identify and select test in the Test Directory. For Clinical tests, use patient name.

- c. Click OK to start test.

*When test screen appears, be sure the Graph Range (time noted at bottom of test graph) is set for a minimum of seven (7) minutes. The Graph Range time can be adjusted under **VIEW** and then **Set Graph Range**.*

*The software will add additional time, if needed, during testing*

- d. If testing with reusable electrodes, Remove prewarmed reusable electrodes from saline cuvette, wipe with a KimWipe and plug into the electrode connector located inside each test well, as appropriate.
- e. If testing with disposable electrodes, plug Disposable Electrode Adapter into the electrode connector located inside each test well, taking care to avoid bending the metal connector, as follows:
  - a) To Insert: Place adapter into Test Well so it lays flat and the left side is covering the words "+ Stir Bar" and "Connector" is partially covered. Gently hold the adaptor flat on the front left side and place fingers on the front and back of the right side. Slide forward until adapter enters the instrument connector and you hear a Click. Do not press down on the right side of the adapter as this may cause bending of the connector.
  - b) To remove: While gently holding the adaptor flat on the left side, place a finger to the front and back of the right piece and slide the adapter to the back of the test well until the connector is removed. Store in a safe environment to protect the metal connector.



- f. Place a prewarmed cuvette with 900  $\mu$ L of diluted sample into the test wells of Channels 1, 2, 3 and 4, as appropriate.
  - a) If testing with reusable electrode, place electrode into test samples with the red wire facing toward the front of the instrument. Press the red electrode wire into test chamber.
  - b) If testing with disposable electrode, place one of the test cuvettes with disposable electrode containing 900  $\mu$ L pre-warmed diluted blood and stir bar in each Whole Blood reaction well. Position electrode fold over adapter slot. To connect with the gold-plated contact points inside adapter slot, firmly press down on the fold until completely inserted into the slot. After insertion, the cuvette should be fully inserted into the well.
- g. Wipe the tip with a KimWipe® and add 100  $\mu$ L CHRONO-LUME® Reagent to the test sample. Dispense directly into the sample and do not allow the blood to flow down the side of the cuvette.

**Pipetting Reminder For All Volumes:** *When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.*

- h. Close the chamber door.
- i. Repeat Step "f" & "g" for each channel.
- j. Press the BASELINE push button for each channel to set Impedance Baseline at 0 and to set Impedance Gain at 20 ohms [using numbers on right side of graph]. Monitor for stability. If not stable after one (1) minute, the BASELINE push button can be reset until the Impedance tracings are stable at the 0 line.
- k. **Incubate with CHRONO-LUME® for two (2) minutes in the test well** during which time stable baselines for aggregation and luminescence should be achieved at the 0 Ohms and 100% marks respectively.
- l. After the sample in Channel 1 has incubated for 2 minutes and stable Impedance and Luminescence baselines have been established on all Channels,

click on **Stop Test** and **Restart Current Test** under **Aggregometer** to start a new screen and to eliminate any artifacts. **DO NOT** click on **Run Test**, as it will be necessary to reenter patient data.

**WARNING:** Use **RESTART CURRENT TEST** only to obtain a clean screen. If used to start a new test, the previous test will be erased.

- m. Press the **BASELINE** push button again on each Channel to show baseline and Gain on the Test Graph.

**NOTE:** Be sure tracing moves to 20 ohms and then back to 0 ohm using the numbers on the right side of the graph.

- n. Once the aggregation tracings return to "0", click on **START** for channel 1, then immediately add the appropriate aggregation reagent. Be sure to wipe the outside of the pipette tip before adding reagent. Dispense directly into the sample and do not allow the reagent to flow down the side of the cuvette. Repeat for remaining channels.
  - 1) 1  $\mu$ L of collagen reagent, equals 1  $\mu$ g/mL final concentration [5  $\mu$ L = 5  $\mu$ g/mL]
  - 2) 10  $\mu$ L of ADP, equals 10  $\mu$ M final concentration [20  $\mu$ L = 20  $\mu$ M]. **NOTE:** Wait 30 minutes from draw to test with ADP.
  - 3) 10  $\mu$ L of Arachidonic Acid equals 0.5 mM.

**NOTE:** Before a test is started, the word **START** is visible in each color-coded bar at the top of the Test Grid. Just before addition of the reagent, Left click on the Color-coded Bar under the appropriate Aggregation tracing # at the top of the Test Grid. A color-coded Start Line will appear momentarily at that time point on the Test Grid. Calculation of the test data will start for both tracings for that Channel and will be shown on the right side of the grid. After clicking, the word **START** will then disappear from the color-coded bars for that Channel.

**NOTE:** Wipe pipette tip with clean KimWipe® before adding reagent. Also, be sure tip is immersed in sample when reagent is added.

**NOTE:** If not completed prior to starting test, Click on **Edit** and **Test Information** to enter Patient Information while test is running. The comments Box can be used for typing notes about the test such as a list of Drugs taken within the past ten (10) days.

- n. While tests are running, prepare one test cuvette for each channel. Gently mix the Whole Blood by end over end inversion 3-4 times. Add 450  $\mu$ L Whole Blood to prewarmed cuvette with 450  $\mu$ L physiological saline and Stir Bar and begin the 5 minute incubation. Dispense directly into the saline and do not allow the blood to flow down the side of the cuvette.

**DO NOT** incubate in the test wells. Only incubate in the incubation wells.

- o. **Stop** the test under **Aggregometer**, after all Aggregation tracings have run for a Minimum of 6 minutes after the addition of the reagent or have reached full amplitude.

- p. Click on **EDIT** and **SET START & STOP TIMES** [*If color-coded line was clicked and there are no artifacts or baseline shifts, this step can be eliminated. Go directly to step r.*]
  - 1) If only calculating Slope and Amplitude:
    - a) A small box will appear with Trace 1 selected. If the Automatic Start Time Feature was used a dotted line will appear on the screen at that point (Start Line) with another dotted line (Stop Line) placed "X" number of minutes after (Test Duration set up on Test Procedure Page).
    - b) If required, Start & Stop times can be moved:
      - I. Individually – Left Click, Hold and Drag to new position. (Be sure Start Line for Aggregation and for Luminescence is placed on stable baseline just before door opening or just after adding reagent. Stop Line for Aggregation should be placed six minutes after Start Time and for Luminescence after the peak.)
      - II. Simultaneously – Place cursor near Start Line. Right click, Hold & Drag Start Line to new position. Stop Line will move in tandem.
    - d) Click on Traces 2 through 8 and repeat steps 1) b and c above.
  - 2) If calculating Slope, Amplitude, Lag Time and Area Under the Curve at the same settings:
    - a) Click on Trace 1
    - b) Click on "Set All" to calculate all parameters with one setting.
    - c) Follow Steps 1) b through d above.
  - 3) To calculate Individual Parameters:
    - a) Click on Trace 1
    - b) Click on parameter (i.e. lag time)
    - c) Follow steps 1) b through d above.

**Exception:** A Luminescence baseline shift may occur with ADP and Arachidonic Acid. Set Luminescence Start Line after the shift.

- q. After setting the Start and Stop Times, Click on **DONE**, then select **CALCULATE RESULTS** under the **EDIT** window. This command will calculate Aggregation in ohms and ATP Release in nmoles. If required, check Duration times to be sure Start & Stop Lines were set correctly. Click on **OK** and calculations will appear in Data Box.
- r. After the calculations have been completed, **SAVE** the test, using the **Save** command under **File** window or the **SAVE** icon. Test can be printed using the **Print** command in **file** window. Use **CASCADE** or **TILE** under **WINDOW** to view and/or close tests before printing.
- s. Remove reusable electrodes from samples and clean by whisking in distilled water. Wipe electrode dry using lintless wipes, such as KimWipes®, being careful not to bend electrode wires. Rinse again in saline and wipe electrode with a lintless wipe. Perform a visual check of

the wires to be certain of cleanliness. Remove the sample(s) from the reaction well and discard in their entirety.

**NOTE:** Do not unplug Electrodes from instrument when cleaning between tests. Hold beaker of water or saline in front of (not above) instrument and bring the electrode to beaker for washing. Leaving electrode plugged in during washing prevents a large shift in baseline for next test.

- t. Remove the test sample(s) including the Disposable electrode from the reaction well and discard in their entirety.
- u. To run additional agonists with luminescence with the same patient sample, click RUN NEXT, then repeat steps "e" through "s"

#### 4. TESTING WITH RISTOCETIN (Do Not add CHRONO-LUME®)

- a. Prewarm two (2) cuvettes for each subject, each with stir bar and 500 µL irrigation grade saline.
- b. Gently mix the Whole Blood by end over end inversion 3-4 times. Add 500 µL Whole Blood added to prewarmed cuvette with 500µL physiological saline and Stir Bar and begin the 5 minute incubation. Dispense directly into the saline and do not allow the blood to flow down the side of the cuvette.
- 1) When testing with reusable electrode, 8 µL of Ristocetin equals a 1 mg/mL final concentration
- 2) When testing with reusable electrode, 2 µL of Ristocetin equals a 0.25 mg/mL final concentration that is used to screen for Type 2B or Platelet-Type von Willebrand Disease.
- 3) When testing with disposable electrode, 3.2 µL of Ristocetin equals a 0.4 mg/mL final concentration.
- 4) When testing with disposable electrode, 1.2 µL of Ristocetin equals a 0.15 mg/mL final concentration.

**NOTE:** At the end of the day, whisk Reusable electrode in water and wipe with a clean KimWipe® being careful not to bend the electrode wires. Then whisk electrodes in 1:10 household bleach dilution. Unplug electrode from instrument and rinse under fresh running deionized or distilled water to thoroughly remove bleach from wires. Dry with a KimWipe® and store in a clean, dry cuvette. The final rinse before storage must be in distilled water to avoid salt deposits.

#### CALCULATIONS:

1. Amplitude – Impedance aggregation results are expressed as ohms (Ω) at six (6) minutes after reagent addition.
2. Slope – Slope is determined by drawing a tangent through the steepest part of the curve. A right triangle is then constructed over an interval of one minute. The height of the triangle is the rate of change of aggregation in one

minute, which is defined as the slope. AGGRO/LINK®8 Software uses a 32-point sliding curve to calculate maximum slope when set at a length of 16 seconds. To change the length of a slope line, click on **EDIT**, then **ADJUST SLOPE LINE**. Click on Traces 1 through 8 and use arrows to adjust slope length up to 99 seconds.

#### REPORTING RESULTS:

1. Report Hematocrit.
2. Report Platelet Count.
3. Report Impedance Aggregation in ohms (Ω).
4. Report ATP Release in nmoles.

#### Reference Ranges with Reusable or Disposable Electrodes:

**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 Whole Blood with <u>Reusable or Disposable</u> Electrodes (Mean +/- 2 SD)			
Reagent	Conc.	Agg. (ohms)	ATP (nmole)
Thrombin	1 Unit	N/A	>0.5
Collagen	1µg/mL	12 - 33	0.43 – 2.2
	2µg/mL	15 - 27	0.5 - 1.7
	5µg/mL	11- 42	0.54 – 2.9
Arachidonic Acid	0.5 mM	7 - 29	0.45 – 2.8
ADP	5µM	5 - 26	0.11 – 2.0
	10µM	6 - 33	0.21 – 2.0

Normal Ranges with Ristocetin in Whole Blood with <u>Reusable</u> Electrodes			
Reagent	Conc.	Agg. (ohms)	ATP (nmole)
Ristocetin	1.0 mg/mL	> 5 Ω < 70 sec. lag time	N/A
Ristocetin	0.25 mg/mL	≤ 5 Ω	N/A

Normal Ranges with Ristocetin in Whole Blood with <u>Disposable Electrodes</u>			
Reagent	Conc.	Agg. (ohms)	ATP (nmole)
Ristocetin	0.4 mg/mL	> 5 $\Omega$ < 70 sec. lag time	N/A
Ristocetin	0.15 mg/mL	$\leq$ 5 $\Omega$	N/A

**Procedures for Abnormal Results:**

1. When Normal Control Test(s) Is Abnormal
  - a. No patient result shall be reported if any reagent does not recover findings within reference range limits when tested with the normal control.
  - b. Repeat both Normal Control and Patient test with new aliquot of frozen reagent or reconstitute new vial of reagent. Be sure each test cuvette contains a stir bar.
2. When Normal Control Test(s) Is Normal
  - a. Repeat abnormal patient test(s) to be sure result is not due to a technical variable. Be sure each test cuvette contains a stir bar.
  - b. If repeated test(s) continue to be abnormal, report the abnormal result(s) and request a retest on another day to confirm findings.

## Platelet Abnormalities

Platelet Aggregation and ATP secretion are clinically significant in the detection and diagnosis of acquired or congenital qualitative platelet defects. The platelet's ability or inability to respond to particular aggregating reagents is the basis for differentiating platelet dysfunctions as shown in the following tables:

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 – 5 μg/mL	1 or 2 μg/mL	5 μg/mL	N	N	A
		R	N			
Ristocetin	0.15 – 1.0 mg/mL	Qualitative  Defect		** A,R,H ***  >70 sec. Lag (vW)	N	Qualitative  Defect

\* Second-wave Inhibited

\*\* Type 2B and Platelet-type von Willebrand increased at low concentration 0.15 / 0.25 mg/mL

\*\*\* To distinguish between von Willebrand & Bernard Soulier, add normal plasma or cyoprecipitate to patient sample, vW patient will respond, Bernard Soulier will not.

\*\*\*\* 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 that it is not necessarily associated with aggregation abnormalities.

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,R,N
Arachidonic Acid	0.5 mM	A	N	A,R	A,R,N
Collagen	1 – 5 µg/mL	R	N	A,R	A,R,N
Ristocetin	0.15 – 1.0 mg/mL	--	--	--	--
Thrombin	1 Unit	N	N	A,R	A,R,N

\* Higher concentrations of any agonist including Thrombin up to 5 Units will induce additional ATP secretion with a Secretion disorder but will not with a Storage Pool Defect.

\*\* Glanzmann's thrombasthenia is classified into three types, depending on the level of GPIIb-IIIa present:

Type 1 (severe) : <5% of normal GPIIb-IIIa levels.

Type 2 (less severe): 10-20% of normal GPIIb-IIIa levels.

Type 3 (variant): normal levels of GPIIb-IIIa, but functionally inactive.

**Key:** A – Absent H – Hyper N – Normal R - Reduced

(Compared to Normal Ranges)

**Reporting Format:**

## PLATELET AGGREGATION STUDIES

DATE \_\_\_\_\_

PHYSICIAN \_\_\_\_\_ TIME BLOOD DRAWN: \_\_\_\_\_

INSTITUTION: \_\_\_\_\_ DEPARTMENT: \_\_\_\_\_

PATIENT \_\_\_\_\_ I.D. \_\_\_\_\_ DATE OF BIRTH: \_\_\_\_\_

AGE: \_\_\_\_\_ SEX: (M) (F) HCT \_\_\_\_\_ PLT CT \_\_\_\_\_ BT \_\_\_\_\_

CLINICAL HISTORY: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_**WHOLE BLOOD AGGREGATION & SECRETION TEST RESULTS (Guideline ONLY)**

AGONIST	NORMAL RANGES (Mean +/- 2 SD)		CONTROL VALUE		PATIENT VALUE		TEST RESULT (Normal, Reduced, Increased)	
	Aggr. (Ohms)	Secr. (nmoles)	Aggr.	Secr.	Aggr.	Secr.	Aggr.	Secr.
ADP, 5 $\mu$ M								
ADP, 10 $\mu$ M								
COLLAGEN, 1 $\mu$ g/mL								
COLLAGEN, 2 $\mu$ g/mL								
COLLAGEN, 5 $\mu$ g/mL								
ARACH. ACID, 0.5mM								
RISTOCETIN, 1.0mg/mL [Reusable Electrode] Lag Phase								
RISTOCETIN, 0.25mg/mL [Reusable Electrode]								
RISTOCETIN, 0.4mg/mL [Disposable Electrode] Lag Phase								
RISTOCETIN, 0.15mg/mL [Disposable Electrode]								
THROMBIN, 1 Unit								

INTERPRETATION: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

TEST PERFORMED BY: \_\_\_\_\_ INTERPRETED BY: \_\_\_\_\_



## INTERPRETATION:

### 1. Impedance Aggregation

Maximum extent aggregation (measured in ohms) is the best method of assessing impedance results with each agonist. There is good correlation between aggregation maximum extent (amplitude) and the amount of protein dissolved from the electrode tip (ADP P=0.97, Thrombin P=0.96, Collagen P=0.97). Aggregation curves in blood 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.

**With Collagen:** Collagen is useful for checking the platelet's general ability to aggregate and is a cost-effective quick screen for monitoring dosage levels of Aspirin. Low Collagen concentration at 1-2 µg/mL is dependent on TxA<sub>2</sub> formation, which is inhibited by aspirin. High Collagen concentration (5 µg/mL) is more dependent on phospholipase C activation, which is not inhibited by aspirin but will show reduction in the presence of some other anti-platelet agents. In addition, the high Collagen concentration (5 µg/mL) serves as an auto-control, allowing the extent of Aspirin response to be determined. Poor aspirin response is defined as ≥ 50% of the platelets still aggregating when the 1 µg/mL is compared to the 5 µg/mL test. Accurate assessment of the anti-platelet effect of Aspirin will be affected if the patient is taking an agent known to affect the phospholipase C pathway. A lag phase of up to a minute is typically seen with this agonist.

**With Arachidonic Acid:** Arachidonic Acid is converted to thromboxane A<sub>2</sub> in the presence of cyclooxygenase. Aspirin inhibits the cyclooxygenase pathway, causing a significant reduction in aggregation with this agonist. Normal aggregation is seen with concentrations of 0.5 mM to 1.0 mM.

**With ADP:** ADP exposes the fibrinogen-binding site on the membrane glycoprotein GPIIb/IIIa complex. In Whole Blood, small amounts of ADP released by RBCs during sampling may cause a desensitizing effect. It is, therefore, recommended that testing with ADP be delayed for 20-30 minutes following venipuncture. Aggregation testing is typically performed in WB with concentrations ranging from 5 µM to 20 µM. P2Y<sub>12</sub> anti-platelet drugs such as Plavix, will inhibit aggregation at ADP concentrations of 5µM to 20 µM

**With Ristocetin:** Used for the detection of von Willebrand Disease (a quantitative or qualitative defect in plasma vW Factor) and Bernard Soulier (a lack of platelet membrane glycoprotein (GPIb). Normal results are seen with concentrations ranging from 0.5 to 1.0 mg/mL [0.4 mg/mL with Disposable Electrodes]. To detect Type 2B or Platelet-Type vW, test for a Hyper-response at a low concentration of 0.25 mg/mL [0.15 mg/mL with Disposable Electrodes]. To distinguish between vW and Bernard Soulier, add normal plasma or cryoprecipitate to patient sample. vW patient will respond, Bernard Soulier will not. A Qualitative defect such as a saw-tooth pattern may be seen with Glanzmann's patients or with subjects taking aspirin.

**NOTE:** The CLSI Document H-58-A reports that the addition of cryoprecipitate (source of normal VWF) to plasma from patients with VWD Type 2B results in no response to low doses of Ristocetin; whereas, patients with pseudo-VWD continue to show enhanced responsiveness."

**While only reported with Optical Aggregometry using PRP samples, this test may be beneficial when testing in Whole Blood.**

**With Epinephrine:** This **agonist is not recommended as a standard agonist for Whole Blood testing clinically**, as fewer than 50% respond. If testing in Whole Blood with Epinephrine is desired, the recommended anti-coagulant is 1.5% trisodium citrate with 2U Heparin per mL of citrate.

### 2. ATP Release with Luminescence

**Thrombin** - is useful for determining the **maximum** amount of ATP that can be secreted from the dense granules. Secretion is independent of thromboxane synthesis. If ATP Release is <0.5 nmole, run a second test with a higher amount of Thrombin (up to 5 Units). If ATP Release increases, consider a Secretion Defect. If ATP Release does not increase, consider Storage Pool Disease.

**Collagen** - is useful for checking the general aggregability of the platelets. Aggregation and secretion are partially dependent on thromboxane synthesis. Secretion to collagen of less than half the secretion to thrombin may be indicative of impaired thromboxane synthesis. Aspirin, and similar drugs, inhibit the production of thromboxane A<sub>2</sub> that, in turn, inhibits aggregation when tested with low concentrations of 0.5 to 2.0 µg/mL and ATP secretion when tested with concentrations up to and including 5 µg/mL.

**Arachidonic Acid** - is most useful in detecting Aspirin-like defects. Aggregation and secretion to Arachidonic Acid is completely dependent on thromboxane synthesis. No aggregation and No secretion to Arachidonic acid confirms impaired thromboxane synthesis. Aggregation but No secretion may be indicative of storage pool deficiency or secretion defect.

**ADP** - ADP has the weakest response in Whole Blood and requires higher concentrations of 10 or 20 µM. Normal ATP release is relatively low (0.1 +/- 0.2 with 5 µM) and is <0.1 with aspirin, Thrombasthenia or Storage Pool defects.

**Ristocetin** - There are no known clinical reasons for measuring release with this agonist.

## PROCEDURE NOTES:

1. It is very important that all reagents are isotonic to avoid red cell lysis. CHRONO-PAR® Reagents are suitable for use in both whole blood and platelet rich plasma.
2. When ADP or Arachidonic Acid is added to a sample containing CHRONO-LUME®, a shift in the Luminescence Baseline may occur. Be sure **Start Line** is placed **after** this shift.
3. Since each reagent requires only micro-volumes of reagent, it is essential that introduction of excess reagent be avoided. Always wipe the outside of the micropipette tip.

4. It is important that the pipette tip be pushed to the bottom of the cuvette before the reagent is injected directly into the sample. DO NOT introduce the reagent above the sample or down the side of the cuvette as the reagent will cling to the side of the cuvette and will not mix with the sample.

#### LIMITATIONS OF THE PROCEDURE:

- Tests should be performed within 3 hours of venipuncture.
- In Whole Blood, small amounts of ADP released by RBCs during sampling may cause a desensitizing effect. It is therefore recommended that testing with ADP be delayed for 20-30 minutes following 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.
- Further Clinical and Laboratory evaluation may be required to confirm diagnosis.
- If platelet count will drop to under  $100 \times 10^9/L$  after dilution, remove 2 mL of blood for testing undiluted with ADP as an additional ATP Standard will need to be run in undiluted blood.

#### PROCEDURE – STEPWISE (PRP/Luminescence Aggregation):

##### Sample

1. Platelet Rich Plasma (PRP)
  - a. Centrifuge sample at approximately 100-170 g for 15 minutes. Be sure brake is set in the OFF position.
  - b. Take off the PRP with a polypropylene transfer pipette and put into a polypropylene plastic tube.

*NOTE: Per CLSI Document H58-A, the pH of the PRP sample can be preserved as follows:*

- *Place PRP in a plastic tube with limited surface area-to-volume ratio (place large volume of PRP in a small tube)*
- *Cap the PRP tube as PRP in uncapped tube undergoes a rise in pH due to diffusion of CO<sub>2</sub> from plasma*
- *Avoid frequent mixing/agitation of PRP*
- *Introduce PRP directly into the tube and don't allow it to flow down the sides of the tube*
- c. Properly label the tube, include the patient's name and sample type. Parafilm or cap the top. Keep at room temperature (20° to 25°C).
- d. It is usual practice to adjust the platelet count in the PRP to be tested to a final count of approximately 250,000 per  $\mu L$ . Use the same donor's PPP to adjust the count.

*NOTE – Platelet counts lower than 50,000/ $\mu L$  may present difficulties in setting optical baseline.*

2. Prepare the Platelet Poor Plasma (PPP)
  - a. Centrifuge sample at approximately 1500 - 2400 g for 20 minutes.
  - b. Take off the PPP with a polypropylene transfer pipette and put into a polypropylene plastic tube.

- c. Properly label the tube, include the patient's name and sample type. Parafilm or cap the top. Keep at room temperature (20° to 25°C).
- d. A single sample of PPP per channel can be used as the reference sample for all tests run with the same patient's blood so the amount of PPP required is only 500  $\mu L$  (0.5 mL) per channel. (The Model 700 requires only one (1) 500  $\mu L$  cuvette of PPP for up to 4 channels with the same subject's sample).

#### Handling Conditions for PRP:

1. Testing can start 30 minutes after venipuncture and continue for about 3 hours after venipuncture.
2. Specimens should be kept at room temperature (20° to 25°C).
3. Gently invert conical tube containing PRP prior to pipetting to ensure an equal distribution of cells.

#### Procedure:

1. The ATP Standard

**NOTE A** - To run patient and control simultaneously, run patient sample in one channel... or two channels if using a 4-channel system and Control in one channel...or two channels if using a 4-channel system.

**NOTE B** - An ATP standard must be run in each channel that will be used for each subject

- a. Prewarm cuvettes and stir bars in the incubation wells. Prepare and prewarm enough cuvettes for the entire aggregation study. See PREPARATION section for further preparation details.
- b. Gently mix the adjusted PRP by end over end inversion 3-4 times before pipetting.
- c. Add 450 $\mu L$  of PRP into prewarmed cuvettes with stir bars (Prepare two (2) cuvettes for each Lumi-channel). Dispense the PRP directly into the test cuvette and do not allow it to flow down the side.
- d. **Incubate for three (3) minutes in Incubation Wells.**

**DO NOT** incubate in the test wells. Only incubate in the incubation wells.

- e. Start AGGRO/LINK®8 program and select **Test Procedure** under **Aggregometer** window. Select number of channels from 1 to 4. Under **Procedure Name**, load **ATP Standard** page. Slope length can be set from 16 to 99 seconds; Chrono-log standard setting is 16 seconds. Check all parameters and click OK.
- f. Select **"Run New Patient"** under **Aggregometer** window. Test Identification Field at top of RUN TEST Screen will read ATP Standard (DATE). Beginning with Channel 1, type in patient name, ID # and other data, or you can type in the information while test is running by clicking on EDIT and TEST INFORMATION.
- g. Click on OK to start the test. When test screen appears, be sure the Graph Range (time noted at bottom of test graph) is set for a minimum of six (6) minutes. The Graph Range time can be adjusted under **VIEW** and then **Set Graph Range**.

**NOTE A**– Patient data can be entered for each Channel or, once entered, can be copied to additional Channels by selecting the appropriate Channel(s) shown in “Copy Data to Selected Channels” and clicking on COPY.

**NOTE B**– Test Identification field at the top of the page is used to identify and select test in the Test Directory. For Clinical tests, use patient name.

- h. Check that test cuvette contains 450 µL PRP and stir bar. Also check for bubbles and be sure to wipe with a KimWipe. Place in the Channel 1 reaction well.
- i. Add 50 µL CHRONO-LUME® Reagent. Dispense directly into the test sample and do not allow reagent to flow down the side of the cuvette. Wipe tip with clean Kim Wipe® before adding.

**Pipetting Reminder For All Volumes:** When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.

- j. Close the chamber door and select GAIN with the SELECT push button.
- k. Using the SET push button, preset the Luminescence GAIN to X0.005 or X 0.01.
- l. Repeat “h” through “k” for additional channels.
- m. **Incubate with CHRONO-LUME® in the test well for 2 minutes** during which time stable luminescence baselines should be achieved at the 100% mark using the numbers on the left side of the graph. After the baselines have stabilized, **Stop Test** and **Restart Current Test**, under **Aggregometer** window (or use icons) to obtain a clean test graph.

**WARNING:** Only use RESTART CURRENT TEST to obtain a clean screen. If used to start a test, the previous test will be erased.

**NOTE:** CHRONO-LUME® is Time and Temperature dependent. To obtain accurate results, be sure to incubate each sample for 2 minute prior to adding reagent.

- n. Draw up 5 µL of ATP Standard into the micropipette. Wipe tip with a clean KimWipe®.
- o. Click start for Channel 1.

**NOTE:** Before each test is started, the word START is visible in each color-coded bar at the top of the Test Grid. **Just before addition of the ATP Standard, Left click on the Color-coded Bar under the appropriate tracing # at the top of the Test Grid.** A color-coded Start Line will appear momentarily at that time point on the Test Grid and calculation of the test data will start for that Channel and will be shown on the right side of the grid. After clicking, the word START will then disappear from the color-coded bar.

- p. Open the test well door on Channel 1 and add 5 µL of ATP Standard. Dispense directly into the test sample and do not allow reagent to flow down the side of the cuvette. Close the test well door.
- q. Observe the luminescence reaction closely. Typically, the reaction peaks and then begins to deteriorate. The aim

is to bring the signal peak to between 60% and 20% using the numbers on the left of the graph. If the peak is less than 60% and before the reaction starts to deteriorate, press the SET push button to increase the Luminescence Gain by one setting. **[Using the SET push button, increase the Luminescence Gain by one step at a time until the tracing peaks between 60% and 20%.]** If the reaction proceeds off scale or goes beyond 20%, do not attempt to decrease the gain to rectify. Instead repeat the test using the appropriate gain setting.

**NOTE A** - Choose to increase the gain at a point when the reaction peak pauses, but before it begins to deteriorate. Increasing the gain too early or too late will result in an improper ATP Standard.

**NOTE B** – If the ATP Standard falls just under 60% (~61,62, 63), it is not absolutely necessary to increase the gain.

- r. Press SELECT push button to return to Main “TEMP, GAIN, SPEED” LCD Screen and to lock in Luminescence Gain setting.
- s. Repeat steps “n” through “r” above for additional channels.
- t. Once all ATP signals start to deteriorate, click on **Aggregometer** and **Stop Test**.

**NOTE:** When test is stopped, the screen will change to the Patient Data View and the ATP Standard will be calculated automatically in percent. In addition, the following Text Box will appear: “Test must be saved to use these results for Luminescence gain.” Save?

YES	NO
-----	----

**Clicking on YES will save the test and automatically plug the ATP Standard calculation into the Luminescence Gain. Select RUN NEXT icon to run additional tests against this gain. To Abandon all ATP standards in this run click on NO and repeat Standard for all Channels. To preserve one (or several) of the ATP standards and rerun in other channels...Click YES to save, then see the instructions after step “y”.**

- u. If necessary to change Start and Stop times, click on EDIT and SET START & STOP TIMES.

**NOTE-** SET START AND STOP TIMES may not be required for Luminescence tracings as the START Line is set automatically at the beginning of the Test Graph (0 seconds) and the STOP Line is set automatically at the end of the test. If adjustments are not needed due to artifacts, proceed to CALCULATE and Click on OK.

- 1) If only calculating Slope and Amplitude:
  - a) A small box will appear with Trace 2 selected. If the Automatic Start Time Feature was used a dotted line will appear on the screen at that point (Start Line) with another dotted line (Stop Line) placed “X” number of minutes after (Test Time set up on procedure page).
  - b) If required, Start & Stop times can be moved:
    - i. **Individually** – Left Click, Hold and Drag to new position. Place Start Line on Stable Baseline before door opening and place Stop Line after the peak.

- ii. Simultaneously – Place cursor near Start Line. Right click, Hold & Drag Start Line to new position. Stop Line will move in tandem.
  - c) Click on traces 4, 6 and 8 and repeat above for each.
- 2) If calculating Slope, Amplitude, Lag Time and Area Under the Curve at the same settings:
  - a) Click on Trace 2
  - b) Click on “Set All” to calculate all parameters with one setting.
  - c) Follow Steps 1) b and c above.
- 3) To calculate Individual Parameters:
  - a) Click on Trace 2
  - b) Click on parameter (i.e. lag time)
  - c) Follow steps 1) b and c above.

**NOTE:** When calculating Lag Time individually, only a start time is displayed.

- v. After setting the Start and Stop Times, Click on DONE, then select CALCULATE RESULTS under the EDIT window. This command will calculate ATP peak as a percentage. Check Duration times to be sure Start & Stop Lines were set correctly. Click on OK and calculations will appear in Data Box.
- w. After the calculations have been completed, SAVE the test, using the SAVE command under FILE or the SAVE icon. Tests can be printed using the PRINT command in the FILE WINDOW. PRINT is for printing one test run per page. Use CASCADE or TILE under WINDOW to view and select tests before printing.
- x. Remove the samples from the reaction well and discard the cuvettes in their entirety.
- y. Keep Luminescence Gain at Final setting for all tests run with that subject’s blood.

**TO PRESERVE VALID ATP STANDARDS IF RERUN NEEDED IN ONE OR MORE CHANNELS:**

- *SAVE the first ATP Standard and CLOSE the test.*
- *Click on RUN NEW Icon and select ATP Standard Test Page*
- *UNCHECK the channels that had a good Standard in the first run [Ex: UNCHECK Channels 2 and 4]*
- *RUN the new ATP Standards in remaining channels [Ex: Channels 1 and 3]*
- *When completed, SAVE the test.*

*Click on RUN NEXT to begin testing with Luminescence. ATP Standard values in all four channels will be carried over.*

## 2. Maximum ATP Release Induced by Thrombin

(Aggregation is not measured with Thrombin at a concentration of 1Unit/mL). It is used to obtain maximum ATP Release. (To measure Aggregation with Thrombin, follow Aggregation testing instructions in Section 3 below and reduce Thrombin concentration to 0.1-0.15 Unit/mL. Sample will aggregate then clot with Thrombin.)

- a. Set-up or select **Test Procedure** under the **Aggregometer** window. Under **Procedure Name**, set up or select Thrombin Page. The Luminescence Gains obtained with the ATP Standard tests will be entered

automatically into the Luminescence Gain for each Channel.

**NOTE** - If testing only one subject at a time in a 4-channel system set up the test page for Thrombin Luminescence Only in one channel and Lumi-Aggregation with agonists in remaining channels and follow the directions for Optical Lumi-Aggregation below for those channels. If testing patient and control, set up test page for Thrombin Luminescence in two of the channels and Collagen Lumi-Aggregation in two channels. With a 2-channel system, run patient in one channel and control in the other

**For Aggregation testing, be sure to place cuvette with 500µL Platelet POOR Plasma [PPP] in appropriate Reference Well(s) for each channel to be used for testing Aggregation. Using front panel switch for each Channel, all Channels can be set to reference its own PPP or to reference the PPP in Channel 1. Each Subject should be tested with their own PPP reference.**

- b. Select number of channels from 1 to 4 and check all parameters. Slope length can be set from 16 to 99 seconds; Chrono-log standard setting is 16 seconds. Click OK.
- c. Now select “Run Same Patient” under **Aggregometer** window. Enter Patient Name and/or ID # in the Test Identification Field at the top of the RUN TEST Screen as tests will be listed in Directory under this information for later extraction. Click OK to start the test.

**NOTE** - *Test Identification field at the top of the page is used to identify and select test in the Test Directory. For Clinical tests, use patient name.*

**NOTE:** *If needed, patient data from Trace 1 can be copied to other tracings. Click on appropriate Trace no. and click on COPY.*

- d. Place one of the prewarmed test cuvettes with 450 µL PRP in each reaction well of the Aggregometer that is being utilized. Be sure to check for bubbles and to wipe the cuvette with a clean KimWipe®.
- e. Add 50 µL CHRONO-LUME® Reagent. Wipe tip with a clean Kim Wipe® before adding. Dispense directly into the test sample and do not allow reagent to flow down the side of the cuvette.

**Pipetting Reminder For All Volumes:** *When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.*

- f. Close the chamber door.
- g. **Incubate with CHRONO-LUME® in the test well for 2 minutes** during which time a stable baseline of luminescence should be achieved at the 100% mark (using the numbers on the left side of the graph).

**NOTE:** *CHRONO-LUME® is Time and Temperature dependent. To obtain accurate results, be sure to incubate each sample for 2 minutes prior to adding reagent.*

- h. After the 2 minute incubation and baselines have stabilized, select **Stop Test** and **Restart Current Test** under **Aggregometer** window. This will eliminate any artifacts from the test.

**WARNING:** Only use **RESTART CURRENT TEST** to obtain a clean screen. If used to start a test, the previous test will be erased.

- i. Draw up 50  $\mu$ L of Thrombin reagent into the pipette tip. Be sure to wipe the tip with a clean KimWipe®.
- j. Click **START** for the appropriate channel.

**NOTE:** Before a test is started, the word **START** is visible in each color-coded bar at the top of the Test Grid. **Just before addition of the reagent, Left click on the Color-coded Bar under the appropriate tracing # at the top of the Test Grid.** A color-coded Start Line will appear momentarily at that time point on the Test Grid and calculation of the test data will start for that Channel and will be shown on the right side of the grid. After clicking, the word **START** will then disappear from the color-coded bar.

- k. Open the chamber door, add the Thrombin and close the chamber door. Be sure to wipe the pipette tip and be sure the pipette tip is down into the sample. Dispense directly into the test sample and do not allow reagent to flow down the side of the cuvette.
- l. Repeat steps "i" through "k" for the other channels (if running Thrombin in multiple Channels) or follow Aggregation instructions below for other agonists.
- m. While tests are running, gently mix PRP by end over end inversion 3-4 times. Prepare one cuvette for each channel for incubation by adding 450  $\mu$ L PRP (The cuvette and stir bar should already be in the incubation wells.) & begin 3 minute incubation. Dispense the PRP directly into the test cuvette and do not allow it to flow down the side.

**DO NOT** incubate in the test wells. Only incubate in the incubation wells.

- n. If only testing Thrombin, once all Thrombin Luminescence tracings begin to deteriorate, select **Stop Test** under **Aggregometer**. If running **Aggregation & Luminescence** tests in other Channels, wait for all Aggregation signals to complete a minimum 6-minute test run or to reach full amplitude before stopping test. Tests cannot be stopped separately.
- o. If necessary to change Start and Stop times, click on **EDIT** and **SET START & STOP TIMES** (or use Icon). Place Start Line on Stable Baseline before door opening and place Stop Line after the Luminescence peak or place Stop Line on Aggregation tracing at full amplitude.

**NOTE – SET START AND STOP TIMES** may not be required for Luminescence tracings as the **START Line** is set automatically at the beginning of the Test Graph (0 seconds) and the **STOP Line** is set automatically at the end of the test. If adjustments are not needed due to artifacts, click on **save**.

- 1) If only calculating Slope and Amplitude:
  - a) A small box will appear with Trace 2 selected. If the **Automatic Start Time Feature** was used, a dotted line will appear on the screen at that point (Start Line) with another dotted line (Stop Line) placed "X" number of minutes after the Start Time,

based on the Duration entered on the Test Procedure Page, or at the end of a Luminescence tracing if test is stopped before.

- b) If required, Start & Stop times can be moved:

*Place Start Line on Stable Baseline before door opening and place Stop Line after Luminescence peak or on Aggregation tracing at full amplitude.*

1. **Individually** – Left Click, Hold and Drag to new position. Place Start Line on Stable Baseline before door opening and place Stop Line after the peak.
2. **Simultaneously** – Place cursor near Start Line. Right click, Hold & Drag Start Line to new position. Stop Line will move in tandem.
- c) If aggregation and/or luminescence were tested in other channels, click on Traces 3 through 8 and repeat steps "a" and "b" above for each.
- 2) If calculating Slope, Amplitude, Lag Time and Area Under the Curve at the same settings:
  - a) Click on appropriate Trace #
  - b) Click on "Set All" to calculate all parameters with one setting.
  - c) Follow Steps 1) "a" through "c".
- 3) To calculate Individual Parameters:
  - a) Click on Trace 2
  - b) Click on parameter (i.e. lag time)
  - c) Follow steps 1) "a" through "c" above.
- l. After setting the Start and Stop Times, Click on **DONE**, then select **CALCULATE RESULTS** under the **EDIT** window. This command will calculate aggregation in percentage and ATP secretion in nMoles. Check Duration times to be sure Start & Stop Lines were set correctly. Click on **OK** and final calculations will appear in Data Box.
- m. After the calculations have been completed, **SAVE** the test, using the **SAVE** command under **FILE** or use the **SAVE** Icon.
- n. Tests can be printed using the **PRINT** command in the **FILE WINDOW**.
- o. Remove the samples from the reaction well and discard the cuvettes in their entirety.

3. PRP Aggregation and Simultaneous ATP Release with Collagen, ADP and Arachidonic Acid

**NOTE:** Be sure a test cuvette **for each channel** (with stir bar and 450  $\mu$ L PRP) has been pre-warmed in incubation well for 3 minutes before starting tests. Dispense the PRP directly into the test cuvette and do not allow it to flow down the inside wall of the cuvette.

**NOTE:** Be sure Aggregation **MODE** is set to **OPTICAL**. Use **MODE** push button to change setting between **OPTICAL** and **IMPEDANCE**. **MODE** push button is disabled until Aggregometer reaches temperature setting and when instrument is in the **SELECT** mode.

- a. Set up or Select Test Procedure under the **Aggregometer** window. [Set-up or load the procedure that corresponds to the reagents and Optical/Lumi methods being used.] Select number of channels from 1 to 4 and check all parameters. The Luminescence Gain value from the ATP Standard will be entered automatically. Slope length can be set from 16 to 99 seconds; Chrono-log standard setting is 16 seconds. Click OK.
- b. Select RUN SAME PATIENT under AGGREGOMETER window or use ICON at top of screen. The information in the Test Identification Field and the Patient Data Fields from the previous tests will be transferred automatically to the new test.

**NOTE** – If needed, patient data from one trace can be copied to other tracings. Click on appropriate Trace No. and Click on COPY.

**NOTE** - Test Identification field at the top of the page is used to identify and select test in the Test Directory. For Clinical tests, use patient name.

- c. Click on OK to start the test.

When test screen appears, but sure the Graph Range (time noted at bottom of test graph) is set for a minimum of six (6) minutes. The Graph Range time can be adjusted under VIEW and then Set Graph Range.

The software will add additional time, if needed, during testing.

- d. For each channel, take preincubated PRP test cuvettes from incubation wells and place in the PRP wells. Wipe the cuvette with a clean KimWipe® and check cuvette for bubbles. For Aggregation testing, be sure to place cuvette with 500uL Platelet POOR Plasma [PPP] in appropriate Reference Well(s) for each channel to be used for testing Aggregation. Using front panel switch for each Channel, all Channels can be set to reference its own PPP or to reference the PPP in Channel 1. Each subject should be tested with his or her own PPP reference.
- e. Wipe tip with a clean KimWipe® before adding 50 µL CHRONO-LUME® Reagent to each test sample. Dispense directly into the test sample and do not allow reagent to flow down the inside wall of the cuvette.

**Pipetting Reminder For All Volumes:** When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.

- f. Close the chamber door.
- g. Repeat steps “e” and “f” for each channel.
- h. Press and hold the **SET BASELINES** pushbutton for each test channel. Each channel is now calibrated for 0 to 100 % light transmission

**NOTE:** Be sure tracing moves to 100% when the button is depressed & returns to 0% when released, using the numbers on the left side of the graph.

- i. **Incubate with CHRONO-LUME® in the test wells for 2 minutes** during which time stable baselines for aggregation and luminescence should be achieved at the 0% and 100% marks respectively.
- j. After the sample in Channel 1 has incubated for 2 minutes and the stable Optical and Luminescence baselines have been established on all Channels, click Stop Test and **Restart Current Test** under Aggregometer window. This will provide a clean test page. **DO NOT** click on **Run Test**, as it will be necessary to reenter patient data.

**WARNING:** Only use **RESTART CURRENT TEST** to obtain a clean screen. If used to start a test, the previous test will be erased.

- k. Press and hold the SET BASELINES pushbutton for each test channel. Each channel is now calibrated for 0 to 100% light transmission.

**NOTE:** Be sure tracing moves to 100% when the button is depressed & returns to 0% when released, using the numbers on the left side of the graph.

**NOTE:** Before a test is started, the word **START** is visible in each color-coded bar at the top of the Test Grid. **Just before addition of the reagent, Left click on the Color-coded Bar under the appropriate tracing # at the top of the Test Grid.** A color-coded Start Line will appear momentarily at that time point on the Test Grid and calculation of the test data will start for both tracings for that Channel and will be shown on the right side of the grid. After clicking, the word **START** will then disappear from the color-coded bar.

- l. Once the aggregation tracings return to 0%, click on start for channel 1, then immediately add the appropriate aggregation reagent. Be sure to wipe the outside of the pipette tip before adding reagent. Dispense directly into the test sample and do not allow reagent to flow down the side of the cuvette. Repeat for the remaining channels.
  - 1) Use **1 µL** of Collagen for **2 µg/mL** final concentration. [2.5 µL for 5µg/mL final concentration].
  - 2) Use **5 µL** of ADP for a final concentration of **10 µM**. [2.5 µL for 5 µM final concentration]
  - 3) Use **5 µL** of Arachidonic Acid for a final concentration of **0.5 mM**.
  - 4) Use 2.5 µL of Epinephrine for a final concentration of **5 µM**.

**NOTE** on Epinephrine: This reagent can be tested without luminescence due to the distinctive bi-phasic pattern.

**NOTE:** Wipe pipette tip with clean KimWipe® before adding reagent. Also, be sure tip is immersed in sample when reagent is added.

- m. While tests are running, gently mix PRP by end over end inversion 3-4 times. Prepare one cuvette for each channel for incubation by adding 450µL PRP. (The cuvette and stir bar should already be incubating) & begin 3 minute incubation. Dispense the PRP directly into the test cuvette and do not allow it to flow down the side.

**DO NOT** incubate in the test wells. Only incubate in the incubation wells.

- n. After all aggregation tracings have run for a minimum of five (5) minutes after the addition of the reagent or have reached full amplitude, click on **Stop Test** under **Aggregometer**.
- o. Click on EDIT and SET START & STOP TIMES *[If color-coded bar was clicked and there are no artifacts or baseline shifts, this step can be eliminated. Go directly to CALCULATE under EDIT or to the CALCULATE icon]*

1) If only calculating Slope and Amplitude:

- a) A small box will appear with Trace 1 selected. If the Automatic Start Time Feature was used, a dotted line will appear on the screen at that point (Start Line) with another dotted line (Stop Line) placed "X" number of minutes after (Test Duration set up on Test Procedure Page).
- b) If required, Start & Stop times can be moved:
  - i. Individually – Left Click, Hold and Drag to new position. (Be sure Start Line for aggregation and for Luminescence is placed on the stable baseline just before opening the door or just after adding the reagent. Stop Line for Aggregation should be placed five (5) minutes after the Start Time and for Luminescence after the peak.)
  - ii. Simultaneously – Place cursor near Start Line. Right click, Hold & Drag Start Line to new position. Stop Line will move in tandem.
- c) Click on Traces 2 through 8 and repeat step b and c above.

1) If calculating Slope, Amplitude, Lag Time and Area Under the Curve at the same settings:

- a) Click on Trace 1
- b) Click on "Set All" to calculate all parameters with one setting.
- c) Follow Steps 1) b through d above.

2) To calculate Individual Parameters:

- a) Click on Trace 1
- b) Click on parameter (i.e. lag time)
- c) Follow steps 1) b through d above.

**EXCEPTION:** A Luminescence baseline shift may occur with **ADP** and **Arachidonic Acid**. Set Luminescence Start Line after the shift.

- p. After setting the Start and Stop Times, Click on DONE, then select CALCULATE RESULTS under the EDIT window. This command will calculate aggregation in percentage and ATP Release in nmoles. Check Duration times to be sure Start & Stop Lines were set correctly. Click on OK and calculations will appear in Data Box.
- q. After the calculations have been completed, SAVE the test, using the SAVE command under FILE or the SAVE icon. Tests can be printed using the PRINT command in the FILE WINDOW. PRINT is for printing one test run per page. Use CASCADE or TILE under WINDOW to view tests before printing.

- r. Remove the samples from the reaction well and discard cuvettes in their entirety. Leave the PPP in the PPP wells, if continuing testing the same subject.
- s. To run additional agonists with the same patient sample, click Run Next, then repeat steps "a" through "r".

#### 4. PRP Aggregation with Ristocetin

Use the procedures in step 3 (skip steps e-g & i), with the following changes (DO NOT add CHRONO-LUME®):

- a. Prewarm two (2) cuvettes for each subject, each with stir bar and 500µL PRP (gently mix PRP by end over end inversion 3-4 times) and incubate for three (3) minutes.

**DO NOT** incubate in the test wells. Only incubate in the incubation wells.

- b. Add **5 µL** of Ristocetin for a final concentration of **1.25 mg/mL**. Dispense directly into the test sample and do not allow reagent to flow down the side of the cuvette.
- c. Add **2 µL** of Ristocetin for a final concentration of **0.5 mg/mL** to screen for vW Type 2B or Platelet-type vW. Dispense directly into the test sample and do not allow reagent to flow down the side of the cuvette.

**Pipetting Reminder For All Volumes:** When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.

- d. Calculate the results for aggregation only. There is no clinical usefulness to measurement of ATP release induced by Ristocetin

#### CALCULATIONS:

- 1. Amplitude – Optical aggregation results are expressed as a percentage of aggregation at a given time interval from reagent addition; aggregation is defined as the difference between the 0% (PRP) baseline and the 100% (PPP) baseline.
- 2. Slope – Slope is determined by drawing a tangent through the steepest part of the curve. A right triangle is then constructed over an interval of one minute. The height of the triangle is the rate of change of aggregation in one minute, which is defined as the slope. AGGRO/LINK®8 Software uses a 32-point sliding curve to calculate slope when set at a length of 16 seconds. To change the length of a slope line, click on **EDIT**, then **ADJUST SLOPE LINE**. Click on Traces 1 through 8 and use arrows to adjust slope length up to 99 seconds.

#### REPORTING RESULTS:

- 1. Report Hematocrit.
- 2. Report Platelet Count.
- 3. Report Platelet Aggregation Amplitude in Percent
- 4. Report ATP Secretion in nmoles.

**Reference 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 $\pm$ 1 SD) [Guideline only]			
Reagent	Conc.	Agg. (%)	ATP (nmole)
Thrombin	1 Unit	N/A	>0.5
Collagen	2 $\mu$ g/mL	70 - 94	0.74 - 1.92
Arachidonic Acid	0.5 mM	74 – 99**	0.56 - 1.40
ADP	5 $\mu$ M	69 - 88	0.41 - 0.63
	10 $\mu$ M	71 - 88	0.5 – 1.06
Epinephrine	5 $\mu$ m	78 - 88	0.40 - 0.52
Ristocetin	1.25 mg/mL	87-102**	N/A

(\*\*  $\pm$  2 SD)

**Procedures for Abnormal Results:**

1. When Normal Control Test(s) Is Abnormal
  - a. No patient result shall be reported if any reagent does not recover findings within reference range limits when tested with the normal control.
  - b. Repeat both Normal Control and Patient test with new aliquot of frozen reagent or reconstitute new vial of reagent. Be sure each test cuvette contains a stir bar.
2. When Normal Control Test(s) Is Normal:
  - a. Repeat abnormal patient test(s) to be sure result is not due to a technical variable. Be sure each test cuvette contains a stir bar.
  - b. If repeated test(s) continue to be abnormal, report the abnormal result(s) and request a retest on another day to confirm findings.



### Platelet Abnormalities

Platelet aggregation and ATP secretion are clinically significant in the detection and diagnosis of acquired or congenital qualitative platelet defects. The platelet's ability or inability to respond to particular aggregating reagents is the basis for differentiating platelet dysfunctions as shown in the tables below:

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	2 – 5 μg/mL	2 μg/mL	5 μg/mL	N	N	A
		R	N			
Epinephrine	5 – 50 μM	R*		N	R *	A
Ristocetin	0.5–1.5 mg/mL	Qualitative Defect		** A,R,H ***	N	Qualitative Defect

\* Second-wave Inhibited

\*\* Type 2B and Platelet-type von Willebrand increased at low concentration 0.2 -0.6 mg/mL

\*\*\* To distinguish between von Willebrand & Bernard Soulier, add normal plasma or cyoprecipitate to patient sample, vW patient will respond, Bernard Soulier will not.

\*\*\*\* 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. 44% had miscellaneous aggregation abnormalities. The authors concluded that SPD is common, heterogeneous and that not necessarily associated with optical (PRP) aggregation abnormalities.

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,R,N
Arachidonic Acid	0.5 mM	A	N	A,R	A,R,N
Collagen	2 – 5 µg/mL	R	N	A,R	A,R,N
Epinephrine	5 – 50µM	A	N	A,R	A,R,N
Ristocetin	0.5 – 1.5 mg/mL	--	--	--	--
Thrombin	1 Unit	N	N	A,R	A,R,N <sup>2</sup>

\* Higher concentrations of any agonist including Thrombin up to 5 Units will induce additional ATP secretion with a Secretion disorder but will not with a Storage Pool Defect. To test with 4 Units Thrombin, reconstitute a new vial with 0.25mL of Irrigation Grade Saline. Add 50µL to 500µL of samples. Normal Controls will show similar ATP Release values at 1 Unit/mL and 4 Units/mL of Thrombin.

\*\* Glanzmann's thrombasthenia is classified into three types, depending on the level of GPIIb-IIIa present: Type 1 (severe) : <5% of normal GPIIb-IIIa levels. Type 2 (less severe): 10-20% of normal GPIIb-IIIa levels. Type 3 (variant): normal levels of GPIIb-IIIa, but functionally inactive.

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

**Reporting Format:****PLATELET AGGREGATION STUDIES**

DATE \_\_\_\_\_

PHYSICIAN \_\_\_\_\_ TIME BLOOD DRAWN: \_\_\_\_\_

INSTITUTION: \_\_\_\_\_ DEPARTMENT: \_\_\_\_\_

PATIENT \_\_\_\_\_ I.D. \_\_\_\_\_ DATE OF BIRTH: \_\_\_\_\_

AGE: \_\_\_\_\_ SEX: (M) (F) HCT \_\_\_\_\_ PLT CT \_\_\_\_\_ BT \_\_\_\_\_

CLINICAL HISTORY: \_\_\_\_\_

**OPTICAL AGGREGATION & SECRETION TEST RESULTS**  
(Dose Response Testing Not Required with Luminescence)

AGONIST	NORMAL RANGES		CONTROL VALUE		PATIENT VALUE		TEST RESULTS (Normal, Reduced, Increased)	
	Aggregation (%)	Secretion (nmoles)	Aggr.	Secr.	Aggr.	Secr.	Aggr.	Secr.
ADP, 5 $\mu$ M								
ADP, 10 $\mu$ M								
ADP, 20 $\mu$ M								
COLLAGEN, 2 $\mu$ g/mL								
COLLAGEN, 5 $\mu$ g/mL								
ARACH. ACID, 0.5 mM								
RISTOCETIN, 1.25 mg/mL								
RISTOCETIN, 0.5 mg/mL								
EPINEPHRINE, 5 $\mu$ M								

INTERPRETATION:

TEST PERFORMED BY: \_\_\_\_\_ INTERPRETED BY: \_\_\_\_\_

## INTERPRETATION:

### 1. Optical Aggregation

- 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.

**With Collagen:** Collagen is useful for checking the platelet's general ability to aggregate. The aspirin effect is reduced aggregation at a 1 µg/mL or 2 µg/mL concentration and normal aggregation at a 5 µg/mL concentration. A lag phase of up to a minute is typically seen with this agonist resulting in ATP release often seen before aggregation.

**With Arachidonic Acid:** Arachidonic Acid is converted to thromboxane A<sub>2</sub> in the presence of cyclooxygenase. Aspirin inhibits the cyclooxygenase pathway, causing a significant reduction in aggregation with this agonist. Responses between no aggregation and the normal range frequently indicate drug ingestion some time during the previous several days. Other defects in thromboxane synthesis will give a similar pattern to cyclooxygenase inhibition. Normal aggregation is seen with concentrations of 0.5 mM to 1.0 mM.

**With ADP:** ADP exposes the fibrinogen-binding site on the membrane glycoprotein GPIIb/IIIa complex. Aggregation testing is typically performed in PRP with concentrations ranging from 1 µM to 10 µM. At the lower concentrations up to 3 µM, a first wave of aggregation will be followed by disaggregation. At the higher concentrations, the first wave of aggregation will blend into the second wave, masking the biphasic wave. It is often necessary to perform dose-response testing with multiple concentrations to obtain a biphasic response (not required with Luminescence). Aspirin effect may be seen with mid-range concentrations such as 5 µM. The anti-platelet drug, Plavix, will inhibit aggregation at ADP concentrations of 5 µM to 20 µM.

**With Epinephrine:** Shape change is not seen with this agonist. Higher concentrations (≥5 µM) produce a biphasic curve with second-wave aggregation dependent on thromboxane A<sub>2</sub> synthesis. "Epinephrine is the least consistent agonist used in the assessment of platelet aggregation and, if the Epinephrine response is the only abnormality seen in testing, one should be very hesitant to make the diagnosis of a 'disorder' based on this result."

**With Ristocetin:** Used for the detection of von Willebrand Disease (a quantitative or qualitative defect in plasma vWF Factor) and Bernard Soulier (a lack of platelet membrane glycoprotein GPIb). Normal results are seen with concentrations ranging from 0.9 to 1.5 mg/mL. To detect Type 2B or Platelet-Type vW, test for a Hyper-response at low concentrations (0.2 – 0.5 mg/mL). The addition of cryoprecipitate (source of normal VWF) to plasma from patients with VWD Type 2B results in no response to low doses of Ristocetin, whereas patients with pseudo-VWD continue to show enhanced responsiveness." To distinguish between vW and Bernard Soulier, add normal plasma or cryoprecipitate to patient sample. vW patient will respond, Bernard Soulier will not. A Qualitative defect may be seen with subjects taking aspirin and a saw-tooth or aggregation-disaggregation pattern with Glanzmann's Thrombasthenia.

### 2. ATP Release with Luminescence

**Thrombin** - is useful for determining the **maximum** amount of ATP secreted from the dense granules. Secretion is independent of thromboxane synthesis. If ATP Release is <0.5 nmoles, run a second test with a higher amount of Thrombin (up to 5 Units). If ATP Release increases, consider a Secretion Defect. If ATP Release does not increase, consider Storage Pool Disease.

**Collagen** - is useful for checking the general aggregability of the platelets. Aggregation and secretion are partially dependent on thromboxane synthesis. Secretion to collagen of less than half the secretion to thrombin may be indicative of impaired thromboxane synthesis. Aspirin, and similar drugs, inhibit the production of thromboxane A<sub>2</sub> which, in turn, inhibits aggregation when tested with low concentrations of 0.5 to 2.0 µg/mL and ATP secretion when tested with concentrations up to and including 5 µg/mL.

**Arachidonic Acid** - is most useful in detecting Aspirin-like defects. Aggregation and secretion to Arachidonic acid is completely dependent on thromboxane synthesis. No aggregation and No secretion to Arachidonic acid confirms impaired thromboxane synthesis. Aggregation but No secretion may be indicative of storage pool deficiency or secretion defect.

**ADP** – exposes the fibrinogen-binding site on the membrane glycoprotein GPIIb/IIIa. Typically used in optical aggregometry to detect second-wave aggregation. Normal ATP release is relatively low (0.3 +/- 0.2 with 5 µM) and is <0.1 with aspirin, Thrombasthenia or Storage Pool defects.

**Ristocetin** - There are no known clinical reasons for measuring ATP Release with this agonist.

#### PROCEDURE NOTES:

1. Since each reagent requires only micro-volumes of reagent, it is essential that introduction of excess reagent be avoided. Always wipe the outside of the micropipette tip.
2. It is important that the pipette tip be pushed to the bottom of the cuvette before the reagent is injected into the sample. **DO NOT** introduce the reagent above the sample in the cuvette since the reagent will cling to the side of the cuvette and will not mix with the sample. Dispense directly into the test sample and do not allow reagent to flow down the side of the cuvette.
3. When ADP or Arachidonic Acid is added to a sample containing CHRONO-LUME®, a shift in the Luminescence Baseline may occur. Be sure Start Line is placed after this shift.

#### LIMITATIONS OF PROCEDURES:

- 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.
- Further Clinical and Laboratory evaluation may be required to confirm diagnosis.
- Red Blood Cells in PRP can inhibit the ability of the Aggregometer to detect changes in light intensity. This may cause the appearance of a decrease in platelet aggregation.

- Hemolysis results in release of nucleotides from the red cells which may cause activation or desensitization of platelets, especially to ADP.
- Lipids in PRP can interfere with light transmission readings & prevent recording of aggregation.
- Icteric samples containing bilirubin of greater than 34.2 umol/L decrease aggregation by ~12.5%.
- Platelet counts below 50,000/ $\mu$ L may cause problems with the setting of optical baseline, preventing the recording of aggregation

## vW CoFactor Assay

### 1. PRINCIPLE:

This procedure is for running the Ristocetin Cofactor Assay using the CHRONO-LOG® vW CoFactor Software Version 3, CHRONO-LOG® Ristocetin Cofactor Assay Kit, and a CHRONO-LOG® Aggregometer with optical capability.

The CHRONO-LOG® Ristocetin CoFactor Assay is used in the quantitative determination of Factor VIII Ristocetin CoFactor activity in citrated plasma.

Ristocetin CoFactor Activity in plasma may be determined by the agglutination of a standardized suspension of platelets in the presence of von Willebrand factor using the antibiotic, Ristocetin. The Ristocetin CoFactor Assay is the in-vitro activity of the von Willebrand Factor which is responsible for the agglutination of platelets in the presence of Ristocetin. Although the platelets play a passive role in such agglutination there is an absolute requirement that the Ristocetin-dependent receptor be intact. von Willebrand disease is associated with a decrease in von Willebrand Factor or Ristocetin CoFactor activity and it is generally accepted that the Ristocetin CoFactor activity is the most useful in-vitro assay for the diagnosis of von Willebrand disease. Levels of Ristocetin CoFactor activity are determined by the ability of the test plasma and Ristocetin to induce aggregation in a standardized platelet suspension.

Following reconstitution, the lyophilized platelets are treated with Ristocetin in the presence of dilutions of a normal standardized human plasma with known amounts of Ristocetin CoFactor activity. A standard curve is prepared, a patient plasma is then used as a source of Ristocetin CoFactor activity in the presence of Ristocetin and reconstituted platelets from which an aggregation pattern is determined and the Ristocetin CoFactor activity interpolated from the standard curve.

### 2. SPECIMEN:

- Patient Preparation: No special Requirements
- Specimen Preparation:
  - Anticoagulant: Buffered sodium citrate, 3.2%.
  - Specimen Collection:
    - Obtain venous blood by clean venipuncture.
    - Immediately mix 9 parts blood with 1 part anticoagulant, mix well by inversion of tube against the stopper.
    - Centrifuge the specimen at 1500 – 2400 g's for 15 minutes.

- Remove plasma from the tube within 60 min. using a plastic pipette and store in a plastic tube.
- Type: One (1) Blue Top Tube is sufficient.
- Handling Conditions:** Test plasma sample within 2 hrs. Otherwise, store frozen at -20° C for up to 2 weeks or at -70° C for up to 6 months and thaw just prior to use.

### 3. EQUIPMENT AND MATERIALS:

- Equipment: CHRONO-LOG® Aggregometer with two or four channels of Optical capability with CHRONO-LOG® vW CoFactor Software and Computer
- Materials:
  - P/N 312 Cuvettes
  - P/N 311 Disposable, Siliconized Stir Bars **or**
  - P/N 313 teflon coated Stir Bars
  - Precision Pipettors: 1 mL, 500  $\mu$ L, 400  $\mu$ L and 50  $\mu$ L – calibrated to deliver  $\pm$ 3% of the selected volume.
  - Plastic transfer pipettes
  - P/N 299 CHRONO-LOG® vW Cofactor Assay Kit
  - P/N 299-6 Hemostasis Reference Plasma (Normal), 1 mL (Control)
  - P/N 299-7 Hemostasis Reference Plasma (Abnormal), 1 mL (Control)
  - P/N 398 Purified Water 15mL

*Should be pyrogen free (ATP free) for reconstituting reagents. Avoid any sterile water for injection that contains benzyl alcohol because it inhibits platelet function. DO NOT use water from the laboratory Millipore filtration system.*

- Lintless wipes, such as KimWipes® - **Gauze squares are NOT suitable**
- Glass tubes with caps (10x75 mm).
- Plastic conical test tubes and caps (for plasma storage).
- Preparation:
  - Aggregometer
    - Turn the CHRONO-LOG® Optical Aggregometer ON.
    - Allow the unit to heat up for 10-15 minutes or until the heater block stabilizes at 37°C.
    - Check to ensure the stirring speed is set to 1200 rpm.
    - Place Optical Inserts in each channel, numerically marked on the back for each channel, to provide proper fitting for P/N 312 cuvette.
    - Place P/N 311 or 313 Stir bars in P/N 312 Cuvettes.
    - Put cuvettes containing stir bar in the incubation wells to warm up.
  - Reagents
    - Ristocetin (7.5 mg/vial):** Store lyophilized Ristocetin at 2° - 8° C before reconstitution. Reconstitute with 0.75 ml (750  $\mu$ L) distilled water at room temperature for a Stock concentration of 10 mg/mL (1 mg/mL Final concentration). Allow to stand at room temperature (20-25° C) with occasional swirling for 30 minutes for complete dissolution. Perform a visual check to be sure that all particulate matter is well dissolved. Keep on ice and in the dark during use. Also, swirl to mix just before

use. Any unused portion may be stored frozen at -20° C for up to 30 days.

- b) **Lyophilized Human Platelets, 6 mL.** Store lyophilized platelets at 2°- 8° C before reconstitution. Reconstitute with 6mL of Tris Buffered Saline. Allow to stand 30 minutes prior to use. Keep at room temperature during testing. Prior to each use, mix by gentle inversion. Stable for 30 days after reconstitution at 2°- 8° C or may be frozen up to 30 days.
- c) **von Willebrand Reference Plasma, Normal.** Store lyophilized plasma at 2 - 8° C before reconstitution Human plasma standardized for Ristocetin Cofactor Activity, 1.0 ml, dried. Reconstitute with 1.0 ml distilled water. Allow to stand 20 minutes at room temperature with occasional swirling. Make certain all particulate matter is well dissolved. Stable for 4 hours at room temperature after reconstitution or may be stored frozen at -20° C for up to 30 days.

*The von Willebrand Reference Plasma used to create the standard has a range of 100 ± 15%, traceable against the WHO International Standards.*

- d) Tris Buffered Saline (TBS), 12 mL. Store at 2°- 8° C. Used for the reconstitution of lyophilized Platelets and dilution of reference and test plasmas. Do not use after expiration date on label.
  - e) Hemostasis Reference Plasma, Normal, 1 mL. Store at 2 - 8° C before reconstitution. Reconstitute individual vials with 1.0 mL high purity water. Allow to stand at room temperature, with occasional swirling, for 15-20 minutes before use. Be certain all particulate matter is well dissolved. Individual Hemostasis proteins vary in their stability; therefore, it is advisable to use this product within 1 hour of reconstitution.
  - f) Hemostasis Reference Plasma, Abnormal, 1 mL. Store at 2 - 8° C before reconstitution. Reconstitute individual vials with 1.0 mL high purity water. Allow to stand at room temperature, with occasional swirling, for 15-20 minutes before use. Be certain all particulate matter is well dissolved. Individual Hemostasis proteins vary in their stability; therefore, it is advisable to use this product within 1 hour of reconstitution.
- 3) **vW CoFactor Software Setup**
- a) Turn on Aggregometer.
  - b) Turn on Computer and Start the vW CoFactor Software, Version 3.
  - c) Click on **EDIT** then **CONFIGURATION**. Select Model 700.
  - d) Select "Number of Channels" (number of optical channels). Click 'OK' to save these settings.
  - e) Click on the box next to "Retain Patient Data Between Tests" if you would like the entered Patient information to remain after saving or abandoning a test.

#### 4) **Micro-Pipettes with 2-Stop Control Button**

##### a) **Description of 2 Stops**

- i. First Stop – The measuring stroke for aspirating and dispensing the selected volume
- ii. Second Stop – To blow out and liquid remaining in the pipette tip after dispensing.

##### b) **Volume Setting**

When adjusting the volume setting from a higher value to a lower value, turn the knob past the desired volume and then back to the required setting.

##### c) **Filling the Pipette Tip**

- i. Press the control button down to the first stop
  - ii. Immerse the pipette tip vertically into the liquid
  - iii. Aspirate and dispense the liquid three times by using the first stop only.
- NOTE: When pipetting Whole Blood, PRP or Platelets do NOT aspirate/dispense 3 times. Only take up the sample one time.*
- iv. Remove the tip slowly from the liquid. For large volumes, wait approximately 3 seconds before removing the tip from the liquid.
  - v. Wipe off the outside of the tip with a lint-free tissue to remove any excess liquid... Taking precaution that the tissue does not touch the tip opening.

##### d) **Dispensing**

- i. Place the pipette tip into the cuvette, so that the end of the tip is immersed in the sample.
- ii. Slightly angle the pipette so that the tip is angled to touch the front wall of the cuvette.
- iii. Press the control button down to the first stop, then press the control button down to the second stop (blow-out) to empty the pipette tip.
- iv. Hold the control button down at the second stop, while keeping the pipette tip at a slight angle pull the pipette tip out of the sample.
- v. Let the control button go, once the pipette tip is completely outside of the cuvette.

#### 4. **Performance Parameters:**

Control plasmas assayed with a normal amount of CoFactor Activity and with an abnormal amount of CoFactor Activity are tested against the Standard Curve before running patient tests.

## **PROCEDURE**

### **1. QUALITY CONTROL:**

Hemostasis Reference Plasmas (P/N 299-6 Normal and P/N 299-7 Abnormal) are available to function as Controls. When used in the place of test plasma, CoFactor Activity should fall within assayed range. If not, repeat the standard curve and control. If the Control is again out, this is indicative of reagent deterioration or technical error. Contact Technical Services at Chrono-log.

If control values do not fall within the established limits, the source of the error must be identified, corrected and documented before patient results are reported.

## 2. PROCEDURE - STEPWISE:

- a. Standard Curve Preparation (must run for each set of assays). Prepare dilutions of Reference Plasma Normal, for a Standard Curve.

***While this procedure is based on running a three (3)-point standard curve with dilutions of 1:2, 1:4 and 1:8, the vW CoFactor Assay Program, Version 3, provides the capability of running up to six (6) points with additional dilutions of: 1:1, 1:16 and 1:32.***

For a 3-point Standard, Label 3 plastic tubes (12 x 75 mm) as 1:2, 1:4, 1:8.

***Pipetting Reminder For All Volumes:*** When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop. When dispensing place the pipette into the sample and hold at a slight angle, so that the tip is angled to touch the 12 o'clock position of the cuvette, then dispense by using the second stop. While holding the second stop down and keeping the tip angled, remove the pipette tip from the cuvette.

**NOTE:** Hand mix plasma or diluted plasma. DO NOT vortex.

- 1) Add 200 µL TBS to each tube being careful to wipe the outside of the tip each time.
- 2) After hand mixing, add 200 µL Reference Plasma, Normal to tube labeled as 1:2. Mix well by hand.
- 3) Using new pipette tip, Transfer 200 µL from 1:2 tube to 1:4 tube. Mix well by hand.
- 4) Using new pipette tip, Transfer 200 µL from 1:4 tube to 1:8 tube. Mix well by hand.

**NOTE - Be sure to wipe the pipette tip before transfer.**

- b. Preparation of Control and Test Plasmas

Prepare Control and Test Plasma dilutions by adding 100 µL of appropriate Test Plasma to 100 µL of TBS in a plastic tube. Mix well by hand. Be sure to wipe the outside of the pipette tip each time.

- c. Preparation of Blank - The Blank is needed for setting the 100% Baseline

Mix well by hand, 250 µL of Tris Buffered Saline and 250 µL reconstituted Platelets in a P/N 312 cuvette. Insert into Channel 1 PPP (reference) well. Be sure to wipe the cuvette with a clean Kim Wipe® and to check for bubbles before inserting into PPP well.

- d. Set the PPP Select switch to 1.

- e. Start vW Cofactor Assay Program

- f. Running the Standard – A standard curve should be prepared on each day of the assay.

- 1) Select STANDARDS from the Menu bar
- 2) Select Create New Standard

- 3) Set Standard Setup as follows (example for setting up a 3-point standard):
  - a) NUMBER OF STANDARDS 3 (minimum 3)
  - b) NUMBER OF REPLICATIONS IN STANDARD 1
  - c) NUMBER OF UNKNOWN REPLICATIONS 1
  - d) CV LIMIT 15% (Coefficients of variation of 15% or less are acceptable).

**NOTE:** CV Limit is only relevant when testing in duplicate.

- e) TEST RUN TIME 5 MINUTES

**NOTE:** Tests will stop automatically at 5 minutes. Tests may be stopped manually when maximum slope is obtained.

- f) INCUBATION TIME 120 SECONDS
- g) PLATELET LOT # enter number on bottle
- h) STANDARD PLASMA LOT # enter number on bottle
- i) RISTOCETIN LOT # enter number on bottle
- j) 100% ACTIVITY = 1:2 DILUTION

*The von Willebrand Reference Plasma used to create the standard has a range of 100% ± 15%, traceable against the WHO International Standards.*

- k) STANDARD DILUTION

1. 1:2
2. 1:4
3. 1:8
4. N/A
5. N/A
6. N/A

- 4) After Standard Setup is completed, select **SAVE AS CURRENT**.
- 5) Select **AGGREGOMETER** from the Menu bar
- 6) Select **RUN STANDARD**: An instruction box for each channel will be displayed on screen.
  - a) For testing with one (1) Channel - Channel 1 instruction box will be set up automatically for running CONCENTRATION 1:2. **NOTE: Once test is completed and saved, Channel 1 instruction box will be set up automatically for running CONCENTRATION 1:4 and so on until all standard points are accepted.**
  - b) For testing with two (2) Channels - Channel 1 instruction box will be set up automatically for running CONCENTRATION 1:2 and Channel 2 instruction box will be set up automatically for running CONCENTRATION 1:4. **NOTE: Once tests are completed and saved, Channel 1 instruction box will be set up automatically for running CONCENTRATION 1:8.**
  - c) For testing with four (4) Channels - Channel 1 instruction box will be set up automatically for running CONCENTRATION 1:2, Channel 2 instruction box will be set up automatically for running CONCENTRATION 1:4, Channel 3 Instruction Box will be set up automatically for running CONCENTRATION 1:8. **NOTE: If running a 4<sup>th</sup> point, Channel 4 instruction box would be set up automatically for running CONCENTRATION 1:16.**

- 7) Prewarm cuvettes (P/N 312) with Stir Bar (P/N 311 or 313) in incubation wells.
- 8) For each test channel (1,2,3 or 4), take one prewarmed cuvette with stir bar and pipette 400  $\mu$ L of reconstituted platelets in each. Incubate for a minimum of 4 minutes in the **incubation wells**.
- 9) After incubation, swirl to mix, and take up 50  $\mu$ L Ristocetin, wipe tip with KimWipe® and add into the prewarmed cuvette with platelets and stir bar. Be sure to wipe test cuvette with KimWipe®, check for bubbles and insert in the PRP well of Channel 1.  
**Click the mouse over the OK.** The Incubation timer for Channel 1 will start running.

**NOTE:** Each Instruction Box will say "Insert Sample with Stir Bar for Incubation and Click 'OK'". Sample includes 400  $\mu$ L Platelets + 50  $\mu$ L Ristocetin + Stir Bar.

**Pipetting Reminder For All Volumes:** When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.

- 10) If using more than one channel, while first sample is incubating, add 50  $\mu$ L Ristocetin into a second prewarmed cuvette with platelets and stir bar. After wiping, check for bubbles and insert cuvette into the PRP well of Channel 2. Click the mouse over the OK. The incubation timer for Channel 2 will start running.
- 11) Repeat above for Channels 3 and 4, if appropriate.
- 12) After the Incubation period is completed in Channel 1, the next instruction reads: "SET BASELINE, ADD PLASMA, CLICK OK" – **When these instructions appear, the blue tracing for Channel 1 will be activated and will start to run across the screen. If more time is needed to start the test, the tracing can be returned to the beginning of the test graph by clicking on the RESET button for Channel 1.**
- 13) **Press the Set Baseline button** on Channel 1 of the Aggregometer

**NOTE:** Be sure tracing moves to 100% when the button is depressed and returns to 0% when released.

- 14) **Mix** well the (1:2) Reference plasma tube...then draw 50  $\mu$ L into the pipette tip.
- 15) **Add Plasma** - Wipe the pipette tip with a clean Kim Wipe before adding plasma to test cuvette. **NOTE: Be sure the pipette tip goes into the sample.**
- 16) **Click on OK** - Click the mouse on OK immediately after adding plasma. **[NOTE: If this step is overlooked, the software will not be able to calculate the slope and the test will need to be repeated.]**
- 17) If you are using 2 or more channels, **Channel 2** Incubation period should be completed or near completion. Follow Steps 13 thru 16 above using the (1:4) Reference plasma tube.
- 18) If you are using a 4-channel Aggregometer, **Channel 3** Incubation period should be completed or near

completion. Follow Step 13 thru 16 above using the (1:8) Reference plasma tube.

- 19) If you are using a 4-channel Aggregometer, and are running a **4-point** standard, **Channel 4** Incubation period should be completed or near completion. Follow Steps 13 thru 16 above using the (1:16) Reference plasma tube.
- 20) **While the test is running, prepare the next test cuvette for that channel by adding 400  $\mu$ L platelets to a prewarmed cuvette with stir bar and place in incubation well.**
- 21) After the test in Channel 1 has run for 5 minutes, the program will calculate the maximum slope of the curve and display it in the Channel 1 Instruction box as MAX SLOPE = XXX. To remove an artifact from calculation, Click on the "Recalc" button, then click the mouse on the color-coded Start Line, which appears on the screen...hold and drag the line to your preferred location on the trace. Then click on the "Recalc" button to recalculate the Slope. This process can be repeated. Once satisfied with the placement of the Start Line and Calculation, Click on the OK button to get to the next message.

**NOTE:** Tests will stop automatically at 5 minutes. Tests may be stopped manually when maximum slope is obtained.

- 22) The next instruction is PRESS OK TO SAVE OR PRESS ABANDON MAX SLOPE = XXX. Click the mouse over the OK to SAVE. ABANDON is only used when there is an error and the test needs to be repeated. If not sure, Click on OK to SAVE. It will be possible to rerun the test after reviewing the Standard Curve.
- 23) Remove the sample from Channel #1 and discard. If using P/N 313 Teflon-coated Stir Bar, retrieve from sample and wash thoroughly. P/N 311 disposable stir bar can be discarded with the test sample.

**NOTE:** If using a single channel Aggregometer, once you've saved the 1:2 test, the software will set up automatically for running the 1:4 test, followed by the 1:8 test. Follow steps 13 thru 16 above.

- 24) After the test in Channel 2 has run for 5 minutes, the program will calculate the maximum slope of the curve and display it in the Channel 2 Instruction box as MAX SLOPE = XXX. To remove an artifact from calculation, Click on the "Recalc" button, then click the mouse on the color-coded Start Line...hold and drag the line to your preferred location on the trace. Then click on the "Recalc" button to recalculate the Slope. This process can be repeated. Click on the OK button to get to the next message.

**NOTE:** Tests will stop automatically at 5 minutes. Tests may be stopped manually when maximum slope is obtained.

- 25) The next instruction is PRESS OK TO SAVE OR PRESS ABANDON MAX SLOPE = XXX. Click the mouse over the OK to SAVE. ABANDON is only used when there is an error and the test needs to be repeated. If not

- sure, click on OK to SAVE. It will be possible to rerun the test after reviewing the Standard Curve.
- 26) Remove the sample from Channel #2 and discard. If using P/N 313 Stir Bar, retrieve from sample and wash thoroughly. P/N 311 disposable stir bar can be discarded with the test sample.

**NOTE:** If using a two-channel Aggregometer, once you've saved the 1:2 and 1:4 tests, the software will set up automatically for running the 1:8 test. Follow steps 13 thru 16 above.

- 27) After the test in Channel 3 has run for 5 minutes, the program will calculate the maximum slope of the curve and display it in the Channel 3 Instruction box as MAX SLOPE = XXX. To remove an artifact from calculation, Click on the "Recalc" button, then click the mouse on the color-coded Start Line...hold and drag the line to your preferred location on the trace. Then click on the "Recalc" button to recalculate the Slope. This process can be repeated. Click on the OK button to get to the next message.

**NOTE:** Tests will stop automatically at 5 minutes. Tests may be stopped manually when maximum slope is obtained.

- 28) The Next Instruction is PRESS OK TO SAVE OR PRESS ABANDON MAX SLOPE = XXX. Click the mouse over the OK. ABANDON is only used when there is an error in the test run and the test needs to be repeated.
- 29) Remove the samples from Channel 3 and discard. If using P/N 313 Stir Bar, retrieve from sample and wash thoroughly. P/N 311 disposable stir bar can be discarded with the test sample.
- 30) Once all standard points are completed and calculated, click the mouse on "M" on the tool bar. This will bring the program back to the main menu. Select STANDARDS and STANDARDS LIST to display the Standard List dialog box. Select VIEW CURRENT to check the standard curve. You can use the Coefficient of Determination ( $r^2$ ) as a measure of how close the points are to the best fit line. The closer the Coefficient of Determination is to "1", the closer the points. A CD of  $\geq .95$  is acceptable to use for running tests.
- After viewing standard, click the mouse on "M" on the toolbar.
  - If the standard is acceptable, go to "g". **RUNNING TESTS** below and continue with running control or patient tests.
  - If the standard needs to be modified, click on **STANDARDS** and **STANDARD LIST**. Click the mouse over the standard listed as "Current". Then click on **MODIFY SELECTED**. The standard will appear on the screen with options to RERUN or DELETE selected dilutions.
  - If DELETE is selected, a minimum of three (3) dilutions must remain after deletion. **DO NOT** select DELETE for a three (3) dilution standard. Select RERUN for the appropriate dilution.

- Click on AGGREGOMETER and select RUN STANDARD. Software will be set automatically for the selected dilution(s).
- Repeat the assay for the dilution to be rerun. Be sure to use the proper Plasma Dilution selected for modification.

g. **Running Tests (Control or Patient) – Can be performed after viewing an acceptable standard.**

- To **Run Tests**, select **AGGREGOMETER** from the Menu bar.
- Select **Run Test**. One instruction box for each channel will be displayed on screen, below the graph. Next to the graph is an information box that can be completed before running the test or while the test is running. However, before the test is started, check that the dilution box is set properly for the plasma dilution being tested, typically 1:2.
- For each channel, take a prewarmed cuvette with stir bar and pipette 400  $\mu$ L of reconstituted platelets in each. Incubate for a minimum of 4 minutes in the incubation well.
- After incubation, swirl to mix and add 50  $\mu$ L Ristocetin into the cuvette. Check cuvette for bubbles, tap out, wipe with KimWipe® and insert it in the PRP well of Channel 1. Click the mouse over the OK for that channel. The Incubation timer will start running. During the 2 minute incubation, mix the appropriate test plasma dilution well.

**Pipetting Reminder For All Volumes:** When filling the pipette tip, pre-rinse the tip by aspirating and dispensing the liquid 3 times using the first stop only. When dispensing, place the pipette tip down into the sample and hold at a slight angle touching the wall of the cuvette with the end of the tip. Press the control button to the second stop to dispense. Keep the control button depressed and the pipette tip angled against the cuvette, while removing the pipette tip from the sample.

- Repeat Step "4" for each channel.
  - After the Incubation period is completed in Channel 1, the next instruction will read:
- 1) SET BASELINE 2) ADD PLASMA 3) PRESS OK.**
- Press the Set Baseline button for Channel 1

**NOTE:** Be sure tracing moves to 100% when the button is depressed and returns to 0% when released.

- Mix well by hand the (1:2) Patient Test or Control Reference plasma tube...then draw 50  $\mu$ L into the pipette.
- Wipe the pipette tip with a clean Kim Wipe and add plasma to test cuvette. Be sure the pipette tip goes into the sample.
- Click the mouse on OK **[NOTE: If this step is overlooked, the software will not be able to calculate the slope and the test will need to be repeated.]** If OK is clicked late, allow the test to finish then select RECALC and move the START like back to the appropriate spot.

The RUNNING TEST message will be in the Instruction box. The test will run for 5 minutes before calculating slope and activity.



- 11) Repeat steps "6" thru "10" above for each channel. Be sure to use appropriate test plasma for each channel.
- 12) After the test(s) has run for 5 minutes, the program will calculate the maximum slope of the curve and display it in the Channel 1 Instruction box as MAX SLOPE = XXX. To remove an artifact from calculation, Click on the "Recalc" button, then click the mouse on the color-coded Start Line...hold and drag the line to your preferred location on the trace. Then click on the "Recalc" button to recalculate the Slope. This process can be repeated. Click on the OK button to get to the next message.

**NOTE:** Tests will stop automatically at 5 minutes. Tests may be stopped manually when maximum slope is obtained.

- 13) The next instruction is PRESS OK TO SAVE OR PRESS ABANDON. The Slope and vW factor activity is displayed in the information box for Channel 1. Click the mouse over the OK to save the test. ABANDON is only used when there is an error in the test run and the test needs to be repeated.
- 14) Repeat steps "12" & "13" for each channel.
- 15) The tests are saved automatically and can be viewed using the Open command or printed using the Print command. Both these commands are found under FILE on the menu bar.

#### h. CALCULATIONS:

Ristocetin CoFactor Activity is calculated automatically with the vW CoFactor Assay Program by plotting the test slope against the standard curve.

#### i. REPORTING RESULTS:

"The diagnosis of vWD should be correlated with a positive clinical history" and is "usually associated with a positive family history."

**NOTE:** When the **Slope** of the test plasma is less than the highest dilution (ie 1:8) or greater than the lowest dilution (ie 1:2) (falls outside the points) on the standard curve, further dilutions should be made until a value is obtained that falls within the points on the standard curve.

#### j. Reference Ranges:

When using the CHRONO-LOG® Cofactor Assay Kit, results of less than 40% are considered abnormal and indicative of von Willebrand disease. However, the individual laboratory utilizing its particular instrument/reagent combination should establish a normal range.

The normal reference intervals vary according to blood type. Type O ranges are lower than other types.

Race is another potential modifier as African American women have higher vWF:Ag levels than Caucasian women, even when blood type was considered. vWF:RCo levels were similar between Caucasian and

African American subjects but the RCo:Ag ratios were lower in African Americans.

#### k. Procedures for Abnormal Results:

Abnormal tests should be repeated for confirmation a complete diagnosis of this disorder should include other factors such as family history, bleeding time, Factor VIII-RAg and VIII:C activity.

#### l. Reporting Format:

The results are reported as Percent of Factor Activity as determined by the intersection of the test sample slope on the best fit line of the standard dilutions. The printed report prints the FACTOR ACTIVITY at the bottom of the dialog box and shows the intersection of the test sample slope on the best-fit line of the standard dilutions. To view the results, the report can be seen by using the Open command or the report can be printed using the Print command. Both these commands are found in FILE on the menu bar.

#### m. PROCEDURE NOTES:

- 1) "Assays should be repeated at least once to confirm any perceived abnormality, and also to exclude VWD should normal VWF results be obtained in clinically strong suspected cases. This is particularly important (i) in case of female individuals, due to VWF variations caused by changes in estrogen level, e.g.: in the menstrual cycle and in pregnancy, and (ii) because of ABO Group differences."
- 2) For Microvolume testing, remove sticky backing and attach P/N 365 Spacer to the outside bottom of the Cuvette after incubation. Reduce test material volumes to 200 µL Platelets + 25 µL Ristocetin + 25 µL Plasma.
- 3) The following technical tips have been developed in various laboratories to improve accuracy:
  - a) Be sure optical circuits are calibrated properly.
  - b) Mix test and control plasmas thoroughly just before use.
  - c) Be sure to use clean, dry KimWipe® to wipe cuvette before placing in test well.
  - d) Use clean tips for each serial dilution & be sure to wipe tips before dispensing.
  - e) See Pipetting Instructions on page 14 for proper pipetting techniques.
  - f) If Aggregometer requires two (2) blanks, prepare both from a batch to avoid platelet count differences.
  - g) If testing for a long period of time, mix blank periodically.

#### n. LIMITATIONS OF THE PROCEDURE:

**The Ristocetin CoFactor Assay is considered by many investigators to be the single most important assay for von Willebrand disease. However, a complete diagnosis and determination of the variant forms of this coagulopathy requires an evaluation of other factors, such as Factor VIII-Rag, VIII-C activity, bleeding time, and family history.**

#### o. POTENTIAL SOURCES OF ERROR:

Potential sources of error include:

1. Incorrect incubation (time, temperature and/or stirring speed)
2. Incorrect preparation and/or handling of the Ristocetin
3. Incorrect preparation and/or handling of the platelets
4. Pipetting error
5. Contaminated Buffer
6. Lipemic Plasma
7. Icteric Plasma

p. **INTERPRETATION OF RESULTS:**

Decreased levels of vWFAg and/or Cofactor Activity in plasma are consistent with the diagnosis of inherited or acquired vWD.

Mild to marked increases in the levels of vWFAg and Cofactor Activity may be observed in a number of conditions, such as:

- Liver Disease
- Uremia
- Vasculitis
- Severe Atherosclerosis
- Acute and Chronic Inflammatory States
- Physical or Emotional Stress
- Estrogen usage

**Type 1 vWD**

- Decreased Cofactor Activity
- Decreased vWFAg
- Normal to decreased VIII:C
- Normal RIPA
- All multimers decreased

**Type 2A**

- Markedly decreased Cofactor Activity
- Decreased vWFAg
- Decreased VIII:C
- Decreased RIPA
- Large and middle multimers decreased.

**Type 2B**

- Decreased Cofactor Activity
- Decreased vWFAg
- Decreased VIII:C
- Increased RIPA
- Large multimers decreased.

**Type 2M**

- Decreased Cofactor Activity
- Decreased vWFAg
- Normal to decreased VIII:C
- Normal RIPA
- Normal multimers; satellite banding may be abnormal

**Type 2N**

- Normal Cofactor Activity
- Normal vWFAg
- Decreased VIII:C
- Normal RIPA
- Normal multimers

**Type 3**

- Markedly decreased to absent Cofactor Activity
- Decreased to absent vWFAg
- Markedly decreased VIII:C
- Absent RIPA
- All multimers absent

## **SAMPLE DISPOSAL**

**Normal precautions, which apply when handling blood, should be taken when disposing of spent samples and used disposable parts. Local laws that apply to the disposal of potential biohazard materials should be observed.**

## **SHUT DOWN PROCEDURE**

**To shut down the system use the following procedure.**

1. First save any open test using the Save command.
2. Exit the AGGRO/LINK® or vW CoFactor Program
3. Turn off the Power Switches of the Computer and the Aggregometer.
4. Place the dust cover over the Aggregometer to keep it clean.

## **MAINTENANCE AND SERVICE**

### **Electrode Assembly**

There is no need to clean the Electrode Assembly with corrosive chemicals. Corrosive chemicals damage the insulation on the Electrode Assembly and make it impossible to set the impedance baseline. Damaged Electrode Assemblies cannot be repaired and must be replaced. A matched set of P/N 369R Electrode Assemblies is required for each channel.

### **Service/Preventive Maintenance**

This Unit does not require Preventive Maintenance; however, yearly calibration is recommended. Calibration and service, if required, should be obtained from the factory or a factory authorized representative.

Outside of the United States and Canada contact your distributor for service. In countries where there is no distributor, contact Chrono-log directly.

### **Cleaning the Aggregometer**

1. Turn off the Aggregometer and any attached peripherals. Disconnect them from the Aggregometer. Unplug the Instrument before cleaning to prevent a shock hazard should any fluid enter the power switch.
2. Use a vacuum cleaner to remove any dust from the slots and vent holes in the Aggregometer. The vent holes are located on the bottom of the Aggregometer.
3. Moisten a soft cleaning cloth with a solution of three parts water and one part liquid detergent.

**NOTE – Do not soak the cloth in the solution; do not let solution drip inside the aggregometer.**

4. Use the moistened cloth to wipe the Aggregometer exterior.

## SELF-CALIBRATION OF OPTICAL CIRCUITS [CANNOT BE PERFORMED UNTIL INSTRUMENT REACHES SET TEMPERATURE]

**Self-Calibration of Optical Circuits** – Available in Instruments Manufactured after November, 1998, or modified to add this feature.

This procedure enables the Optical wells (PRP & PPP) to be calibrated automatically with the push of a button and verifies that the instrument's optical circuits are operating properly. During the performance of this procedure, the light intensity of the LEDs are adjusted and set to the proper calibrated voltage.

Use a stop watch or timer since some of the steps must be timed.

The calibration switch located on the front of the instrument is key activated. This is to prevent unauthorized use or accidental initialization of the procedure. DO NOT LEAVE THE KEY IN THE SWITCH WHEN NOT PERFORMING THE CALIBRATION PROCEDURE. The key should be stored in a safe location accessible only to personnel authorized by laboratory management. If the key is lost, a replacement key may be purchased from the factory. Please note your Key # here \_\_\_\_\_.

Failure to follow this procedure exactly may result in an inaccurate calibration. The Post Calibration Test checks the accuracy of the calibration and enables the operator to have a record of the results. This calibration procedure is not a substitute for the recommended yearly instrument calibration and PM.

### Preparation:

1. Turn on the unit and let it heat up until the heater blocks stabilize at 37°C.
2. For instruments requiring recabling from Impedance to Optical, check to be sure instrument is cabled for Optical mode. For instruments with Optical Small/Large Switches on the front panel, be sure switch is set to "Small" for each channel. If required, place Optical inserts in the Heater Blocks. Inserts for each channel are marked on the back with #1, #2 etc to reflect the proper channel. For Model 700 instruments, be sure the MODE button is set to Optical.
3. **For instruments that have PPP Select switches, be sure that the switches are set to each Channel's PPP sample, not to Channel #1 PPP.**

**NOTE: ADDITIONAL STEPS REQUIRED FOR CHRONO-LOG® INSTRUMENTS THAT HAVE A PPP REFERENCE SWITCH ALLOWING MULTIPLE CHANNELS TO REFERENCE THE PPP IN CHANNEL 1. [See "Additional Post Calibration test for Instruments with PPP Select Switches" on Page 44]**

4. Take three (3) new cuvettes (P/N 312) and pipette 500 µL of water into each. Incubate the cuvettes for a minimum of three (3) minutes in the incubation wells.
5. Make sure that the cuvettes are clean and there are no fingerprints on the bottom half. Different cuvettes will give slightly different outputs, so visually inspect several cuvettes and pick the pair that seems to be the most free from blemishes. The fewer blemishes in the cuvette the more

accurate the calibration.

**NOTE:** Be sure to always wipe cuvettes with Kim Wipe® before placing in test or reference wells.

6. After the three (3) minute incubation, check cuvettes for air bubbles. Eliminate bubbles by tapping the cuvette.

**For Computer** - Turn on computer and Start AGGRO/LINK®8 for Windows® program. Under the AGGREGOMETER window, select or set-up a Test Procedure page for Optical mode. Click on OK, then select RUN NEW PATIENT under AGGREGOMETER and Click on OK. Type, "Optical Calibration Pre", in Test Identification box at top of screen. Click on OK and test will begin running.

### Calibration Procedure:

#### Cuvette Selection

1. **Ensure there are no large imperfections in the cuvettes**
  - a. With a KimWipe wipe one prewarmed cuvette with water and place in the PRP well of Channel 1 and press Set Baseline. Be sure tracings reaches a full 100% of graph before releasing.
  - b. With tracing stable at 0%, slowly turn the cuvette to look for any large spikes [ $> 5\%$ ] in the tracing. If there are large fluctuations, discard the cuvette and repeat with another. If there are no large fluctuations, turn the cuvette so the tracing returns to 0% line and mark the top front center of the cuvette with a dark Alignment Mark.
  - c. Repeat steps a and b above until there are 3 marked cuvettes.
2. **Ensure that two selected cuvettes match**
  - a. Wipe one marked cuvette with a KimWipe and place in the PRP well of Channel 1, with the Alignment Mark facing front and center.
  - b. Press **and hold** the Set Baseline button for Channel 1. The trace will go down to near 100% on the graph. After seeing a few seconds of stability, release the Set Baseline button and the tracing will go to 0% on the graph.
  - c. Wipe the second water cuvette with a KimWipe and, with the Alignment Mark facing front and center, place in the PPP well of Channel 1. The trace will drop to or near 100% on the graph. **Note the position where the tracing stops (Ex: 98%)**
  - d. Keeping the Alignment Mark facing front and center, swap the cuvettes (ie: take the cuvette from the PRP well and place it in the PPP well. Take the cuvette from the PPP well and place it in the PRP well). **Note the position where the tracing stops (Ex: 97%).**
  - e. If the difference between the two numbers ( 98% and 97%), is  $> 4\%$ , repeat Steps a to d above, using one original water cuvette and an additional marked cuvette, until there are two matched cuvettes with a difference of  $\leq 4\%$ . Mark one with an "R" to indicate PRP and one with a "P" to indicate PPP.

#### Pre-Calibration Test

**NOTE:** Throughout the entire procedure, the Water Cuvette used in PRP well should only be used in PRP test wells and the Water Cuvette used in PPP well should only be used in PPP reference wells. Be sure to use the Alignment Mark on front top of each

cuvette to ensure consistent placement of cuvettes into test and reference wells.

1. With AGGRO/LINK®8 for Windows® software, select RUN NEXT. Click on OK. Type "Optical Calibration Pre Test" in Test Identification box at top of screen. Click on OK and test will begin running with a clean screen.
2. Using the two matched cuvettes and keeping the Alignment Marks front and center, place the "R" cuvette in Channel 1 **PRP** well ... leave the Channel 1 **PPP** well empty. Press and hold the Channel 1 Set Baseline Button and check that the tracing moves to 100% on the graph. Hold for a few seconds.
3. Release the Set Baseline Button and check that the tracing moves to 0% on the graph. Allow to run for a few seconds.
4. Keeping the Alignment Mark front and center, place the "P" cuvette in Sample 1 **PPP** well. Check the tracing on the computer screen (or chart recorder). The tracing should move near 100% on the graph. Note the position where the tracing stops (Ex: 97%) and allow to run for a few seconds.
5. Repeat steps 1 & 2 for each channel, being sure to use the appropriate Set Baseline button for each individual channel.
6. If tracings are >4% from 0% or 100% on the graph, proceed with calibration steps below.
7. Remove cuvettes from the test well before starting calibration.
8. Save and Print Test for files.

#### Calibration

**NOTE:** CALIBRATE ALL TEST CHANNELS, EVEN IF ONLY ONE CHANNEL IS OUT OF TOLERANCE. THIS WILL ENSURE THAT ALL LEDs ARE SET TO THE PROPER INTENSITY.

1. With AGGRO/LINK®8 for Windows® software, select RUN NEXT. Click on OK. Type "Optical Calibration" in Test Identification box at top of screen. Click on OK and test will begin running with a clean screen.
2. Place the key in the calibrate switch, turn the key clockwise 90° and keep it in this position.
3. Keeping the Alignment Marks front and center, place the "R" water cuvette in Sample 1 PRP well and the "P" water cuvette in Sample 1 PPP well. Set the timer for one minute but do not start. Press the Set Baseline button for Sample 1, hold for a second then release. Start the timer as it may take up to a minute for the channel to calibrate. During this minute DO NOT touch or move the samples. Moving the samples may cause inaccuracies in the calibration. Wait for the entire minute before proceeding to the next step.

**NOTE:** Disregard traces during calibration.

4. After waiting one minute, and when tracing stabilizes in Channel 1 ... Repeat step 3 for each channel using the "R" cuvette in the corresponding PRP test well and the "P" cuvette in the corresponding PPP test well, being certain to use each channel's individual Set Baseline button.
5. Once step 3 is completed for every channel, turn the calibrate switch counter-clockwise 90° and **remove the key from the switch**. Calibration is now completed.

#### Post Calibration test

1. With or AGGRO/LINK®8 for Windows® software, select RUN NEXT. Click on OK. Type "Optical Calibration Post" in Test

Identification box at top of screen. Click on OK and test will begin running with a clean screen.

2. Place the cuvette of water marked "R" in Channel 1 **PRP** well and leave the Channel 1 PPP well empty. Press and hold the Channel 1 Set Baseline Button for a few seconds and check that the tracing moves to 100% on the graph. Release the Set Baseline Button and check that the tracing moves to 0% on the graph. Allow to run for a few seconds.
3. Place the cuvette of water marked "P" in Sample 1 **PPP** well. Check the tracing on the computer screen (or chart recorder). The tracing should move near 100% on the graph. Allow to run for a few seconds.
4. Repeat steps 2 & 3 for each channel.

**NOTE:** If the tracings are > 4% from 0% or 100% on the graph, this may indicate that Auto-Calibration was not set properly. Recheck the two water cuvettes to be sure they match and then repeat the entire calibration procedure. Be sure to wipe the cuvettes with a KimWipe® each time they are placed into a test or reference well. If after the second attempt the results are > 4%, the system may need Service. Contact Chrono-log Service Department at 1-800-247-6665 for further assistance.

5. Save and print test for files.

**Additional Post Calibration test for Instruments with PPP Select Switches** - This section provides for the ability to check each channel's calibration against the Channel 1 PPP Reference.

1. With AGGRO/LINK®8 for Windows® software, select RUN NEXT. Click on OK. Type "Optical Calibration vs Ch 1" in Test Identification box at top of screen. Click on OK and test will begin running with a clean screen.
2. **Be sure all PPP/Reference Select Switches are set to each Channel's PPP sample, not to Channel #1 PPP.**
3. Place the PRP cuvette of water marked "R" in Channel 1 **PRP** well and leave the Channel 1 PPP well empty. Press and hold the Channel 1 Set Baseline Button for a few seconds and check that the tracing moves to 100% on the graph. Release the Set Baseline Button and check that the tracing moves to 0% on the graph. Allow to run for a few seconds.
4. Place the cuvette of water marked "P" in Channel 1 **PPP** well. Check the tracing on the computer screen (or chart recorder). The tracing should move near 100% on the graph. Note the position where the tracing stops (Ex: 97%). Allow to run for a few seconds.
5. Leave the cuvette of water marked "P" in Channel 1 **PPP** well.
6. Place the PRP cuvette of water marked "R" in Channel 2 **PRP** well. Press and hold the Channel 2 Set Baseline Button for a few seconds and check that the tracing moves to 100% on the graph. Release the Set Baseline Button and check that the tracing moves to 0% on the graph. Allow to run for a few seconds.
7. Set the Channel 2 PRP Reference Switch to the "1" position. Check the Channel 2 tracing on the computer screen (or chart recorder). The tracing should move near 100% on the graph. Note the position where the tracing stops (Ex: 97%). Allow to run for a few seconds.
8. Repeat steps 5 and 6 for each additional channel.

**NOTE:** If the tracings are > 4% from 0% or 100% on the graph, this may indicate that Auto-Calibration was not set properly. Recheck the two water cuvettes to be sure they match and then repeat the entire calibration procedure. Be sure to wipe the cuvettes with a KimWipe® each time they are placed into a test or reference well.

If, after the second attempt, the results are still >4%, the system may need service. Contact Chrono-log Service Department at 1-800-247-6665 for further assistance.

9. After completion, print out the graphs, mark the date and mark the water tests for pre-calibration and post calibration. Store the graph for reference and store the key in a safe location.
10. Save and print test for files.

### Discussion

The clear water samples allow the maximum amount of light through. Each instrument is adjusted at the factory for a set voltage with water samples. The automatic calibration procedure resets the voltage while the water samples are in the wells. The water samples have equal optical density and should allow the same amount of light to pass through the samples. This will result in a tracing at 100% of the test graph. This tracing is used to check that the calibration is set properly.

Sometimes the difference is caused by variations in the cuvettes. If the baseline is not within 4% of the 100% baseline, change the water samples and run the procedure again.

The PRE-calibration, Calibration and POST- Calibration steps described above will catch calibration errors, including conditions where one channel has matching wells that do not match the remaining channels. Calibration with empty wells is one instance that would create this problem. Faults may occur at any time due to spills, broken glass, etc. Daily Quality Control checks can be performed to prevent false readings.

Contact Chrono-log Service Department at 1-800-247-6665 to inquire about instrument service if the tracing is still > 4% after following this procedure.

### Troubleshooting Guide

Many factors will affect the performance and reproducibility of tests run on the Aggregometer. These can be broken down into several categories; Instrument failure or Calibration problems, Sample and Materials problems, and Operator technique related problems. Below is a brief list of common problems.

#### Optical Aggregation

- ☐ Missing stir bar
- ☐ Bubbles in sample blocking optical path
- ☐ Missing rubber spacer, sample volume too low
- ☐ Platelet count- PRP below 50K, PPP above 3K
- ☐ Platelet count- mismatch between PPP samples for each
- ☐ Cuvette dirty, scratched
- ☐ Stirring RPM setting mismatch
- ☐ Stirring motor failure
- ☐ Inconsistent pipetting; force, position, tip too short.

#### Impedance Aggregation

- ☐ Missing stir bar
- ☐ Electrode dirty
- ☐ Electrode platinum alloy wires bent
- ☐ Electrode plug wires broken
- ☐ Electrode coating worn, solder joints exposed
- ☐ Front panel controls improperly adjusted

- ☐ Saline, incorrect type, inhibiting aggregation.
- ☐ Inconsistent pipetting force, position, or tip too short.
- ☐ Incorrect brand of reagent; CHRONO-PAR® reagents are specially formulated for whole blood
- ☐ Stirring RPM setting mismatch
- ☐ Stirring motor failure

#### Luminescence, ATP Release Measurements

- ☐ Baseline offset – ATP contamination in sample or diluent
- ☐ Baseline offset - spill in well or calibration problem
- ☐ Baseline offset - light leak, septum defective (Ca+/PICA)
- ☐ ATP standard or release poor - Improper reagent preparation or incubation time.
- ☐ Unexpected release - sample diluted with water instead of saline.

If these suggestions do not solve the problem, then contact your local distributor to inquire about service.

### AGGRO/LINK®8 FOR WINDOWS

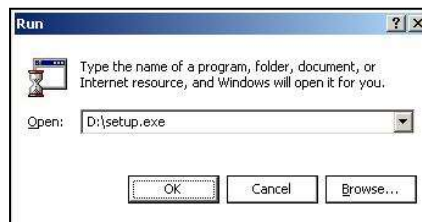
#### INTRODUCTION

The AGGRO/LINK®8 is Windows driven; that is, the operator uses a mouse to select functions from a pull-down menu. The operator should be familiar with using Windows and using the mouse, before running the AGGRO/LINK®8 program. For information on Windows and mouse operation see the Windows Instruction Manual.

The AGGRO/LINK®8 Control Software is a program designed to allow operation of the Aggregometer's Computer Interface with a Windows-based PC running Windows 7 or higher. It provides for aggregation recording, calculation of maximum slope, amplitude of curves, area under the curve, measurement of lag time, plotting and storage. The Software is designed for a Windows-based PC with a CD drive, a hard disk drive, Windows Version 98 or higher and a Serial Port or USB Port for connecting to the Aggregometer.

Where necessary, the program asks for operator responses to obtain the information required to perform the functions. However, in order to facilitate operation, a variety of parameters are assigned initially from the keyboard and stored in a file. This eliminates the need to request these parameters repeatedly during operation.

The AGGRO/LINK®8 program should never be installed to a removable type drive, such as a CD or a removable hard drive. These devices can be easily write protected and may have their drive identification vary each time they are removed and installed causing the program to fail.



This manual describes each command available in the AGGRO/LINK®8 Program. However, since some commands are

only available when a document (test) is Open. The manual will note which commands are available when a document is open with **Available only in Document Menu**. If the command is always available, the statement **Available in the Main Menu and Document Menu** will follow the command.

The data can be printed on a standard color printer. The data may also be copied or transferred to a disk using the 'Move' function.

When using the 'MOVE' function, the files should never be copied or transferred from one directory to another on a single removable floppy drive. Due to the limited amount of space on floppy disks, there may be insufficient disk space to create the required files. The function could fail to complete without moving all of the selected files.

To install the software in the computer, follow the procedures shown on the following page. The software will create a sub-directory named AGGROLINK8 in your systems PROGRAM FILES directory and a sub-directory within AGGROLINK8™ named DATA. The AGGROLINK®8 sub-directory contains the AGGRO/LINK®8 program files and the DATA sub-directory; the DATA sub-directory contains the test data and the test data directory file.

## STARTING AGGRO/LINK®8

Left click the mouse on the Windows START button, move the mouse pointer to 'Programs'. When the programs menu is displayed move to the 'AGGROLINK8' program group and select AGGROLINK8 from its program listing.

The first time the software is started, the following screen is displayed.

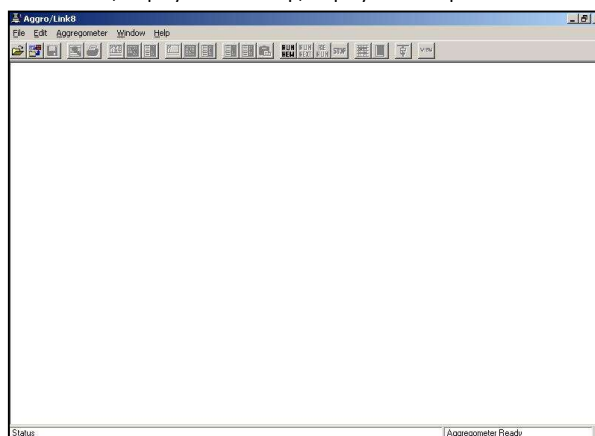


After selecting 2 or 4 channels, click OK, then continue with the directions below.

After starting the AGGRO/LINK®8 software, the Program screen is displayed.

In the menu bar are the titles of each command group. To select a command group, the operator left clicks the mouse over the title. A menu window listing all the options available under that category will be displayed.

There are two possible menu bars that may be displayed. The first menu bar, displayed at start up, displays menu options that are



available when there are no test documents open. The second menu bar displays all of the main menu options, plus additional menu choices for manipulating test data. This second menu is only activated if a test document is open or a test is being run.

A detailed description of each command group follows.

For simplicity, we will describe the menu items as they will appear with an open document, indicating which functions are available only when a document is open and which are available at all times.

## FILE

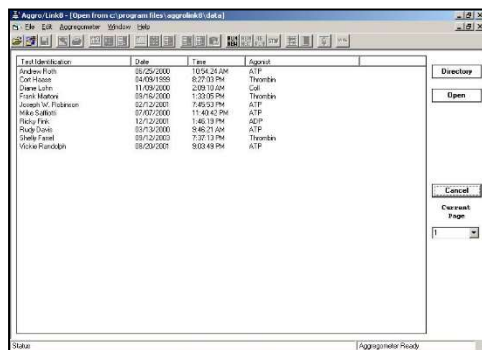
The FILE command group allows the operator to perform basic file functions. When the FILE group is selected from the menu bar, the sub-menu shown below is displayed. To select an option in the FILE Group, left click the mouse over the desired command.

Open	Ctrl+O
Close	Ctrl+C
Save	Ctrl+S
Move	Ctrl+M
Create Merge	
Export	Ctrl+E
Print	Ctrl+P
Print Preview	
Print Setup...	Ctrl+U
Exit	Ctrl+X

OPEN - Open an existing document

**Available in Main Menu and Document Menu.**

This command is used to open an existing test. When this command is selected, a dialog box, will be displayed on the screen.



The test Directory dialog box consists of a test file list, command buttons and page select controls. The test file list will contain the list of tests available to open. Tests are selected to be opened in the following manner:

#### Selecting a single file:

To select a single file to open, left click the mouse over the file name. A highlight bar will appear over the file name selected. Left click the mouse on the 'Open' button or simply double left click the mouse over the file name to open the desired test.

#### Selecting multiple files:

There are two options for selecting multiple files to open. Select groups of files by left clicking the mouse on the first file to be opened. Then while holding down the 'SHIFT' key, left click the mouse on the last file in the group to be opened. Multiple files can also be selected by holding down the 'CONTROL' key and left clicking the mouse on each file to be opened. After selecting the files, left click the mouse on the 'Open' button to open the files.

The maximum number of files that can be open at one time is ten.

To change the directory, left click the mouse over the 'Directory' button. A dialog box will appear.



Type in the name of the new directory or left click the mouse on the 'Browse' button. Left clicking the mouse on the 'Browse' button will display a dialog box.

A highlight bar will appear over the chosen directory. Left click the mouse over the desired directory and then left click the mouse on the 'OK' button to change to this directory.

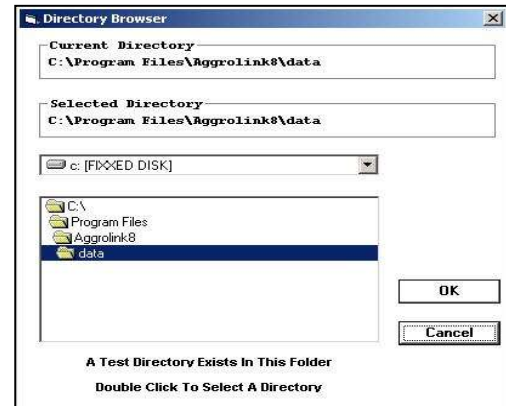
To change the disk drive, left click the mouse over the arrow next to the drives box. A pull-down box with the names of the available drives will be displayed. Left click the mouse over the desired drive to change. The floppy drives must have a diskette before the system will change to that drive.

To cancel the command, click the mouse over the 'Cancel' button. The document box will close.

**CLOSE** – Closes the active document

**Available only in Document Menu.**

This command will close the currently active document. A



message prompt will be displayed about saving the file if the current test has not been saved.

**SAVE** – Saves the currently active document

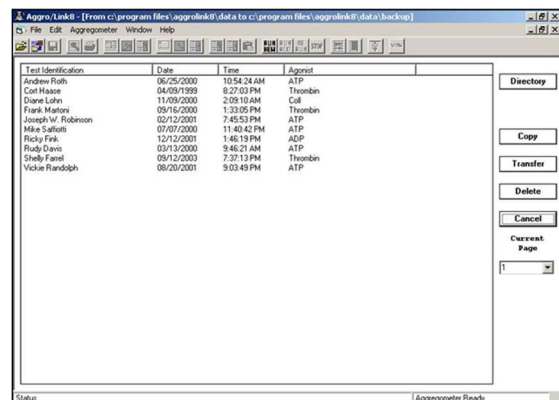
**Available only in Document Menu.**

This command saves the currently active document to our AGGROLINK8 Data Directory with the name given as TEST IDENTIFICATION.

**MOVE** – Copy, transfer or delete selected files

**Available in Main Menu and Document Menu.**

This command is used for file management. When this command is selected a dialog box will be displayed.

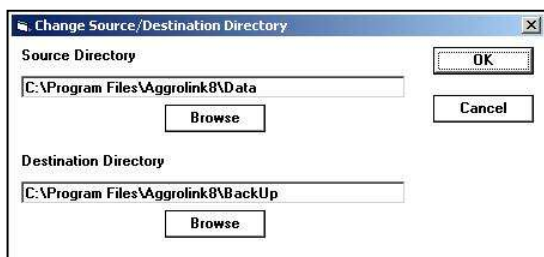


**NOTE:** When using the 'MOVE' function, the files should never be copied or transferred from one directory to another on a single removable floppy drive. Due to the limited amount of space on floppy disks, there may be insufficient disk space to create the required files. The function could fail to complete without moving all of the selected files.

This dialog box allows for copying, transferring or deleting selected files. Files are selected in the same manner as they are in the 'Open' dialog box.

The 'Directory' button operation is similar to the 'Open' dialog box directory button. The difference is that with the 'Move' dialog box there are two directories to select. One directory is used as the 'Source' directory and will contain the files to be maneuvered. The second directory is the 'Destination' directory, and is used to receive files chosen to copy or transfer. A dialog box will be shown when the 'Directory' button is selected from the 'Move' dialog box.

The TRANSFER and DELETE functions are disabled if any previously saved tests are currently open. To TRANSFER or DELETE test files, close all test files currently open for view.



The 'Browse' button operation is identical in functionality to the 'Open' dialog directory/browse function.

To use a 'Move' function, select the files to be manipulated and left click the mouse on the desired function's button.

To cancel the command, left click the mouse over the 'Cancel' button. The document box will close.

The Page Selection (Current Page) box allows the operator to select which page of tests in shown in the file list box. As many as 1,000 tests will be shown at one time in the file list box. To negotiate through the list, select the page to view from the Current Page pull down box.

**CREATE MERGE** – Creates a blank document for pasting curves

**Available in Main Menu and Document Menu.**

This command creates a blank document to be used for the purpose of merging test curves and data from multiple test documents. As many as four samples and their associated tracings can be copied onto any one merge document. Once the curves are copied onto the merge document they can be edited and saved in the same manner as any other AGGRO/LINK®8 document.

**EXPORT - Export test data for use with another program**

**Available only in File Menu.**

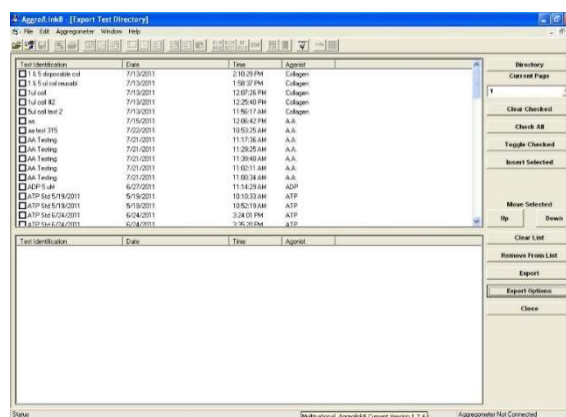
Use this command to export a saved test to ASCII format, ACSII format in MS Word , MS Excel or as a relation data base for use with programs such as MS Access.

To export tests in any given format, select Export from the File menu. The following dialog screen is displayed.

This dialog screen consists of two main windows.

- The top window displays all of the tests currently saved in AGGRO/LINK®8 format.

- The bottom window displays a list of tests that have been



selected for exportation.

To select a test for export, check the box next to the test name and insert the test into the list of tests to be exported. There are command buttons on the right to assist in selecting/deselecting tests for exportation.

### Command Buttons:

The following command buttons are used for selecting/deselecting:

**Directory:** This button displays the default AGGRO/LINK®8 directory box. This will allow you to change the directory where the original AGGRO/LINK tests can be found.

**Current Page:** Allows selection of any page of test results when there are more tests then permitted to be displayed on the test page.

**Clear Checked:** Clears check marks from tests selected to export.

**Check All:** Sets all check marks, selecting all tests currently listed for the page displayed.

**Toggle Checked:** Inserts all check marks.

**Insert Selected:** Inserts the selected tests into the bottom window for exportation. You can then manipulate the order and format in which the tests shall be exported.

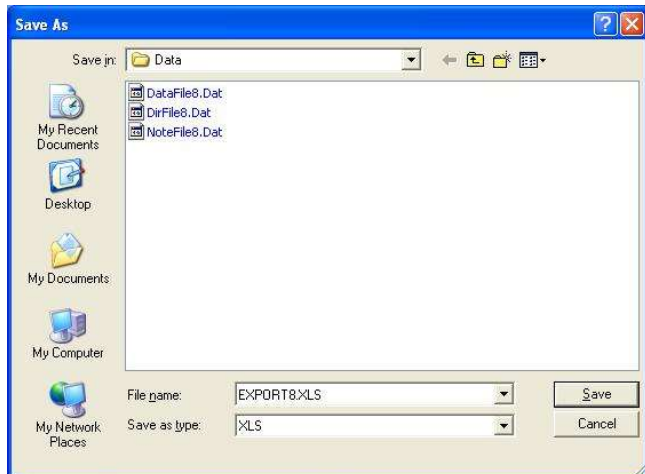
The following command buttons are used to set the order and format of the tests selected to be imported.

**Up/Down:** Use these buttons after selecting a test in the bottom selection window to move that test up or down in the list.

**Clear List:** Removes all tests from the bottom selection window.

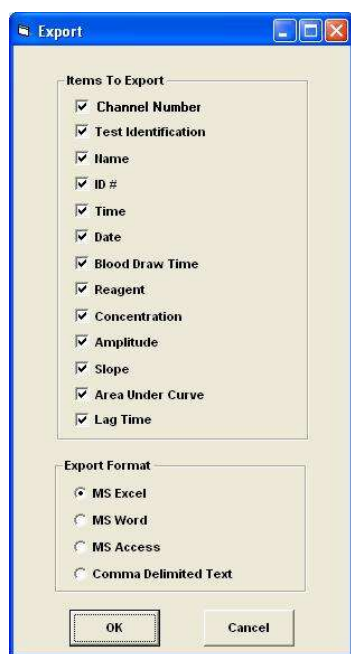


**Export:** Export the selected tests. This will display the dialog screen below.



The default name given to the exported file is EXPORT8. The extension will vary based on the output type selected. The extensions used by AGGRO/LINK8® are .DEL for delimited, .DOC for delimited in Word format, .XLS for MS Excel and .MDB for MS Access or other relational database programs. The name of the file may be changed to whatever is suitable. The extension cannot be changed. You may also select the location to where the exported file will be saved. By default the file is saved to the DATA subdirectory of where the AGGRO/LINK8® program is installed. After selecting a name or accepting the default name, choose SAVE and the export file will be generated.

**Export Options:** The information fields to export and the format in which it is to be exported are selectable by using the Options dialog box shown below:



This dialog screen consists of two fields, Items to Export and Export Format. In the Items to Export field, you select the

specific information that you want included in the export file. In the Export Format field, you select the type of output file desired. Choose OK to store the settings.

**Close:** This will close the export dialog.

**EXPORT ASCII WITH DATA POINTS – Export test data for use with another program.**

**Available only in File Menu.**

With this command, the open test file is exported to a data file under a name entered by the user. When this command is selected, a dialog box will be displayed on the screen.

Enter the name of the file where the exported data is to be stored in the File Name Box. The channel number for that curve is automatically added to the end of the file name you enter.

To change the directory where the export file is to be saved, left click the mouse over the directory name. A highlight bar will appear over the chosen directory. Then left click the mouse over the 'OK' button.

The export files are formatted in ASCII, delimited with commas (,) between data and CR/LF between records. Each file is formatted as follows:

**Channel #, Sample #  
Last name, First name, ID, Test Date**

**Maximum amplitude, Max slope, Area Under Curve, Lag Time**

**Start time min, Start time secs, End time min, End time secs  
Concentration, Units of concentration, Reagent, Gain  
Units for data points**

**First Data Point**

**Last Data Point**

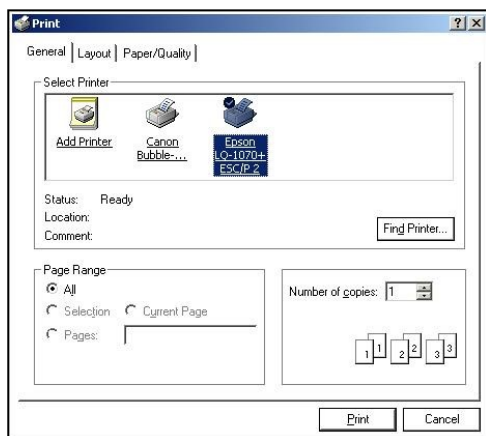
The exported data is in actual units or % of scale, as appropriate

To cancel the command, click the mouse over the cancel box. The document box will disappear.

**PRINT - Print the active test**

**Available only in Document Menu.**

This command is used to print the active file. When this command is selected, a dialogue box will be displayed on the screen. Check the Windows manual for a detailed description of the printer dialog box used by this version of Windows.



If a printer with the Windows Print Manager or the printer's setup disk hasn't been installed, this should be done before trying to print with the AGGRO/LINK®8 software.

The Print Range section is not used by this program. The Number of Copies section is used to determine how many copies of the selected document are printed. The default is one.

Once these settings are correct, click the mouse over the 'PRINT' button. The document box will disappear from the display and the test will begin printing.

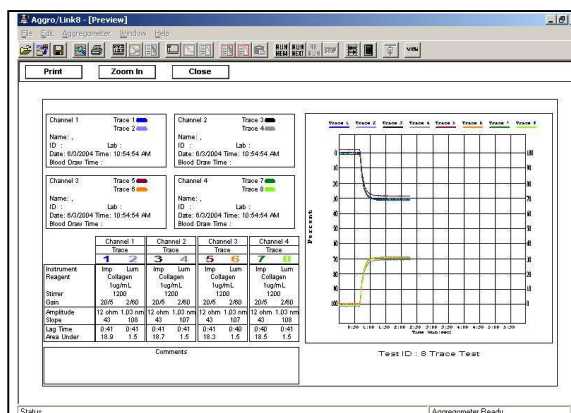
To cancel the command, left click the mouse over the cancel box. The document box will disappear from the display.

### PRINT PREVIEW - Preview full pages

**Available only in Document Menu.**

Use this command to view the active test as it would appear when printed.

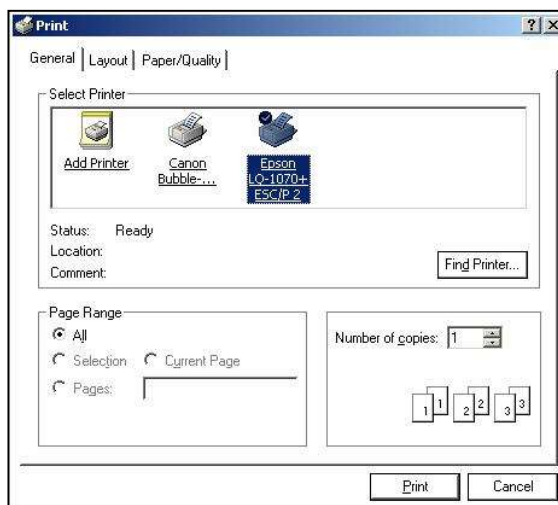
When this command is chosen, the main window will be replaced with a Print Preview window in which the active test will be displayed in its printed format. The Print Preview Toolbar offers options to zoom in and out of the page and initiate the print job.



### PRINT SETUP - Change the printer and/or printer options

**Available in Main Menu and Document Menu.**

This command is used to change the printer and/or the printer options. Only printers that were installed using the Windows Print Manager are accessible with this command. When this command is selected, a dialog box will be displayed on the screen.



In this box the operator can change the printer, the paper and orientation of the printing.

To change the Printer, Paper Size or Paper Source, left click the mouse over the appropriate box. All the available options will be displayed. To choose one of these options, left click the mouse on it. That option will appear in the dialog box.

Various printers will display different options unique to that printer.

Once the changes are made, left click the mouse on the 'OK' button to close the Print Setup dialog box.

To cancel the command, left click the mouse over the cancel box. The document box will disappear from the display.

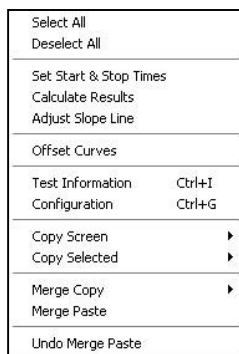
### EXIT - This command will close all open documents, prompting to save changes, and end the program.

**Available in main menu and document menu.**

Choosing this command will terminate the program. If a test is running, you will be prompted to stop the test before the program will allow it to be closed. A message prompt will appear about saving any unsaved or edited documents that are open.

### EDIT

The edit command group allows the operator to perform editing functions on an active or previously edited test file. When the EDIT group is selected from the menu bar, a menu is displayed:



#### SELECT ALL - Select all curves for editing

*Available only in Document Menu.*

This command selects all the curves to be active for the Copy commands. An active curve is highlighted on the screen.

#### DESELECT ALL - Deselect all curves

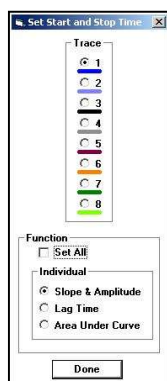
*Available only in Document Menu.*

This command selects all the curves to be deactivated. When curves are deselected, the highlight on the curve is removed.

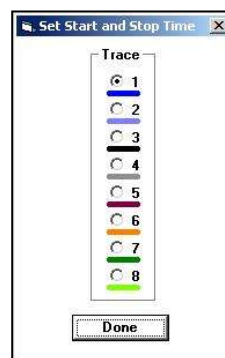
#### SET START AND STOP TIME - Set Start/Stop time for calculation

*Available only in Document Menu.*

This command is used to set the starting and ending points on the test curve for the calculation of the slope, amplitude, lag time and area under curve; thus eliminating any artifacts caused by the set-up and reagent injection from these calculations. When this command is selected, a dialogue box will be displayed on the screen.



When the Display Area Under Curve/Lag Time Calculations check box (located in the configuration dialog box) is unchecked a dialog box is shown.



Simultaneous setting of all positions for all functions can be accomplished by checking the SET ALL check box. Setting this option will set the Area Under Curve start/stop lines and the Lag Time calculation start time to the Slope/Amplitude start/stop times respectively.

When the dialog box is first displayed, channel one is selected and the active function will be Slope & Amplitude. The channels must be set one at a time. To change from channel to channel, click the mouse key on the circle next to the channel indication.

For the Slope & Amplitude and Area Under Curve functions, two vertical lines will be displayed on the graph. For the Lag Time function one line will be displayed on the graph. The color of these lines will match the color of the channel selected in the dialogue box.

For the Slope & Amplitude and Area Under Curve functions, the start line is on the left and the stop time is on the right. The distance between the start and stop lines corresponds to the test duration in the Test Procedure settings.

These lines may be moved individually by left clicking the mouse in the general area of the line to be moved. The line closest to the mouse will snap to the location of the mouse pointer and may then be positioned by moving the mouse while holding down the left button. Both lines may be moved in unison by right clicking the mouse in the general area of either the start or the stop line and then positioning the lines at the desired location.

For the Lag Time function, only a start line is displayed. This line is moved by left clicking the mouse in the general area of the line. The line will snap to the location of the mouse pointer and may then be positioned by moving the mouse while holding down the left button.

When the time setting is completed for all signals, click the mouse over the 'Done' button.

**CALCULATE RESULTS** - Compute maximum slope and amplitude, lag time and area under curve.

*Available only in Document Menu.*

This command is used to calculate the maximum Slope and Amplitude, lag time and area under curve in the selected area of the active curves. When this command is selected a dialog will be displayed on the screen.

Trace	1	2	3	4	5	6	7	8
Instrument	Imp	Lum	Imp	Lum	Imp	Lum	Imp	Lum
Gain	20/5	2/60	20/5	2/60	20/5	2/60	20/5	2/60
<b>Slope And Amplitude</b>								
Start	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Duration	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
<b>Lag Time</b>								
Start	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Area Under Curve</b>								
Start	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Duration	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

When the Display Area Under Curve/Lag Time Calculations check box (located in the Configuration Dialog box) is unchecked a dialog box is shown.

In the gain box is the gain setting of the curve. For example, an impedance curve would have a gain setting of 20/5, which means the gain is twenty ohms for five divisions and a Luminescence curve would have a gain setting of 2/74, which means that two nanomoles of ATP is equal to seventy-four percent of scale. The optical channels do not use gains for calculation. The procedure in the Aggregometer section of this Instruction Manual will explain what value to enter into the gain box.

The Slope & Amplitude and Area Under Curve calculations will have a start time and a duration text box for each channel.

The Lag Time calculation has only a start time text box for each channel.

The start box contains the start point of the curve where the calculation begins. The start point should be set to exclude the baseline setting and the spikes caused by the injection of reagent from the calculation. The starting time can be changed from the keyboard or by using the set Start and Stop times command. The start time can also be automatically set while the test is running by left clicking the mouse on the channel identifier above the test grid. After clicking on the channel identifier, a line indicating the start time position will momentarily flash on the test grid.

In the duration box will be the test length. The test length is the time duration from the starting time, not from the beginning of the graph. The portion of the curve before the starting time and past the duration from the starting time will not be included in the calculations.

When the information in the Calculate Results dialogue box is correct, click the mouse over the 'OK' button. The maximum slope and amplitude of each test will be

calculated and displayed on the test screen. The AGGRO/LINK® Software calculates the results as follows:

- **OPTICAL**  
Amplitude in percent  
Slope in percent per minute  
Lag Time in minutes and seconds  
Area Under the Curve in Percent\*time
- **IMPEDANCE**  
Amplitude in ohms  
Slope in ohms per minute  
Lag Time in minutes and seconds  
Area Under the Curve in ohms\*time
- **LUMINESCENCE**  
Amplitude in nanomoles.  
Slope in percent per minute  
Lag Time in minutes and seconds  
Area Under the Curve in percent\*time, nM\*time

To cancel the command, left click the mouse over the 'Cancel' button. The document box will disappear from the display.

#### ADJUST SLOPE LINE – Change slope calculation time

*Available only in document menu.*

This command allows for adjusting slope measurement time from 16 to 99 seconds. When this command is chosen, a dialog box is shown.



To adjust the slope line for a particular channel, select that channel by left clicking the mouse on the appropriate channel designation. Then, by using the scroll bar below the channel selection frame, adjust the slope time value up or down until the desired slope time is reached. The slope line will redraw itself as the value is changed. When satisfactory slope values are reached, left click the mouse on the 'OK' button to close the dialog box. After changing these values, save the test file or the changes will be lost.

To cancel the command, left click the mouse over the cancel box. The document box will disappear from the display.

#### OFFSET CURVES – Change the physical start time of a test Channel *Available only in Document Menu.*

This command allows for the shifting of curves along the test time line. This command is useful for correcting test start time offsets that can occur between running individual channels. When this command is chosen a dialog box is shown.

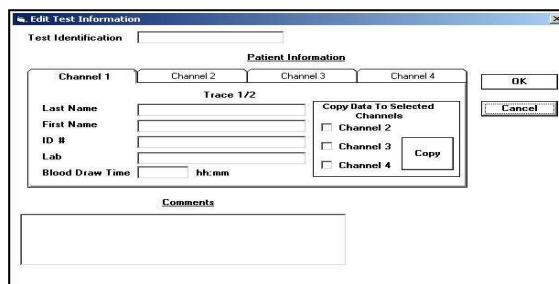
To use this function select the trace to be shifted by selecting the appropriate trace from the trace option group and then moving the curve with the slider bar. A colored highlight bar corresponding to the channel selected will appear over the slider. The curve is redrawn as the slider is moved. When the trace positions are satisfactory left click the mouse on the 'OK' button.

To cancel the command, left click the mouse over the 'Cancel' button. The document box will disappear from the display.

#### TEST INFORMATION - Change patient and/or test information *Available in Main Menu and Document Menu.*

This command is for editing the patient information for the test. When this command is selected a dialog box will be displayed on the screen.

This dialog box allows test information to be entered either while the test is running or after it is complete. The patient data can be unique for each tracing or shared among tracings by using the Copy Data command. To copy the current displayed data to another trace, select the trace numbers to receive data. This is done by setting the



appropriate check box, and left clicking the COPY button to copy the data. The check boxes will clear once the data is copied. Select each trace by left clicking the mouse on the desired Trace tab.

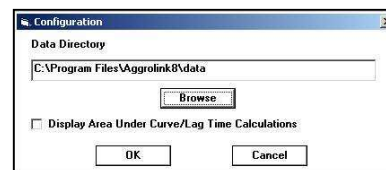
Using the mouse, left click on the tab of the channel for which information is to be entered, then enter any of the text boxes and edit the text using the keyboard. The comment field is generic for all channels on the current document.

After entering the test information left click the 'OK' button to store the information and close the dialog box.

To cancel the command, left click the mouse over the 'Cancel' button. The document box will disappear from the display.

#### CONFIGURATION - Change the program configuration *Available in Main Menu and Document Menu.*

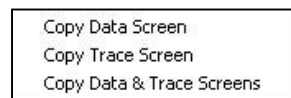
This command is for editing the configuration of the program. When this command is selected a dialog box will be displayed on the screen.



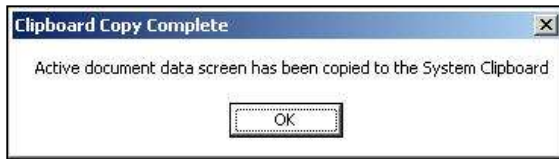
The data directory shows the location where the test is stored. The location can be changed by either typing a new location or using the browser button and selecting a new directory.

Check the Display Area Under Curve/Lag Time Calculations box to display the results. To disable the display of the calculations, leave this box unchecked.

#### COPY SCREEN-Copies the trace screen, the data screen, or both to the system clipboard *Available only in Document Menu.*



Copy Data Screen – Copies data screen for all channels to the system clipboard.



Copy Trace Screen – Copies trace screen for all channels to the system clipboard.



Copy Data & Trace Screen - Copies data and trace screen for all channels to the system clipboard.

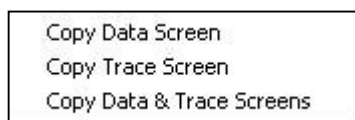


Selecting this command will copy the test grid and all curves into the system clipboard. After activating this command, a message box will appear.

The test grid and curves can now be copied into any application that supports graphical pasting, such as Excel, Paint or WORD for Windows.

Left click the mouse on "OK" to close the message box.

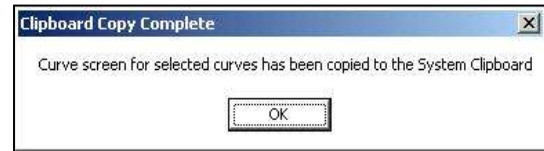
**COPY SELECTED – Copies the trace screen, the data screen or both, for selected channels to the system Clipboard**  
*Available only in Document Menu.*



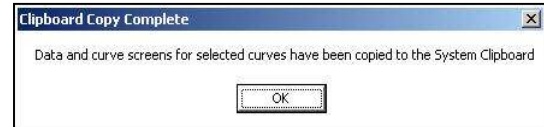
Copy Data Screen – Copies data screen for selected channels to the system clipboard.



Copy Trace Screen – Copies trace screen for selected channels to the system clipboard.



Copy Data & Trace Screen - Copies data and trace screen for selected channels to the system clipboard.

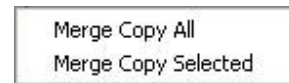


Selecting this command will copy the entire document, data, grid and curves to the system clipboard. After activating this command, a message box will appear.

The data, grid and curves can now be copied into any application that supports graphical pasting, such as Excel, Paint or WORD for Windows.

Left click the mouse on "OK" to close the message box.

**MERGE COPY - Copies curves and data to an internal clipboard for use in creating MERGE Documents.**  
*Available only in Document Menu.*



MERGE COPY ALL - Copies all test data for the active document to an internal clipboard. Once the data is in the internal clipboard it may be copied onto a Merge Document.

MERGE COPY SELECTED - Copies all selected test data for the active document to an internal clipboard. Once the data is in the internal clipboard it may be copied onto a Merge Document. Double click the tracing(s) to select.

**MERGE PASTE - Pastes test data acquired using one of the Merge Copy menu commands.** Up to four test curves, acquired from any document, can be pasted onto a single merge document. The curves will be added sequentially to the channels list. All test information, including any previous calculations, is included.

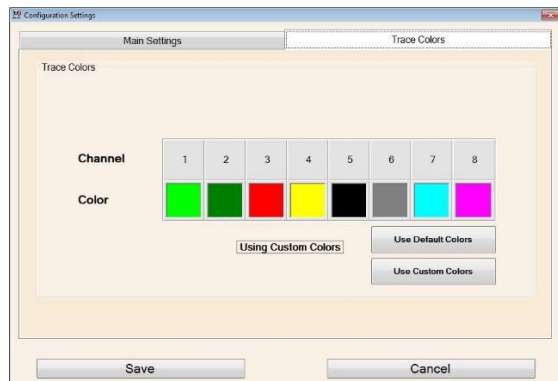
*Available only in a MERGE document.*

**UNDO MERGE PASTE - Undo last MERGE PASTE operation**  
**Available only in a MERGE document.**  
Removes previously pasted curves and data from a MERGE document.

**CUSTOM COLOR - Allows the user to switch back and forth between custom and default trace colors.** Basic Windows colors can be used, or Custom colors can be mixed and saved. the trace colors will remain set until they are changed and saved. Tests completed in older version software are compatible with the new software, and will still



appear as standard colors if opened in any older software package.



At any time during the operation of the program, the Custom Color Dialog Setup can be active to allow for individual user selected trace colors. To select custom colors, Click on the “Use Custom Colors” button and select the channel. A dialog will appear allowing selection of system defined or custom colors.



Custom Colors can be created by selecting “Define Custom Colors”. The following will appear.



Select the desired color and click OK. The selected color will appear in the color box below the indicated channel. Repeat for additional channels then select SAVE or to discard changes click CANCEL

## AGGREGOMETER

Connect	
Test Procedure	Ctrl+T
Run New Patient	Ctrl+N
Run Same Patient	Ctrl+A
Restart Current Test	Ctrl+R
Stop Test	

**CONNECT** - Establish connection with Aggregometer

**Available in Main Menu and Document Menu.**

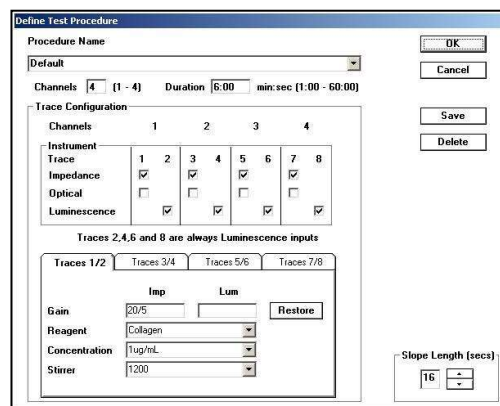
This command is for establishing communication with the Aggregometer. This command is to be used when the program has been running without the Aggregometer, and a function requiring the Aggregometer, such as run test, is to be performed.

If connection to the Aggregometer is successful the status message in the lower right corner of the screen will display the message “Aggregometer Ready”. If there is a problem connecting, a message box describing the problem will be displayed.

**TEST PROCEDURE** - Create or load a test procedure

**Available in Main Menu and Document Menu.**

This command is for establishing the test procedure configuration. When this command is selected, the program will display a document box.



Procedure Name - Each test procedure can be assigned a name and saved. Once the procedure is set to the proper configuration, enter the Procedure Name box and type the name of the procedure. To save the procedure, click the mouse over the ‘OK’ button. To cancel the settings, click the mouse over the cancel box. The document box will disappear from the display.

To load a previously saved procedure, click the mouse over the arrow next to the Procedure Name box. A list of all the previously saved procedures will be displayed. Click the mouse over the desired file and that test procedure setting will be displayed.

The Delete function is for deleting any previously saved test procedure. To delete a saved procedure, select this procedure setting. Left click the mouse over the ‘Delete’ button. The program will prompt with a confirmation box asking to confirm the deletion. Left click the mouse on the

'Yes' button to delete or left click the mouse on the 'No' button to cancel the deletion.

**NOTE-** An ATP Standard test procedure is pre-set in the software. Simply change number of standard channels to run ATP Standard

**Channels** - This box is for defining the number of channels on the instrument to be used for the test. This may be any number of channels between 1 and 4. Left click the mouse over the input box and type the number of channels being used on the instrument.

**Duration** - This box is for defining the duration of the test. For Impedance aggregation we recommend a test duration of 6 minutes and 5 minutes for optical. The duration is important for the slope and amplitude calculations. If six minutes is defined, it will not calculate beyond six minutes from the starting point, unless the position of the start and stop times are edited after the test is run.

To set the test duration, click the mouse over the Duration box. Using the keyboard, type the duration of the test in minutes and seconds.

**Trace Configuration** - The trace configuration frame contains editable fields for describing the specific attributes of each trace being run in the test. The input channels are labeled 1-4 across the top with the corresponding traces labeled 1 & 2, 3 & 4, 5 & 6 and 7 & 8. Traces 1, 3, 5 and 7 are selectable as either Impedance or Optical traces, while 2, 4, 6 and 8 are ALWAYS Luminescence.

Use the tabs marked Traces 1/2, Traces 3/4, Traces 5/6 and Traces 7/8 to select the reagent, concentration and stirrer speed.

**Reagent** - The reagent box allows the entry of the reagent type being used for the test. When a test is run and saved, the data on the reagent will be saved with the test data. Click the mouse over the arrow next to the box and select the reagent to be used or type the reagent type using the keyboard.

**Concentration** - The Concentration box allows entry of the reagent concentration for the test. When the test is saved, the concentration information is also saved with the test data. Click the mouse over the arrow next to the box and select the concentration to be used or type the reagent concentration using the keyboard.

**Stirrer** - The Stirrer box records stirring speed for the test. When the test is saved, the stirring speed is also saved with the test data. Click the mouse over the Stirrer box and enter the stirring speed using the keyboard.

**Gain** - The Gain box allows the entry of a gain setting used during the calculation. The gain normally applies to the luminescence or impedance channels and is stored along with the test data when the test is saved. The gains will be set automatically. The gain for the Impedance is set to 20/5. The lumi gain will be set using information from the ATP Standard that is run in the beginning of the assay. There is no Gain setting for the Optical method. The gain is set manually.

**Slope Length** - The slope length scroll bar and text box are used for varying the time range used to calculate the slope. This can be set to any value from 16 to 99.

**OK** - Will close the Define Test Procedure dialog box and save any changes that were made to the procedure list.

**Cancel** - Will close the Define Test Procedure dialog box and abandon any changes that were made to the procedure list.

**Save** - Will save changes made to the procedure list.

**Delete** - Will delete the currently selected procedure from the procedure list. You must always leave at least one procedure on the list.

#### **RUN NEW PATIENT - Run a test**

##### **Available in Document Menu.**

This command is used to run a test. When this command is selected, the program will display a document box.

**Test Identification** - This field is used to identify the test group. (In a Clinical Lab, use the patient name)

**Channel Tabs** - These fields, one for each channel, are used to uniquely identify the test information for a particular trace.

The patient data can be unique for each channel or shared among channels by using the Copy Data command. To copy the current displayed data to another channel, select the channel numbers to receive data. This is done by setting the appropriate check box, and left clicking the COPY button to copy the data. The check boxes will clear once the data is copied. Select each channel by left clicking the mouse on the desired channel tab.

To enter the patient Name, ID number, Lab and Blood Draw Time in the box provided, left click the mouse over the box and enter the data using the keyboard.

If the test procedure information needs to be changed, click the mouse over the EDIT box and edit the Test Procedure as described in the Test Procedure description.



When the information is correct, left click the mouse over the OK box. The display will show a graph with the test tracing starting at the left margin.

To cancel the command, left click the mouse over the 'Cancel' button. The document box will disappear from the display.

**RUN SAME PATIENT** – Starts a new test using the currently active document's patient information

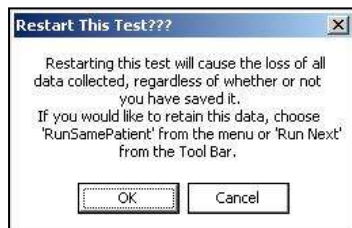
**Available only in Document Menu.**

The Run Same Patient command allows for starting a new test using a currently open document test information.

**RESTART CURRENT TEST** - Starts the test over again

**Available only in Document Menu.**

The Restart Test command is used to restart a test. When selected, the data points of the previous test will be discarded, the Patient Information will remain as entered. This command is useful for discarding artifacts caused by baseline setting. DO NOT use between tests.



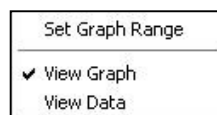
**STOP TEST** - Stop test in progress

**Available only in Document Menu.**

The Stop Test command is only valid when there is a test in progress. When the Stop test command is executed, the program will stop collecting data from the AGGRO/LINK® interface. The file should either be saved to disk, closed, or the test should be restarted using the Restart Test command.

## VIEW

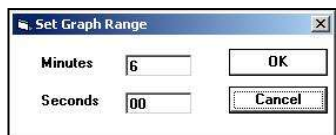
The VIEW command group provides commands for changing the display format of the active document. When the View group is selected from the menu bar, a menu is displayed.



**SET GRAPH RANGE** – Change the time range of the graph

**Available only in Document Menu.**

This command is used to set the graph range from 1 to 60 minutes. When this command is selected the Set Graph Range dialog box is shown.



Left click the mouse over the text box for minutes or seconds and enter the desired range using the keyboard. Once the time is entered, left click the mouse on the 'OK' button to close the dialog box and resize the screen.

To cancel the command, left click the mouse over the 'Cancel' button. The document box will disappear from the display.

**VIEW GRAPH**– Display the graph only

**Available only in Document Menu.**

This command is used to display the graph area. When selected, the graph will occupy the entire document screen.

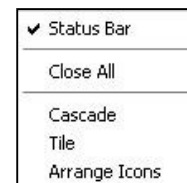
**VIEW DATA**– Display the data of the document.

**Available only in Document Menu.**

**This command is used to display the data of the document.**

## WINDOW

The WINDOW command group allows for the configuration of the window on the screen. When the WINDOW group is selected from the menu bar, the following sub-menu is displayed:



**STATUS BAR** - Show or hide status bar

**Available in Main Menu and Document Menu.**

This is a switch used to show or hide the status bar at the bottom of the file screen. When the status bar is on, a check will be next to the command in the menu box.



The status bar gives a brief description of each command and shows the status of the Aggregometer.

**CLOSE ALL** - Close all open test documents.

**Available only in Document Menu.**

This command closes all open test documents, prompting the user if the test has not been saved.

**CASCADE** - Arrange the windows so that they overlap.

**Available only in Document Menu.**

This command will cause all the currently open files to overlap each other. They are overlapped in a cascading fashion so that the title bars of each window can be seen by the operator. The active window is always placed on top.

**TILE** - Arrange the windows in non-overlapping tiles.

**Available only in Document Menu.**

This command will cause all the currently open files to be arranged as individual tiles on the monitor. The active window is indicated by a highlighted title bar.

ARRANGE ICONS – Arranges minimized test document across lower left corner of screen.

## HELP

AGGRO/LINK® Help	Ctrl+H
About AGGRO/LINK®	

The HELP command group allows for access to installed AGGRO/LINK®8 Help Files and to the About AGGRO/LINK®8 dialog which displays the AGGRO/LINK® version number.

## HOW DO I...

### START FOR THE FIRST TIME

1. After all the connections are made, turn on the Aggregometer.
2. Turn on the computer.
3. Start the AGGRO/LINK®8 program.
4. Select **EDIT, CONFIGURATION**.
5. Set applicable configuration for your system. Click **OK** when complete.
6. Select **AGGREGOMETER, TEST PROCEDURE**.
7. Setup the test procedure page to conform to your system and the test being run. Each test procedure page can be named and saved for future retrieval.
8. The program is now setup and you are ready to run Aggregation tests.

### RUN A NEW PATIENT

1. Select **AGGREGOMETER, RUN A NEW PATIENT**; or click on **RUN NEW** icon on the tool bar.
2. Enter test identification. This will be the file name for the test when it is saved. The test identification should be a recognizable name so the test can be easily retrieved in the future. (In a Clinical Lab, use the patient's name)
3. Enter patient information. If all channels are for the same patient, mark the **"USE CHANNEL 1 DATA FOR ALL CHANNELS"** Box. If more than one patient is being run, click on the tab for each channel and enter the appropriate information.
4. Check that the test procedure is correct. If not, select **EDIT** to edit the procedure.
5. Select **OK**. The test tracings will now be running. Start the test as instructed in the instrument test procedures. If you need to obtain a clear screen, select **AGGREGOMETER, STOP TEST**; or select **STOP** Icon on the tool bar. Then select **AGGREGOMETER, RESTART CURRENT TEST**; or select **RERUN** Icon on the tool bar. This is for eliminating artifacts caused during setting of the baseline and not for running a new test.

### RUN A NEW TEST ON THE SAME PATIENT(S)

1. Select **AGGREGOMETER, RUN SAME PATIENT**; or select **RUN NEXT** Icon on the tool bar.
2. Enter test identification. This will be the file name of the test when it is saved. The test identification should be a recognizable name so the test can be easily retrieved in the future. (In a Clinical Lab, use the patient's name)
3. Check that the test procedure is correct. If not, select **EDIT** to edit the procedure.

4. Select **OK**. The test tracings will now be running. Start the test as instructed in the instrument test procedures. If you need to obtain a clear screen, select **AGGREGOMETER, STOP TEST**; or select **STOP** Icon on the tool bar. Then select **AGGREGOMETER, RESTART CURRENT TEST**; or select **RERUN** Icon on the tool bar. This is for eliminating artifacts caused during setting of the baseline and not for running a new test.

### CALCULATE RESULTS

**NOTE:** START TIME can be set automatically. Immediately before the addition of the reagent, left click on the Color-Coded Bar under the trace number at the top of the Test Graph. A color-coded line will appear at that time point. To check Start & Stop Times at the completion of the Test, click on **EDIT** then **SET START & STOP TIMES**.

The Calculate Results command can be used on a running test, after the test has been stopped or on a saved test that has been opened.

1. Select **EDIT, SET START AND STOP TIMES**; or select the Icon on the tool bar.
2. Move the start line to the start of the test, usually on the stable baseline, just before the addition of the reagent. Move the stop line to the end of the test.
3. Select **EDIT, CALCULATE RESULTS**; or select **CALCULATE RESULTS** icon on the tool bar.
4. Check the settings and select **OK**. The results calculation will be marked on the test data.

### CREATE A MERGE DOCUMENT

1. Select **FILE, CREATE MERGE**. This will create a Merge Document
2. Open the files that contain the curves to be merged.
3. To copy all the curves from a file to send to a Merge Document:
  - a. Activate the chart where the curves to be copied are located.
  - b. Select **EDIT, MERGE COPY**.
  - c. Select **MERGE COPY ALL**.
  - d. Activate the Merge Document
  - e. Select **EDIT, MERGE PASTE**.
4. To copy only selected curves from a file to a merge document:
  - a. Activate the chart where the curves to be copied are located.
  - b. Select the curve to be copied by double clicking on the curve. When selected, the curve will be highlighted.
  - c. Select **EDIT, MERGE COPY**.
  - d. Select **MERGE COPY SELECTED**.
  - e. Activate the Merge Document
5. Select **EDIT, MERGE PASTE**.  
If other curves are to be added to the Merge Document, repeat steps 2 and 3. Up to eight tracings can be put onto a merge document.
6. The **EDIT, OFFSET CURVES FEATURE** can be used to shift the curves horizontally along the time axis. This enables curves from different documents to be aligned.

### UNINSTALL AGGRO/LINK®8 PROGRAM.

The built-in uninstall program for AGGRO/LINK® will not remove data directories or data files. To uninstall AGGRO/LINK®8, left

click the mouse on the Windows "START" button and select "SETTINGS" and then "CONTROL PANEL". When the Control panel appears double left click on the "ADD/REMOVE PROGRAMS" icon. When the Add/Remove dialog box appears, select "AGGRO/LINK®8" and left click on the "ADD/REMOVE" button. When prompted to continue, left click on 'YES' to uninstall or 'NO' to cancel uninstall. The application path and data files must be removed manually to Windows recycle bin or permanently deleted.

## vW COFACTOR ASSAY

### Introduction

The CHRONO-LOG® von Willebrand Assay Software for Windows Version 3 is designed to operate with a one (1), two (2) or four (4) channel Aggregometer connected to an AGGRO/LINK® Interface or with an Aggregometer having the computer interface option and a Windows compatible computer. The von Willebrand Software is a compiled program designed to be run in Microsoft Windows. The operator should be familiar with using Windows and using the mouse before running the von Willebrand Software. For information on Windows and mouse operation, see the Windows Instruction Manual.

The software calculates a best fit standard curve for two (2) to six (6) points down to 3% of Factor Activity and slope. The coefficient of determination for the standard curve is reported on screen. The standard curve can be viewed and modified by rerunning or deleting a serial dilution point on the curve. Standard curves are stored for future reference.

The program gives on screen instructions for running the test and standard dilutions. The program allows for replications of both test and standard curve dilutions, calculates coefficient of variation between replications and reports unacceptable values.

Test results are adjusted automatically for dilution factor of the sample. The test results are given in percent of von Willebrand activity. A print command allows for batch printing of reports. The reports are printed in standard size (8.5" X 11").

### General Description

The vW Software Assay for Windows program is Windows driven, that is, the operator uses a mouse to select functions to be performed from a pull-down window. The operator should be familiar with Windows program and using the mouse before running the vW CoFactor Assay for Windows program. For information on the Windows and mouse operation, see the Windows instruction manual.

The vW CoFactor Assay Setup automatically creates a vW CoFactor Assay program group.

A vW Cofactor Icon can be placed on the desktop using the following procedure:

1. Choose "My Computer"
2. Choose "C:" Drive
3. Choose "Program Files"
4. Choose "vW CoFactor Assay V3"

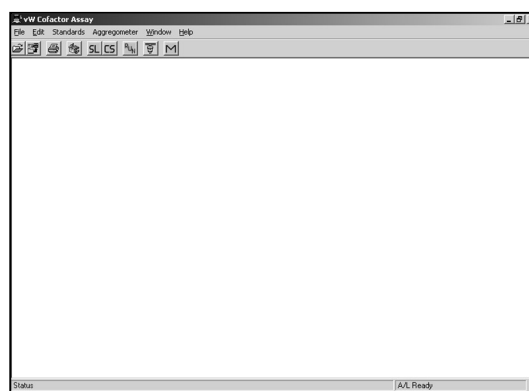
5. Highlight "vW CoFactor Assay V3" Icon
6. Right click the mouse on the highlighted Icon.
7. Select "Create Shortcut". This will create an Icon titled "Shortcut to vW CoFactor Assay V3".
8. Drag this new Icon onto the desktop.

These steps will work provided that the program was installed in the default directory. For additional information on the Windows Operation, use your Windows Help File.

### Starting vW CoFactor Assay

Activate vW CoFactor Assay program group using the mouse. The group is active when the title bar is highlighted. program.

After starting vW CoFactor Assay, the following document box is displayed:



In the menu bar are the titles of each command group. To select a command group, the operator clicks the mouse over the title. A menu window listing all the options available under that category will be displayed.

### File

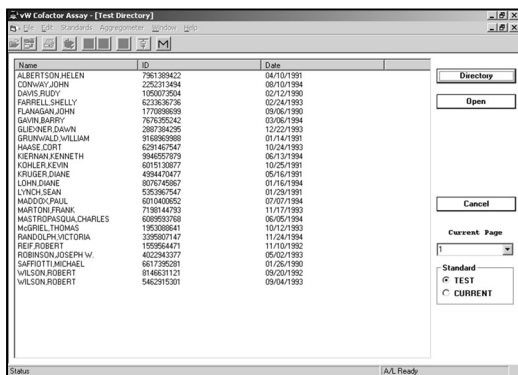
The File command group allows the operator to perform basic file functions. When the File group is selected, the submenu shown below is displayed. To select an option in the File group, click the mouse over the desired command.



1. Open (View Test)

This command is used to open a file that has been saved on disk. With this command, the files can be listed by alphabetical order of the names, by patient ID

number or by test dates. When selected, the following dialog box will be displayed.



The Standard box allows the test to be viewed with the standard that was originally used or to be viewed with the current standard in memory. To view the test with the standard with which the test was saved, select Test. To view the test with the standard presently in memory, select Current.

The test directory dialog box consists of a test file list, command buttons and page select controls. The test file list will contain the list of tests available to open. Test are selected to be opened in the following manner:

#### Selecting a single file:

To select a single file to open, left click the mouse over the file name. A highlight bar will appear over the file name. You may then left click the mouse on the Open button or simply double left click the mouse over the file name to open the desired test.

#### Selecting multiple files:

To select multiple files to open, you have two options. You may select groups of files by left clicking the mouse on the first file you wish to open, then, while holding down the 'SHIFT' key, left click the mouse on the last file in the group you wish to open. You may also select multiple files by holding down the 'CONTROL' key and left clicking the mouse on the desired files. After selecting the files, left click the mouse on the Open button to open the files. You are limited to a maximum of twenty open files at one time.

Command buttons:

#### Directory

Left clicking the mouse on the Directory button will display a [DIRECTORYOPEN.htm](#) directory dialog that will allow you to change the directory path. The default directory for vW Cofactor Assay Ver. 3 is C:\programfiles\vwCofactorAssayV3\Data.

#### Open:

Left clicking the mouse on the OPEN button will open all selected tests in the file list box.

#### Cancel:

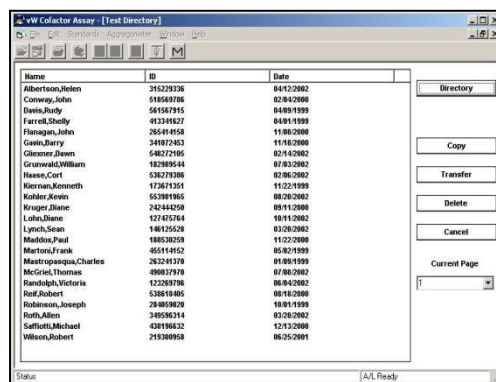
Left clicking the mouse on the Cancel button will close the Test Directory dialog box.

#### Page Selection: (Current Page)

This pull down box allows you to select which page of tests is shown in the file list box. As many as 1000 tests will be shown at one time in the file list box. To negotiate through the list, select the page you want to view from the Current Page pull down box.

## 2. Move

This selection is used to copy previously saved tests to diskettes, to transfer tests from Hard Drive to diskette or from one diskette to another, or to delete a file from Hard disk or diskette. When selected, the dialog box below will be displayed.



The Test Directory dialog box consists of a test file list, command buttons and page select controls. The test file list will contain the list of all saved tests in the current data directory. Tests are selected for Copy, Transfer or Delete in the following manner:

#### Selecting a single file:

To select a single file, left click the mouse over the file name. A highlight bar will appear over the file name. You may then left click the mouse on the Copy, Transfer or Delete button.

#### Selecting multiple files:

When selecting multiple files to Copy, Transfer or Delete, you have two options. You may select groups of files by left clicking the mouse on the first file you wish to Copy, Transfer or Delete, then, while holding down the 'SHIFT' key, left click the mouse on the last file in the group you wish to Copy, Transfer or Delete. You may also select multiple files by holding down the 'CONTROL' key and left clicking the mouse on the desired files. After selecting the files, left click the mouse on the Copy, Transfer or Delete button to Copy, Transfer or Delete the files.

Command buttons:

#### Directory:

Left clicking the mouse on the *Directory* button will display a directory dialog box. You can then change the source directory where test files will be copied, transferred or deleted from and the

destination directory where the test will be copied. Example:

To copy or transfer a test from the previous software version to the correct directory, set the source directory to “C:\vwcofw\vwtest” and the destination directory to “C:\programfiles\vwcofactorassayv3\data”.

**Copy:**

Copies selected test(s) to the Destination Directory. The source file will remain in your Source Directory.

**Transfer:**

Moves selected test(s) to the Destination Directory. The source file will then be deleted from your Source Directory.

**Delete:**

Deletes selected test(s) from the Source Directory.

**Cancel:**

Left clicking the mouse on the Cancel button will close the Test Directory dialog box.

**Page Selection: (Current Page)**

This pull down box allows you to select which page of tests is shown in the file list box. As many as 1000 tests will be shown at one time in the file list box. To negotiate through the list, select the page you want to view from the Current Page pull down box.

### 3. Batch Print

This command is for selecting files to print. When this command is selected, a dialogue box, shown below, will be displayed.



If you haven't installed the printer with the Windows Print Manager, you should do so before you try printing with the vW Cofactor Assay software.

The Print Directory dialog box consists of a test file list, command buttons and page select controls.

The test file list will contain the list of all saved tests in the current data directory. Test are selected for Printing in the following manner:

**Selecting a single file:**

To select a single file, left click the mouse over the file name. A highlight bar will appear over the file name. You may then left click the mouse on the Print button.

**Selecting multiple files:**

When selecting multiple files to Print, you have two options. You may select groups of files by left clicking the mouse on the first file you wish to Print, then, while holding down the 'SHIFT' key, left click the mouse on the last file in the group you wish to Print. You may also select multiple files by holding down the 'CONTROL' key and left clicking the mouse on the desired files. After selecting the files, left click the mouse on the Print button to print the files.

**Command buttons:**

**Directory:**

Left clicking the mouse on the *Directory* button will display a directory dialog box. You can then change the directory paths that determine where test files to be printed are located.

**Print:**

Prints the selected test(s)

**Cancel:**

Left clicking the mouse on the Cancel button will close the Test Directory dialog box.

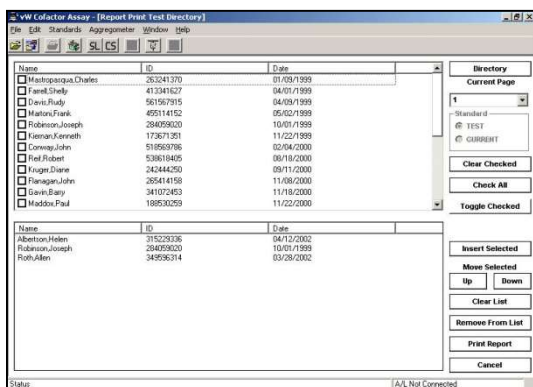
**Page Selection: (Current Page)**

This pull down box allows you to select which page of tests is shown in the file list box. As many as 1000 tests will be shown at one time in the file list box. To navigate through the list, select the page you want to view from the Current Page pull down box.

To Cancel, click the mouse on the Cancel box.

### 4. Report Print

This command will print tests in a report format. When this command is selected from the **FILE MENU** the Report Print dialog box is displayed.



This dialog box is used to select, arrange and print multiple tests in a report format.

Tests are selected by checking the accompanying box next to the test description.

Up to four tests per page can be printed. The order can be set to control the final print arrangement.

As with the normal print format, the tests can be printed with the original standard or with the currently select standard.

The following commands are available on the Report Print dialog:

#### Directory:

Use this command to change the directory from which to print tests. Tests in a single report must come from the same directory.

Changing the directory will remove all previously selected tests from the report list.

#### Current Page:

Use this command to change the current page of tests to view.

#### Standard:

Use this command to select the standard that will be viewed with each test. You may select the original standard with which the test was run or the current standard may be selected.

#### Clear Checked:

Use this command to clear all selections.

#### Check All:

Use this command to select all tests on the current page.

#### Toggle Checked:

Use this command to toggle all check boxes.

#### Insert Selected:

Use this command to place all selected tests into the report list.

#### Move Selected:

Use the **UP** or **Down** commands to move a selected the report list for positioning.

#### Clear List:

Use this command to remove all tests from the report list.

#### Remove From List:

Use this command to remove an individual test from the report list.

#### Print Report:

Use this Command to print the tests in the report list.

#### Cancel:

Use this command to cancel report printing and close the **Report Print** dialog box.

### 5. Print Setup

Use this command to select a printer and printer connection. This command displays the Print dialog box, where you specify the printer and its connection.

The Print dialog box appearance will vary depending on the operating system you are running.

### 6. Exit

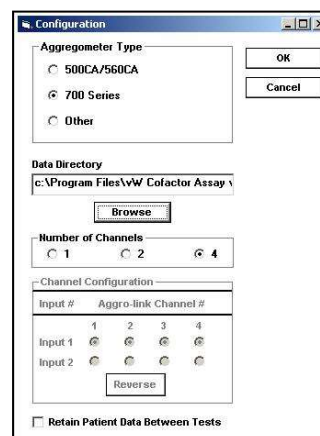
This command causes the program to exit this session of vW Cofactor Assay and return to the Windows program manager.

### Edit

The Edit function is used to edit the configuration settings, such as instrument type, File directory and number of channels. When the edit group is selected from the menu bar, the menu shown below is displayed.



Use this command to change the configuration of the vW Cofactor Assay software. When this command is selected, the Configuration dialog box is displayed:



This dialog has the following controls:

**Aggregometer Type:**

Select the type of Aggregometer being used.

**Data Directory:**

Type in the name of the directory where you would like to store vW Cofactor Assay test files.

**Browse:**

Left click this command button to browse directories.

**Number of Channels:**

Select the number of optical channels available on the Aggregometer that is being used.

**Channel Configuration:**

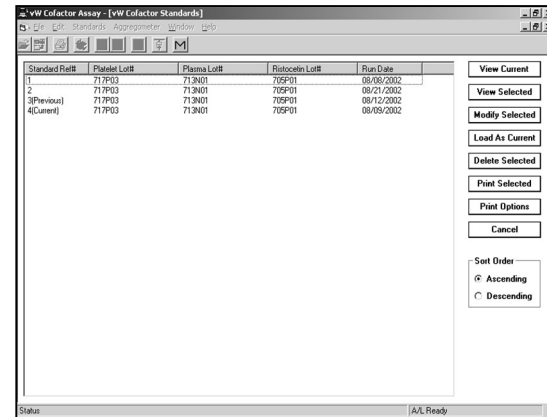
Available for 1 or 2 Optical channel configurations.

Describes which channel on the AGGRO/LINK® Interface will be used as the data source for a particular screen sample. Select a unique input port for each channel. The port numbers correspond to the jacks labeled on the back of the AGGRO/LINK® interface unit.  
On 4 channel units the input channel coincides with the screen tracing, ex. 1 = 1, 2 = 2 etc...

**Retain Patient Data Between Tests:**

Select this checkbox if you would like the entered patient information to remain after saving or abandoning a test.

When finished making changes to the configuration, left click the mouse on the OK button to accept the changes and close the dialog box. Left clicking the mouse on the Cancel button disregards any changes and closes the dialog box.



This dialog allows you to manipulate the currently stored standards.

**View Current:**

Displays the currently loaded standard.

**View Selected:**

Views the selected standard. Only one standard may be viewed at a time. Left click the mouse on the standard to select.

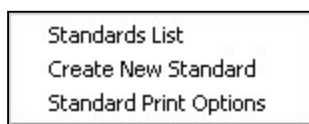
**Modify Selected:**

Displays the Modify Standard dialog box. When the Modify Standard dialog box is displayed, certain attributes of an existing standard may be modified, deleted or rerun. The Modify Standard dialog box is explained in detail along with Create Standard dialog box in the next section.

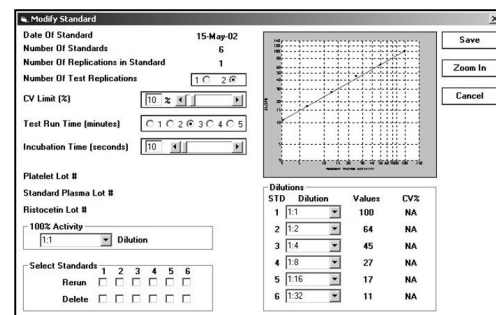
The Modify Standard box, below, is almost identical to the Create Standard dialog box and its entry fields.

## Standards

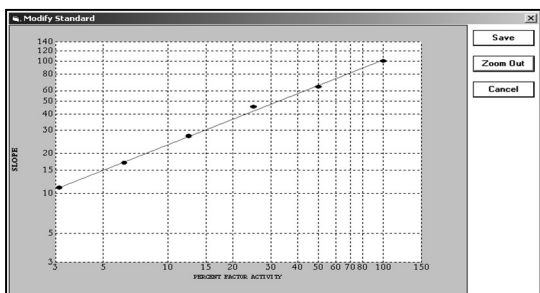
This should be the first selection after installation. Without a standard curve, you cannot run a test sample. When the Standards group is selected from the menu bar, the menu shown below is displayed. To select an option in the Standards group, click the mouse over the desired command.



1. Standards List – This selection displays the Standards List dialog. When this selection is chosen the following dialog is displayed.



The Modify Standard dialog allows for an expanded view of the existing standard by left clicking on the ZOOM button. Shown below is how the dialog box will look when zoomed.



#### Load As Current:

Loads the selected standard as the current standard. This is the standard used when running an actual test. Only one standard may be selected as current at one time.

#### Delete Selected:

Deletes selected standards from memory.

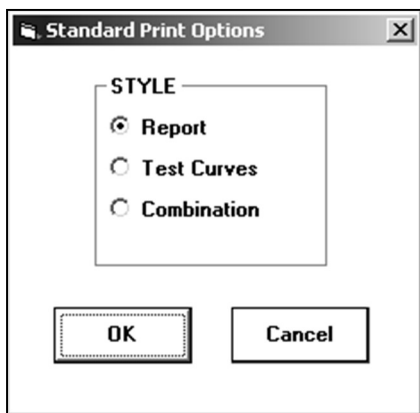
#### Print Selected:

Prints all selected standards in the format indicated by Print Options.

#### Print Options:

Allows selection of print format used when printing standards.

There are 3 formats that can be selected from as shown in the Print Options Dialog shown below.



- 1) Report: Prints standard line only.
- 2) Test Curves: Prints associated curves only.
- 3) Combination: Prints standard line and associated curves.

#### Cancel:

Closes the Standard List dialog box

#### Sort Order:

Allows selection of sort order, either ascending or descending.

2. Create New Standard -- This selection is used when a new standard curve is to be run. When selected, the following dialog box appears allowing entry of specifics for the standard to be created.

STD	Dilution
1	1:1
2	1:2
3	1:4
4	1:8
5	1:16
6	1:32

This selection is used when a new standard curve is to be run. After setting the specifics for the new standard, click the mouse over the Save As Current button. Once the settings are saved, the previous standard curve and settings are saved in memory automatically. Now you must run the new standard dilutions. If you do not want to run and want to keep the present standard curve, click the mouse over the Cancel button. You can restore the previous standard by selecting the 'Previous' standard in the standards list dialog and loading it using the Load As Current command in the Standard List dialog box.

Below is a description of the individual fields for Creating New Standard or Modifying an existing one:

- a. Number of Standards – Select the number of standard dilutions to be used for calculation of the standard curve. Check the procedures of the Ristocetin Cofactor Assay Kit being used and enter the number of recommended dilutions from the Assay Kit instructions. The default setting for the Number of Standards is four.
- b. Number of Standard Replications – If a Replication is desired for each Standard Dilution, select 2 on this line. If you do not want to run a replication test for Standard Dilution, select 1 on the line.
- c. Number of Test Replications – If a replication is desired for each Test Sample, select 2 on this line. If you do not want to run a replication test for Test Sample, select 1 on this line.
- d. CV Limit – Select or Enter the maximum desired Coefficient of Variation between two replications. If the CV Value entered is exceeded, you will be issued a warning and can decide to accept or reject the test. If you are set to run a single test with no replicate, the CV Value is not used.
- e. Test Run Time in Minutes – Select the desired test time on this line. You may enter from one to five minutes in whole minute increments. Once



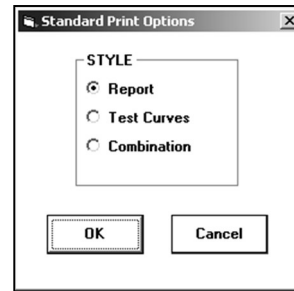
the test reaches the entered time the test will end.

- f. Incubation Time In Seconds – Select or Enter the desired Incubation Time of the sample on this line. You can set the incubation time from zero to nine hundred ninety nine seconds. Once the sample has incubated to the desired setting, an optional alarm will sound (requires speakers) and a message indicating the next step will be displayed.
- g. Platelet Lot # - Enter the Lot Number of the Lyophilized Platelets used for running the standard curve and test samples. This entry is for record keeping only and does not have an effect on the results.
- h. Standard Plasma Lot # - Enter the lot number of the Plasma used for Running the standard curve. This entry is for record keeping only and does not have an effect on the results.
- i. Ristocetin Lot # - Enter the lot number of the Ristocetin used for running the standard curve and test samples. This entry is for record keeping only and does not have an effect on the results.
- j. 100% Activity Dilution – Enter the dilution ratio which represents 100% activity (typically 1:2). As this entry is used in the calculation of Test Samples Cofactor Activity, the proper ratio must be entered to obtain accurate test results.
- k. STD Dilution – Enter the Dilution factor for each of the standards being used to calculate the Standard Curve under the Dilution column. They should be entered in serial dilution order, with the first standard being undiluted or the least diluted and the last standard being the most diluted. Check the Assay Kit Instructions for the recommended Standard Dilutions for running the standard curve.
- l. Enter Standard's # to Rerun – This selection will be displayed only when in the Modify Standard screen. If a standard dilution is abnormal or when running Replicates and the CV is over the limit, you can select to rerun that dilution only. The standard curve will then be recalculated with the new standard dilution and the previous standard dilutions.  
**NOTE:** When this option is selected, the Original Standard is saved in the Standards List as "Previous" while the Modified Standard is saved as "Current".
- m. Enter Standard's # to Delete – This selection will be displayed only when in the Modify Standard screen. If a standard dilution is abnormal or when running replicates or the CV is over the limit, you can select to delete that dilution. The standard curve will then be recalculated with the remaining standard dilutions. DO NOT use delete with a 3 pt Standard.  
**NOTE:** When this option is selected, the Original Standard is saved in the Standards List as "Previous" while the Modified Standard is saved as "Current".

### 3. Standard Print Options:

Allows selection of print format used when printing standards.

There are 3 formats that can be selected from as shown in the Print Options Dialog shown below.



- 1) Report: Prints standard line only.
- 2) Test Curves: Prints associated curves only.
- 3) Combination: Prints standard line and associated curves.

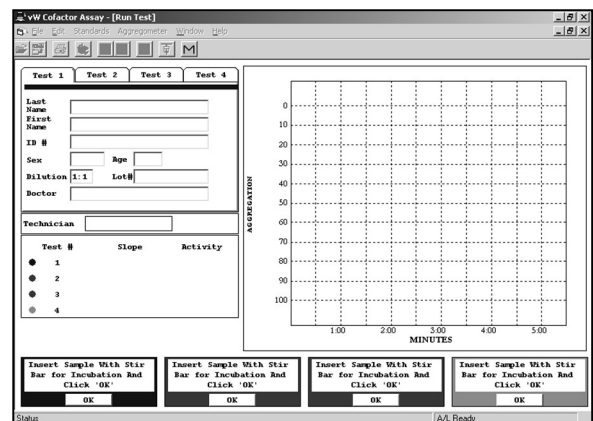
### Aggregometer

The Aggregometer selection is used for Run Test, Run Standard or Connect to AGGRO/LINK®. In Run Test, the program enters a routine for running the Patient Test Sample. A Test Sample cannot be run unless there is a standard curve in memory. In Run Standard, the program enters a routine for running the Standard Dilutions to calculate the Standard Curve.

When the Aggregometer group is selected, the menu below is displayed.



1. Run Test – The Run Test selection will cause the screen shown below to be displayed. The following screen is set for a four channel Aggregometer.



When not running test replications, there will be 1 control box and one information tab box for each channel.

If the system is set to run duplicate tests, the screen will display two test channels but only one information tab box for each set of replications.

The control boxes are arranged vertically across the bottom of the screen. These boxes provide instructions for running the test.

The first instruction given is “Insert Sample With Stir Bar For Incubation And Click OK”. Insert the test sample that is formulated in accordance with the assay procedures. Be sure there is a blank reference sample in the PPP well. After the samples are inserted, press the OK using the mouse.

The next message will be “Incubating” with a clock that will count incubation time. The clock will start at the time set for incubation and count down to zero.

The next message will be:

- 1) “Set Baseline” – Press the Aggregometer’s Set Baseline switch on the channel that corresponds to the message.
- 2) “Add Plasma” – Add the test plasma as specified in the Assay Kit Instructions.
- 3) “Click OK” – The click OK using the mouse.

The next message will be “Running test (X)”. [(X) is the channel number to which the message corresponds.] The test will run from one to five minutes depending on the Run Test Time entered during the standard setup.

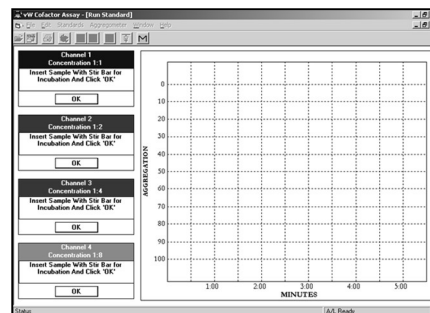
At the completion of the test, the program will respond with “Press OK to continue or RECALC to recalculate”. If you want to save this test, press OK. If the slope calculation is incorrect due to an artifact in the curve, press the “Recalc” button. The start line for the corresponding test will then appear on the test grid. Drag the start line just past the artifact. Press “Recalc” and the slope will be recalculated only on the part of the curve to the right of the start line. Continue this process until slope is calculated accurately.

After the slope calculation is accepted, the program will respond with “OK to save or ABANDON”. If you want to save this test, press OK. If the test is not satisfactory and you want to rerun it, press ABANDON. The program will loop back to the beginning of the procedure and the instruction box will display the first step.

After the test has been saved, the computer will automatically calculate the factor activity and all information will be saved to disk. You can print a hard copy of the test using the print command on the main menu.

## 2. Run Standard

The Run Standard selection will cause the following screen to be displayed. The screen shown is configured for use with a four channel Aggregometer. The screen will display one control box for each channel available.



If the procedure is set to run duplicate Standard Samples, the instruction box will have two of each dilution, referring to one sample as Replication 1 and the other sample as Replication 2.

The first message in the instruction box is “Insert Sample with Stir Bar For Incubation And Click OK”. Insert the Standard Sample that is formulated in accordance with the assay procedures. Be sure there is a blank reference sample in the PPP well. After the samples are inserted, click the mouse on OK.

The next message will be “Incubating” with a clock that will count incubation time. The clock will start at the time set for incubation and count down to zero.

The next message will be :

- a. "Set Baseline" - Press the Aggregometer’s Set Baseline switch on the channel that corresponds to the message.
- b. "Add Plasma" - Add the test plasma as specified in the Assay Kit Instructions.
- c. "Click 'OK' " - Then click OK using the mouse.

The next message will be “Running test (X) Press OK to End”. The test will run from one to five minutes depending on the Test Run Time entered during the standard setup.

At the completion of the test, the program will respond with “Press OK to continue or RECALC to Recalculate”. If you want to save this test, press OK. If the slope calculation is incorrect due to an artifact in the curve, press the 'Recalc' button. The start line for the corresponding test will then appear on the test grid.

Drag the start line just past the artifact. Press "Recalc" and the slope will be recalculated only on the part of the curve to the right of the start line. You may continue this process until a satisfactory slope is achieved.

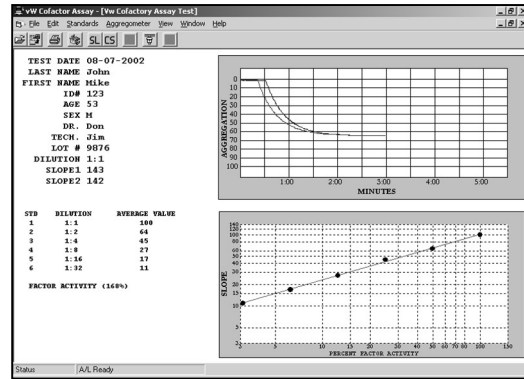
After the slope calculation is accepted, the program will respond with “OK to save or ABANDON Max Slope”. If you want to save this test, press OK. If the test is not satisfactory and you want to rerun it, press ABANDON. The program will loop back to the

beginning of the procedure and the instruction box will display the first step.

After the test has been saved, the computer will automatically calculate the factor activity and all information will be saved to disk. You can print a hard copy of this test using the Print Standard option on the Standard List dialog.

### 3. Connect – Establish connection with Aggregometer

This command is for establishing communication with the Aggregometer. This command is to be used when the program has been running without communication being established and a function requiring the communication, such as run test, is to be performed.



## Window

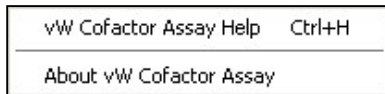
When the Windows group is selected from the menu bar, the menu shown below is displayed.



Selecting or deselecting the status bar Item will display or hide the status bar located across the bottom of the screen.

## Help

When the Help group is selected from the menu bar, the menu shown below is displayed.



vW Cofactor Assay Help - Displays the vW Cofactor Assay programs help file.

About vW Cofactor Assay - Displays Version Information for the vW Cofactor Assay program

## View

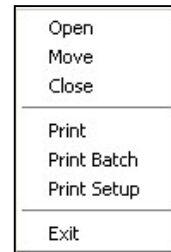
The View Menu Group is only available when a test is open.

Previously run and saved tests can be viewed using the OPEN command from the FILE Menu Group. See the FILE menu group Description earlier in this manual for details on opening tests and use of the Test List dialog.

Below is an example of an opened test document:

When a test is opened for viewing there are several additional menu options available. Each is described below.

With an open test document the FILE menu options will appear as shown below:



The additions to the normal FILE menu options are:

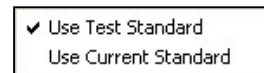
### Close:

Closes the currently active test document.

### Print:

Prints the currently active test document.

An additional menu group labeled 'VIEW' will appear as shown below.

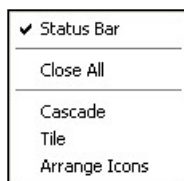


These menu options allow for switching the viewed test reference standard between the standard reference it was run against and the current standard reference.

Use Test Standard - Uses the standard that was loaded when the test was run.

Use Current Standard - Uses the currently loaded standard.

With an open test document the WINDOW menu options will appear as shown below.



The additions to the normal WINDOW menu options are:

Close All - Close all open test documents.

Cascade - Arranges all open test documents in a cascade fashion.

Tile - Arranges all open test documents in a tiles fashion.

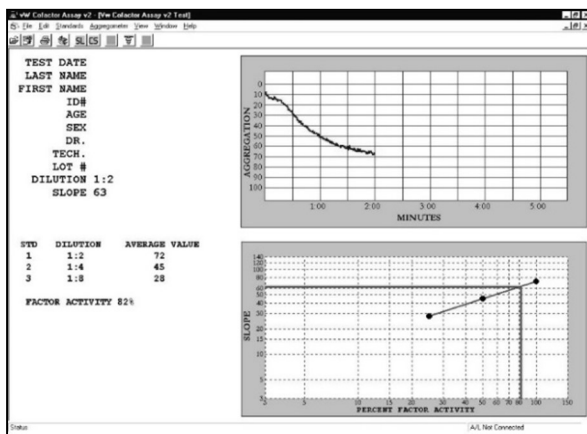
Arrange Icons - Arranges all minimized test documents

## Results

The results are reported as Percent of Factor Activity as determined by the Intersection of the test sample slope on the best fit line of the standard dilutions. The Print Report Command prints the Factor Activity at the bottom of the dialog box and shows the intersection of the test sample slope on the best fit line of the standard dilutions.

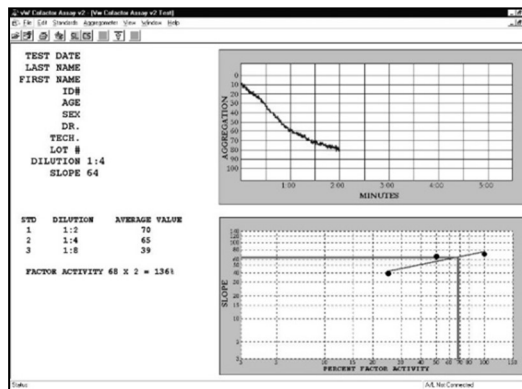
In this section there are three examples for reporting results. In the first example, the factor activity is 82%. This is determined by taking the slope of the test, which is 63, and intersecting the best fit line of the standard curve at 82% factor activity.

This is shown on the graph of the best fit curve. In this example, the test sample was run at the same dilution as the 100% standard dilution.



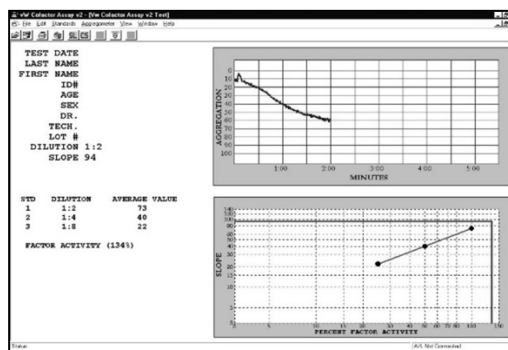
Sometimes, there is a need to run the test sample at a different dilution than the 100% standard dilution. In the second example, the test sample was diluted 1:4 and the 100% standard was diluted 1:2. The slope of the test, which is 64, intersects the best fit line of the standard curve at 68% factor activity. However, since the test sample is twice as diluted as the 100% standard dilution, the factor activity is multiplied by 2, as shown in the calculated Factor Activity at the

bottom of the dialog box. This calculation is done automatically by the program.



The third example shows a test with a slope that is greater than the slope of the 100% standard dilution. Therefore, the intersection point is beyond the best fit line. The program will calculate FACTOR ACTIVITY based on the best fit line continuing linearly to the intersection point. Test results outside the limits of the best fit curve may be inaccurate.

Parenthesis are placed around the calculated FACTOR ACTIVITY to indicate that the results are outside the limits of the best fit curve.



When using the CHRONO-LOG® Cofactor Assay Kit, results of less than 40% are considered abnormal and indicative of von Willebrand disease. However, a normal range should be established by the individual laboratory utilizing its particular instrument/reagent combination.

Although the Ristocetin Cofactor Assay is considered by many investigators to be the most functional test for von Willebrand disease, a complete diagnosis of this disorder should include other factors such as family history, bleeding time, Factor VIII-Rag and VIII:C activity.

If using a kit other than the CHRONO-LOG® Cofactor Assay Kit, check the insert of the reagent kit for expected results.

## DATA BACKUP

For every folder designated to save tests, a pair of data files will be created. This pair will have the same time and date stamp. To back-up the data, it is recommended to use the CHRONO-LOG® software and the File Move feature.

It is recommended to install and run the CHRONO-LOG® software as Administrator for all Windows logins. This will allow common folders for all test data and prevent misplaced data. When run as Standard user with Windows W7, W8 and W10, each user login will have their own set of data folders and not have access to the others. By default these would be located in virtual store:

**Users\user name\appdata\local\virtual store\program files\AGGROLINK8\data.**

Alternative backup for IT personnel.

It is also possible to use Microsoft Windows to backup the data. It is useful to search for the files by partial name such as datafile\*, datafile8, dirfile8. Windows will locate the accessible datafile folders. The folders should be backed up rather than copying the .dat files from the folders since they all have files with the same names but unique contents. Please exercise care with the files to prevent data loss by overwriting the contents of one folder with another.

vW CoFactor Assay data files.

Data files for vW CoFactor Assay are saved in the install folder (standards and .INI file), and the tests are saved in the end user defined data folders.

Data files associated with programs:

**AGGRO/LINK®8** – datafile8.dat, dirfile8.dat, notefile8.dat (not present on older versions)

**vW CoFactor Assay** – vwstd.dat, vwdir.dat, vwtestS.dat, vwtestD.dat

## BIBLIOGRAPHY

Abbate R, Boddi M, Prisco D and Gensini GF: Ability of whole blood aggregometer to detect platelet hyperaggregability. *Am J Clin Pathol* Vol. 91, No. 2 (159-164) February 1989.

Abbate R, Favilla S, Boddi M, Costanzo G and Prisco D: Factors influencing platelet aggregation in whole blood. *Am J Clin Pathol* Vol. 86 No. 1 (91-96) 1986.

Abbate R. and Boddi M: Which test(s) for platelet aggregation? *Proceedings of a European Conference*, Editors: G.V.R. Born, G.G. Neri Serneri, 91-102, Florence, 26-28 March 1987.

Abbate R, Boddi M, Favilla S, Costanzo G, Panicia R, Gensini GF: Whole blood aggregometer in the assessment of platelet hyperaggregability. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Agarwal KC, Buckley RS and Parks Jr RE: Role of plasma adenosine in the antiplatelet action of HL 725, a potent inhibitor of cAMP phosphodiesterase: species differences. *Thromb. Res.* 47 (191-200) 1987.

Allain JP, Cooper HA, Wagner RH, et al: *J. Lab. Clin. Med.* 85:318, 1975

Apitz-Castro R, Escalante J, Vargas R and Jain MK: Ajoene, the antiplatelet principle of garlic, synergistically potentiates the antiaggregatory action of prostacyclin, forskolin, indomethacin and dipyridamole on human platelets. *Thromb. Res.* 42 (303-311) 1986.

Badimon L, Badimon JJ, Galvez A, Turitto V and Fuster V: Platelet interaction with vessel wall and collagen in pigs with homozygous von willebrand's disease associated with abnormal collagen aggregation. *Thrombosis & Haemostasis* 61 (1) 57-64 (1989).

Balduini CL, Bertolino G, Polino G, Gamba G, Sinigaglia F and Ascari E: The effect of red blood cells on platelet aggregation in normal subjects and myelofibrotic patients. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Bandi E, Thaiss H, Choi S, Kunzer W and Sutor AH: Platelet function test in citrated whole blood application and interpretation in pediatric patients. *Auszug aus: Abstracts-Alexander-Schmidt-Gedachnis- Vorlesung 31. Jahrestag der Gesellschaft fur Thrombose-und Hamostaseforschung (GTH) Freiburg i. Br., 18.-21. Februar 1987.*

Biagi G, Coccheri S, Laschi R, Pasquinelli G, Preda P, Fortunato G and Milanese G: Prostanoid production in blood and platelet ultrastructure after administration of indobufene in humans. *Thromb Haemostas* THHADQ 62 (1) 556 (1989) August 19, 1989.

Bizios R, Lai L, Fenton II JW, Sonder SA and Malik AB: Thrombin-induced aggregation of lymphocytes: non-enzymic induction by an hirudin-blocked thrombin exosite. *Thromb. Res.* 38 (425-431) 1985.

Born GVR. Quantitative investigations into the aggregation of blood platelets. *J Physiol Lond* 162, 67 (1962).

Brinkhous KM, Graham JC, Cooper HA, Allain JP and Wagner RH; *Thromb. Res.* 6:267, 1975.

Bult H, Wechsung E, Houvenaghel A and Herman AG: Prostanoids and hemostasis in chickens: anti-aggregating activity of prostaglandins E1 and E2, but not of prostacyclin and prostaglandin D2. *Prostaglandins Vol.* 21, No.6 (1045-1058) June 1981.

Cardinal DC and Flower RJ: The electronic aggregometer: a novel device for assessing platelet behavior in blood. *Jnl. of Pharmac. Meth.* 3 (135-158) 1980.

Catalano M, Belletti S, Russo U and Libretti A: Can the time interval between sampling and testing influence whole blood platelet aggregation? *Thromb Haemostas THHADQ* 62 (1) 114 (1989) August 19, 1989.

Catalfamo JL, Dodds WJ and Fenton II JW: Leupeptin inhibits Ca<sup>2+</sup> mobilization in thrombin-stimulated platelets. *BLOOD*, Vol 72, No. 5, Suppl 1, November 1988.

Challen A, Branch WJ and Cummings JH: Quantitation of platelet mass during aggregation in the electronic (Wellcome) whole blood aggregometer. *Jnl. of Pharmac. Meth.* 8 (115-122) 1982.

Challen AD, Branch WJ and Cummings JH: The effect of aspirin and linoleic acid on platelet aggregation, platelet fatty acid

composition and haemostasis in man. Human Nutrition: Clinical Nutrition 37C (197- 208) 1983.

Challen AD, Branch WJ and Cummings JH: The effect of pectin and wheat bran on platelet function and haemostasis in man. Human Nutrition: Clinical Nutrition 37C (209-217) 1983.

Chamone DAF, Ivany-Silva M, Cassaro C, Bellotti G, Massumoto C and Hoshikawa-Fujimura AY: Guarana (Paullinia cupana) inhibits aggregation in whole blood. Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Chang S, Sanjo K, Idezuke Y, Tanabe A, Yatomi Y, Nakajima K and Kariya T: Effects of Sclerosants for esophageal varices on platelet aggregation. Thromb Haemostas THHADQ 62 1) 560 (1989) August 19, 1989.

Chen ZM and Liu YO: Evidence for ADP release in U46619-stimulated human aequorin-loaded platelets. Thromb Haemostas THHADQ 62 (1) 106 (1989) August 19, 1989.

Chiang TM, Wojcikiewicz RJH, Kang AH and Fain JN: Phosphorylation of the outer surface of platelets enhances the effects of collagen on aggregation, ATP release, calcium translocation and phosphoinositide hydrolysis. Thrombosis Res. 50; 719-731, 1988.

CLSI Document H58-A, Vol. 28 No. 31. Platelet Function Testing by Aggregometry; Approved Guideline.

Coeffier E, Joseph D and Vargaftig BB: Platelet-leukocyte interaction: Activation of rabbit platelets by FMLP-stimulated neutrophils. Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Colgan SP, Thrall MAH and Gasper PW: Platelet aggregation and ATP secretion in whole blood of normal cats and cats homozygous and heterozygous for chediak-higashi syndrome. Blood Cells 15:596-599 (1989)

Cosgriff TM, Canonico PG, Hodgson L, Parrish D, Chapman T, Huggins JW, Gong ZJ, Xiang LB and Hsiang CH: Ribavirin: Studies of the effects of the antiviral drug on platelet function. Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Craveri A, Colombo L, Lanfredini M, Citella C, Picollo S, De Angeli B, Milanesi G and Lavezzari M: Effect of indobufen on whole blood platelet aggregation recorded in the morning in patients with ischaemic heart disease. The Jnl of International Medical Research 1989; 17: 381-387.

Davis RB, McKinney M\*, Boyd DG\* and Jones CC\*: Effects of exercise and exercise conditioning on blood platelet function. BLOOD, VOL 72, No. 5, Suppl 1, Abstract 1192, November 1988.

Dawicki DD, Agarwal KC and Parks Jr RE: Potentiation of the antiplatelet action of adenosine in whole blood by dipyridamole or dilazep and the cAMP phosphodiesterase inhibitor, RA 233. Thromb. Res., 43, (161-175) 1986.

Dawicki DD, Agarwal KC and Parks Jr RE: Role of adenosine uptake and metabolism by blood cells in the antiplatelet actions of dipyridamole, dilazep and nitrobenzylthioinosine. Biochem. Pharmac. Vol. 34, No. 22 (3965-3972) 1985.

de la Cruz JP, Bellido I, Camara S and Sanchez de la Cuesta F: Aspirin sex difference on platelet aggregation in human whole blood: Influence of estradiol and thromboxane levels. Rev. Farmacol Clin. Exp 1987; 4(2): 109-113.

de la Cruz JP, Bellido I, Camara S, Martos F and Sanchez de la Cuesta F: Effects of acetylsalicylic acid on platelet aggregation in male and female whole blood: an in vitro study. Scand J Haematol 1986; 36:394- 397.

de la Cruz JP, Pavia J, Bellido I and Sanchez de la Cuesta F: Effects of triflusal and acetylsalicylic acid on platelet aggregation in human whole blood: Influence of red blood cells and leukocytes. Meth and Find Exptl Clin Pharmacol 1988; 10(6): 363-367.

de la Cruz JP, Pavia J, Garcia-Arnes J. and Sanchez de la Cuesta F: Effects of Triflusal and acetylsalicylic acid on platelet aggregation in whole blood of diabetic patients. Eur J. Haematol 1988;40:232-236.

de la Cruz Cortes JP, Martos Crespo E, Bellido Estevez I and Sanchez de la Cuesta F: Inhibicion in vitro con dipiridamol de la agregacion plaquetaria inducida por ADP en sangre total y plasma. Rev Farmacol Clin Exp 1985; 2(1): 51-53.

de la Cruz JP, Camara S, Bellido I, Carasco T & Sanchez de la Cuesta F: Platelet aggregation in human blood after chronic administration of aspirin. Thromb. Res. 46; 133-140, 1987.

de la Cruz JP, Pavia J, Bellido I, Gonzalez MC and Sanchez de la Cuesta F: Platelet Antiaggregatory effect of triflusal in human whole blood. Meth and Find Exptl Clin Pharmacol 1988, 10(4): 273-277.

Del Maschio A, Chen ZM, Evangelists V, Rajtar G, Cerletti C and de Gaetano G: Effects of activated polymorphonuclear leukocytes on platelet function. Thromb Haemostas THHADQ 62 (1) 283 (1989) August 19, 1989.

Del Maschio A, Albors M, Bucchi F, Tomasiak M, Bertele V, Cerletti C and de Gaetano G: Human polymorphonuclear leukocyte activation induced by platelet activating factor (PAF). Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Diodati J, Theroux P, Latour JG, Lacoste L and Waters D: La nitroglycerine a doses therapeutiques inhibe l'agregation plaquettaire chez l'humain. Journee de la recherche 20 mai 1988.

Diodati J, Theroux P, Latour JG, Lacoste L, Pelletier GB, Roy D and Waters DD: Nitroglycerin at therapeutic doses inhibits platelet aggregation in man. Journal American College Cardiol II: 54A, 1988.

Doerger PT, Glueck HI, van der Bel-Kahn J, Taylor A and Goldman L: The comparative effects of the argon, nd:yag, and argon-pumped dye lasers on human platelets and erythrocytes in vitro. Lasers in Surgery and Medicine 5:457-468 (1985)

Doerger PT, Glueck HI and McGill M: The effects of argon laser on in vitro aggregation of platelets in platelet rich plasma and whole blood. Thromb. Res. 50; 657-667, 1988.

Dorn GW: Distinct platelet thromboxane A2/prostaglandin H2 receptor subtypes. A radioligand binding study of human platelets. J. Clin. Invest, Volume 84, December 1989, 1883-1891.

Dutrillaux F, Lecompte T and Samama M: Impairment of platelet behaviour by isosorbide dinitrate. *Thrombosis & Haemostasis* 61 (1) 152 (1989).

Ehrlich YH, Snider RM, Kornecki E, Garfield MG and Lenox RH: Modulation of neuronal signal transduction systems by extracellular ATP. *J. Neurochem.*, Vol. 50, No. 1, (295-301) 1988.

Erne P, Schachter M, Fabbro D, Miles CMMM and Sever PS: Calcium transients in human platelets monitored by aequorin, fura-1 and quin-2: Effects of protein kinase C activation and inhibition. *Biochemical and Biophysical Research Communications*. Vol. 145, No. 1, 1987, Pg. 66-72, May 29, 1987.

Favaloro EJ, Koutts J: Laboratory Assays for von Willebrand Factor: Relative Contribution to the Diagnosis of von Willebrand's Disease. *Pathology*, 29, 1997.

Favier R, Lecompte T, Morel MC, Potevin F, Benoit P, Boucheix C, Kaplan C and Samama M: Calcium rise in human platelets elicited by anti-CD9 and -CD41 murine monoclonal antibodies. *Thrombosis Research*, May 1989.

Feinman RD, Lubowsky J, Charo I, and Zabinski MP: The lumi-aggregometer: A new instrument for simultaneous measurement of aggregation and secretion. *J Lab Clin Med* 90, 125, (1977).

Flower RJ and Cardinal DC: Use of a novel platelet aggregometer to study the generation by, and actions of, prostacyclin in whole blood. *Prostacyclin*, Chap. 20, edited by Vane JR and Bergstrom S, Raven Press, NY c 1979.

Forsythe LT, Jackson ML, Meric SM: Whole blood platelet aggregation in uremic dogs. *Am J Vet Res*, Vol 50, No. 10, pgs. 1754-1757, Oct. 1989.

Fujimoto T, Fujimura K and Kuramoto A: Measurement of platelet ionized calcium in patients with myeloproliferative disorders by aequorin method. *Thromb Haemostas, THHADQ* 58-1 (1-676) July 6, 1987.

Galante A, Romitelli S, Sommariva L, Iellamo F and Lenci R: Effects of sodium citrate concentration, of time and temperature incubation on platelet aggregation of blood by using impedance aggregometer. *G.I. Pat. Clin.*, Vol. II, No. 3, (57-59), September 1987.

Galante A, Sommariva L, Pocobelli D, Iellamo F, Romitelli S, Ruscitti G, Lenci R and Cannata D: L' aggregazione piastrinica con metodo impedenziometrico nel sangue in toto e nel plasma ricco in piastrine. Risultati di uno studio su soggetti normali. *MEDICINA* Vol. 77, N. 11 (503-507) Novembre 1986.

Galante A, Lenci R and Romitelli S: Platelet aggregation on PRP with optical method and by using impedance aggregometer. *G.I. Pat. Clin.*, Vol. II, No. 3, (49-52), September 1987.

Galvez A, Badimon L, Badimon JJ and Fuster V: Electrical aggregometry in whole blood from human, pig and rabbit. *Thromb. & Haemos*. 56-2 (128- 132) 1986.

George JN, Nurden AT and Phillips DR: *N. Eng. J. Med.* 311:1084, 1984.

Gewirtz H, Steiner M, Sasken H and Most AS: Measurement by electrical impedance aggregometry of porcine platelets response to selected physiological agonists. *Proc. of the Soc. Exp. Biol. & Med.*, 179, (324- 330) 1985.

Glazier J: Measurement of platelet aggregation in whole blood. *ACPR* (26-30) April 1987.

Gordge MP, Dodd NJ, Rylance PB and Weston MJ: An assessment of whole blood impedance aggregometry using blood from normal subjects and haemodialysis patients. *Thromb. Res*. 36 (17-27) 1984.

Gresele P, Arnout J, Janssens W, Deckmyn H, Lemmens J and Vermynen J: BM 13.177, A selective blocker of platelet and vessel wall thromboxane receptors, is active in man. *Lancet* (991-994) May 5, 1984.

Gresele P, Zoja C, Deckmyn H, Arnout J, Vermynen J and Verstraete M: Dipyridamole inhibits platelet aggregation in whole blood. *Thromb Haemostas* 50-4 (852-856) 1983.

Gresele P, Vezza R and Nenci GG: Inhibition of cytoplasmic calcium movements in stimulated platelets by picotamide. *Thromb Haemostas THHADQ* 62 (1) 552 (1989) August 19, 1989.

Gresele P, Deckmyn H, Arnout J, Zoja C and Vermynen J: Lack of synergism between dazoxiben and dipyridamole following administration to man. *Thromb. Res*. 37 (231-236) 1985.

Gresele P, Arnout J, Deckmyn H and Vermynen J: Mechanism of the antiplatelet action of dipyridamole in whole blood: Modulation of adenosine concentration and activity. *Thromb. & Haemos*. 55-1 (12-18) 1986.

Gresele P, Deckmyn H, Arnout J and Vermynen J: Platelet inhibitory activity of prostacyclin in the presence of erythrocytes as studied with the impedance aggregometer. *BR Jnl. Haem.* 57 (171-173) 1984.

Gresele P, Arnout J, Deckmyn H and Vermynen J: Combining antiplatelet agents: Potentiation between aspirin and dipyridamole. *Lancet* (937-938) April 20, 1985.

Gresele P, Van Houtte E, Arnout J, Deckmyn H and Vermynen J: Thromboxane synthase inhibition combined with thromboxane receptor blockade: A step forward in antithrombotic strategy? *Thromb Haemostas* 52-3 (364) 1984.

Hansen JB, Olsen JO, Wilsgard L and Osterud B: The effect of cod liver oil intake on the stimulation of blood cells. *Thromb Haemostas THHADQ* 58-1 (1-676) July 6, 1987.

Hardart GE, Sullivan GW, Carper and Mandell GL: Adenosine inhibits human neutrophil (PMN) bactericidal function. *Clinical Research*, Vol. 37, No. 1, 1989.

Hardeman MR and Vreeken J: Transient aggregation resistance of human platelet-rich plasma; a neglected in vitro phenomenon with physiological impact. *Thromb Haemostas, THHADQ* 58-1 (1-676) July 6, 1987.

Hattori A, Nagayama R, Fuse I, Takeshige T, Takizawa S and Shibata A: Further characterization of platelet release mechanism

defect with defective A23187 aggregation. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Heptner W: Platelet aggregation in citrated whole blood determined by impedance in the Chrono-log whole blood aggregometer. Auszug aus: Abstracts-Alexander-Schmidt-Gedachnis-Vorlesung 31. Jahrestag der Gesellschaft fur Thrombose- und Hamostaseforschung (GTH) Freiburg i.Br., 18.-21. Februar 1987.

Heptner W, Suarez JR and Lutgendorf V: Studies on platelet aggregation by impedance aggregometry and ATP secretion in non-anticoagulant blood. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Herman AG and Bult H: Red blood cell lysis may influence platelet aggregation in whole blood aggregometer. *Thromb Haemostas* 58-1 (1-676) July 6, 1987.

Hornstra EG, Hennissen AH, Kalafusz R and Tan DTS: The antithrombotic effect of palm oil is correlated with its contents of Vitamin E. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Hossmann V, Schafer HJ, Auel H and Etti H: Treatment of advanced stages of peripheral obliterative disease with the thromboxane receptor antagonist BM 13.177: A placebo-controlled double blind study. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Houwelingen AC, Hennissen A, Verbeek-Schippers F, Simonson T, Fischer S and Horstra G: Influence of dietary fish on platelet function and thromboxane formation. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Hung TC, Butter DB, Graham MF, Lewis JH, Zhao JQ and Haubold AD: Platelet alterations in shear flow: Dose response of prostacyclin on human platelets. *Trans. Am. Soc. Artif. Intern. Organs*, Vol. XXIX (436-441) 1983.

Ingerman-Wojenski CM and Silver MJ: A quick method for screening platelet dysfunctions using the whole blood lumi-aggregometer. *Thromb Haemos*. 51-2 (154-156) 1984.

Ingerman-Wojenski CM, Smith JB and Silver MJ: Difficulty in detecting inhibition of platelet aggregation by the impedance method. *Thromb. Res*. 28 (427-432) 1982.

Ingerman-Wojenski CM, Smith JB and Silver MJ: Evaluation of electrical aggregometry: Comparison with optical aggregometry, secretion of ATP, and accumulation of radiolabeled platelets. *Jnl. Lab. & Clin. Med.*, Vol. 101, No. 1, (44-52) January, 1983.

Ingerman-Wojenski CM and Silver MJ: Model system to study interaction of platelets with damaged arterial wall. II. Inhibition of smooth muscle cell proliferation by dipyrindamole and AH-P719. *Exper and Molec Pathology* 48, (116-134) 1988.

Ingerman-Wojenski CM: Simultaneous measurement of platelet aggregation and the release reaction in platelet-rich plasma and in whole blood. *Jnl. Med. Tech*. 1:9 (697-701) Sept. 1984.

Ingerman-Wojenski CM, Smith JB and Silver MJ: Use of the whole-blood aggregometer to study the aggregation of "giant" platelets. *Thromb. Res*. 27 (371-376) 1982.

Ingham Medical Center, Lansing, MI.

Isobe J and Mizuno A: Impedance platelet aggregometry using whole blood as a test of vascular function. *Thromb Haemostas* THHADQ 62 (1) 446 (1989) August 19, 1989.

Jackson ML, Searcy GP and Olexson DW: The effect of oral phenylbutazone on whole blood platelet aggregation in the dog. *Can J Comp Med* 49 (271- 277) 1985.

Jeremy JY, Barradas MA, Mikhailidis DP and Dandona P: An investigation into the effects of nifedipine and nimodipine on platelet function and vascular prostacyclin synthesis. *Drugs Exptl. Clin. Res*. XI (9) (645- 651) 1985.

Johnson MF and Davis RB: Effects of blood dilution on electrical impedance aggregometry. *Thromb. Res*. 42 (855-857) 1986.

Johnson PC, Ware JA and Salzman EW: Concurrent measurement of platelet ionized calcium concentration and aggregation. Studies with the lumi-aggregometer. *Thromb. Res*. 40-4 (435-443) 1985.

Jones RJ, Delamothe AP, Curtis LD, Machin SJ and Betteridge DJ: Measurement of platelet aggregation in diabetics using the new electronic platelet aggregometer. *Diabetic Medicine*, Vol. 2, (105-109) 1985.

Joseph R, Welch KMA, D'Andrea G and Levine SR: Epinephrine does not induce platelet aggregation in citrated whole blood. *Thromb. Res*. 45 (871-872) 1987.

Kaack MB, Dowling KJ, Patterson GM and Roberts JA: Immunology of pyelonephritis VIII. *E. coli* causes granulocytic aggregation and renal ischemia. *The Journal of Urology*, Vol. 136 (1117-1122) November 1986.

Kini RM and Evans HJ: Correlation between the enzymatic activity, anticoagulant and antiplatelet effects of phospholipase A2 isoenzymes from *naja nigricollis* venom. *Thrombosis & Haemostasis*, 60 (2) 170-173 1988.

Kini RM and Evans HJ: Mechanism of platelet effects of cardiotoxins from *Naja nigricollis* *crawshawii* (spitting cobra) snake venom. *Thromb. Res*. 52; 185-195, 1988.

Kornecki E, Lenox RH, Hardwick DH, Bergdahl JA and Ehrlich YH: Interactions of the alkyl-ester-phospholipid, platelet activating factor (PAF) with platelets, neural cells, and the psychotropic drugs triazolobenzodiazepines. *Molecular Mechanisms of Neuronal Responsive-ness Advances in Experimental Medicine & Biology*. Vol 221 Editor YH Erlich, RM Lennox E, Kornecki, WO Barres. Plenum Press New York 1987.

Kornecki E and Ehrlich YH: Neuroregulatory and neuropathological actions of the ether-phospholipid platelet-activating factor. *Science*, Vol. 240 (1792-1794) June, 1988.

Kornecki E and Ehrlich YH: Platelet activating factor (PAF) stimulates calcium uptake in intact neural cells. Abstract Transactions of the Amer. Soc. for Neurochemistry, Vol. 17, Number 1, March, 1986.



Lacoste L, Latour JG, Theroux P and Waters DD: Hyperagregabilite plaquettaire et leucocytaire dans l'angine instable. Med Sci. (Suppl 1): 39A 1988.

Lages B and Weiss HJ: Time dependence of aequorin-indicate calcium levels in stimulated and unstimulated platelets: Evidence for multiple aequorin environments in platelets. Thromb & Haemostasis, (Stuttgart) 62(4) 1094-1099 (1989).

Lages B and Weiss HJ: Time dependence of aequorin-indicated calcium signals in stimulated and unstimulated platelets. Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Latour JG, Lacoste L, Theroux P, Leger-Gauthier C and Waters DD: Platelet hyperaggregability in patients with unstable angina. Clin. Invest, Med II: D77 1988.

Lecrubier C, Girard P, Noire P and Samama M: Comparative study of platelet aggregation measured with 3 different methods (photometric and impedance) in ticlopidine treated patients. Agents & Actions Supplements, Vol. 15 (60-67) 1984.

Lefer AM, Tsao P, Aoki N and Palladino MS, Jr: Mediation of cardio-protection by transforming growth factor-B. Science, Vol. 249, 6 July 1990, pages 61-64.

Lehmann K, Groscurth P, Vollenweider I, von Felten A and Rhyner K: Morphologic alterations of blood cells in the impedance aggregometer. Blood Cells 11 (325-336) 1985.

Libersan D, Dumont L, Chartrand C, Levy E and Garceau D: Experimental evidences that the calcium antagonist, benzothiazepine analog, TA-3090 inhibits platelet aggregation. The FASEB Journal, Abstracts 3341-5632, Volume 4, Number 4, February 28, 1990.

Lifshitz MS and De Cresce RP: The Chrono-log Whole-Blood Lumi-Aggregation System. Laboratory Medicine Vol 18, No 12 (858-860) December, 1987.

Liu S and Sylvester DM: Mechanisms of antiplatelet effect of tetramethyl-pyrazine. The FASEB Journal, Abstracts 3341-5632, Vol. 4, Number 4, February 28, 1990.

Mackie IJ, Bihari D and Machin SJ: Increased platelet aggregability in native whole blood in the adult respiratory distress syndrome (ARDS). Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Mackie IJ and Machin SJ: Platelet aggregation measurement. MLW (19-22) May 1984.

Mackie IJ, Jones R and Machin SJ: Platelet impedance aggregation in whole blood and its inhibition by antiplatelet drugs. J Clin Pathol 37 (874-878) 1984.

Mammen EF, Koets MH, Washington BC, Wolk LW, Brown JM, Burdick M, Selik NR and Wilson RF: Hemostasis changes during cardiopulmonary bypass surgery. Sem. in Thromb. & Hemos. Vol. 11, No. 3, (281-292) 1985.

Mannucci L, Redaelli R and Tremoli E: Effects of aggregating agents and of blood cells on the aggregation of whole blood by impedance technique. Thrombosis Research 52; 143-151. 1988.

Mannucci L, Redaelli R and Tremoli E: Effects of blood cells on platelet aggregation by impedance method. Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Mascelli MA, Worley S, Veriabo NJ, Lance ET, Mack S, Schaible T, Weisman HF and Jordan RE: Rapid Assessment of Platelet Function With a Modified Whole-Blood Aggregometer in Percutaneous Transluminal Coronary Angioplasty Patients Receiving Anti-GP IIB/IIIa Therapy. Circulation Vol. 96, No. 11, December 2, 1997.

Matsuno K, Katabami F, Koyama M, Abe K, Sakurada K, Miyazaki T, Ozasa S, Saitoh H, Maekawa I and Matsumiya H: Platelet-activating factor (PAF)-induced intracellular Ca<sup>2+</sup> mobilization in human platelets. Thromb Haemostas, THHADQ, 58-1 (1-676) July 6, 1987.

McManama G, Lindon JN, Kloczewiak M, Smith MA, Ware JA, Hawiger J, Merrill EW and Salzman EW: Platelet aggregation by fibrinogen polymers crosslinked across the E domain. BLOOD, Vol 68, No. 2 (363-371) 1986.

Mikhailidis DP, Barradas MA, Epemolu O and Dandona P: Ethanol ingestion inhibits human whole blood platelet impedance aggregation. Am J Clin Pathol, Vol. 88, No. 3, (342-345) 1987.

Mikhailidis DP, Barradas MA and Dandona P: Platelet aggregation in diabetics using the new electronic platelet aggregometer. Diabetic Medicine 2 (411-412) 1985.

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

Miller JL, Kupinski JM, Castella A, Ruggeri ZM. Von Willebrand factor binds to platelets and induces aggregation in platelet-type but not type IIB von Willebrand disease. J Clin Invest. 1983;72;1532-1542.

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

Morel MC, Lecompte T, Champeiz P, Favier R, Potevin F, Samama M, Salmon C and Kaplan C: PL2-49, a monoclonal antibody against glycoprotein IIb which is a platelet activator. British Journal of Haematology, 1989, 71, 57-63.

Mori K, Suzuki S, Sugai K, Akutsu Y, Ishikawa M, Sakai H and Hiwatashi K: Intracellular Ca<sup>++</sup> mobilization in gray platelet syndrome. Electronmicroscopic studies on aequorin loaded platelets. Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Morisato DK and Grainick HR.: Blood 55:9, 1980

Moreno A, de la Cruz JP, Garcia Campos J and Sanchez de la Cuesta F: Effect of dipyridamole + ASA on the retine vascular pattern of estreptozotocin-diabetic rats. Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.

Musumeci V, Cremona G, Baroni S, Bisbano A, Tutinelli F and Zuppi C: Inhibitory interference of red cells in the measurement of whole blood platelet aggregation by the impedance method. Thromb. Res. 45 (95-100) 1987.

Nagayama R, Hattori, Fuse I, Takeshige T. Takizawa S and Shibata A: Platelet ionized calcium mobilization (aequorin method) in

patients with primary platelet dysfunctions. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987

Nakano T, Terawaki A and Arita H: Measurement of thromboxane A<sub>2</sub>-induced elevation of ionized calcium in collagen-stimulated platelets with the photoprotein, aequorin. *J. Biochem*: 99-4 (1285-1288) 1986.

Nelson SD, Moriarty AJ, Hughes R and Balnave K: Transitory influence of fibrinogen/fibrin degradation products (FDPs) on platelet aggregation. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Nguyen P, Beduchaud MP, Droulle G, and Potron G: Difficulties of Interpretation of platelet aggregation in whole blood: A comparison between two techniques. *Thromb Haemostas* THHADQ 61 (1) 358 (1989) August 19, 1989.

Nichols WL, Kaese SE, Gastineau DA, Otteman LA and Bowie EJW: Bernard- Soulier syndrome: Whole blood diagnostic assays of platelets. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

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.

Nidorf SM, Sturm M, Strophair J, Kendrew PJ and Taylor RR: Whole blood aggregation, thromboxane release and the lyso derivative of platelet activating factor in myocardial infarction and unstable angina. *Cardiovascular Research* Volume XXIII, No. 4, pages 273-278 April 1989.

Okazaki M, Zhang L, Suzuki M and Sakamoto K: The measurement of platelet aggregation and ATP-release in mice with liver damage induced by carbon tetrachloride (CCl<sub>4</sub>) using a whole blood aggregometer. *Japan J. Pharmacol.* 48,407-415 (1989)

Okuma M, Kanaji K, Sensaki S and Uchino H: Effects of diltiazem on cytoplasmic calcium ion concentration and Arachidonic acid metabolism in human platelets and neutrophils. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Okuma M, Kanaji K, Sensaki S and Uchino H: Effects of diltiazem on cytoplasmic calcium ion concentrations and Arachidonic acid metabolism in activated human platelets. *Arzneimittel-Forschung/Drug Research* 39 (1), 372-375 (1989).

Olson JD, Brockway WJ, Fass DN, Magnuson MA and Bowie EJW: *Am. J. Clin. Path.* 63:210, 1975.

Oura Y, Sakiyama N, Ueshima R, Higuchi M, Kakishita E and Nagai K: Effect of platelet activating factor (PAF) on the collagen induced platelet aggregation in whole blood. *Thromb Haemostas*, THHADQ 58-1 (1- 676) July 6, 1987.

Ozaki Y, Matsumoto Y, Tatomi Y, Higashihara M, Kariya T and Shoji K: Effects of five anion channel blockers on thrombin-and ionomycin- activated platelet functions. *Biochemical Pharmacology*, Vol. 38, No. 13 pp. 2147-2152, 1989.

Ozaki Y and Kume S: Functional responses of aequorin-loaded human neutrophils. Comparison with fura-2-loaded cells, *Biochimica et Biophysica Acta*, 972 (1988) 113-119 Elsevier.

Parks Jr RE, Dawicki DD, Agarwal KC, Chen SF and Stoeckler JD: Role of nucleoside transport in drug action. The adenosine deaminase inhibitor, deoxycytosine, and the antiplatelet drugs, dipyridamole and diltiazem. *Annals NY Acad. Sciences* 451 (188-203) 1985.

Perzborn E: The effect of calcium entry blockers on intracellular free calcium (CA) i increase and ATP secretion during collagen induced platelet aggregation. *Thromb Haemostas* THHADQ 62 (1) 407 (1989) August 19, 1989.

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

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

Ramsey R and Evatt BL: *Am. J. Clin. Path.* 72:996, 1979.

Rand ML, Hennissen AAHM and Hornstra G: Effects of dietary sunflowerseed oil and marine oil on platelet membrane fluidity, arterial thrombosis, and platelet responses in rats. *Atherosclerosis*, 62 (267-276) 1986.

Rao AK, Kowalska MA and Disa Jyoti: Impaired cytoplasmic ionized calcium mobilization in inherited platelet secretion defects. *BLOOD*, Vol 74, No. 2 (August 1), 1989: pp 664-672.

Riess H, Braun G, Brehm G and Hiller E: Critical evaluation of platelet aggregation in whole human blood. *Am J Clin Pathol*, Vol. 85, No. 1, (50-56) 1986.

Russell-Smith NC, Flower RJ, and Cardinal DC: Measuring platelet and leucocyte aggregation/adhesion responses in very small volumes of whole blood. *Jnl. of Pharmac. Meth.* 6 (315-333) 1981.

Saba SR, Saba HI and Morelli GA: Effect of collagen and citrate on heparin-induced platelet anti-aggregatory activity. *Thromb. Haemostas*, THHADQ 58 (1), 1-676, July 6, 1987.

Saitoh M, Ware JA, Watkins SC, Slayter HS and Salzman EW: Localization of aequorin in human platelets by autoradiography and immuno- histochemistry. *THHADQ* 62 (1) 1-647 (1989).

Saitoh M, Ware JA, Watkins SC, Slayter HS and Salzman EW: Localization of aequorin in human platelets by autoradiography and immunohisto-chemistry. *Thromb Haemostas* THHADQ 62 (1) 485 (1989)

Saitoh M, Ware JA and Salzman EW: Relation of fibrinogen binding to changes in cytoplasmic CA<sup>2+</sup> in activated platelets. *Thromb Haemostas* THHADQ 62 (1) 98 (1989) August 19, 1989.

Salzman EW, Ware A and Johnson PC: Cytoplasmic ionized calcium in blood platelets. *Thromb. & Haemos.* No 1, Vol 54, July 14, 1985.

Salzman EW and Ware JA: Ionized calcium as in intracellular messenger in blood platelets. *Progress in Hemostasis & Thrombosis*, Volume 9, (177-202), 1989.

Salzman EW, Johnson P and Ware JA: Measurement of platelet ionized calcium. *Nouv Rev Fr Hematol*: 27 (205-209) 1985.

Sathiropas P, Marbet GA, Sahaphong S, Duckert F: Detection of small inhibitory effects of acetylsalicylic acid (ASA) by platelet impedance aggregometry in whole blood. *Thrombosis Research* 51: 55-62, 1988.

Saxon DJ and Hammond CE: Platelet response in hypercholesterolemic blood to thrombin-induced aggregation. *The FASEB Journal*, Abstracts 3341-5632, Volume 4, Number 4, February 28, 1990.

Schafer AI, Maas AK, Ware JA, Johnson PC, Rittenhouse SE and Salzman EW: Platelet protein phosphorylation, elevation of cytosolic calcium, and inositol phospholipid breakdown in platelet activation induced by plasmin. *J. Clin. Invest.*, Vol. 78 (73-79) July 1986.

Schincio PC, Fusaro A, Bazzan M, Pannocchia A, Pileri A and Tamponi G: Interference of monoclonal immunoglobulins on platelets and whole blood aggregation. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Schincio PC, Fusaro A, Marranca R, Battaglio S, Bazzan M, Pannocchia A, Tamponi G and Pileri A: Paraproteinaemias and platelet aggregation: role of whole blood aggregometry. *Thrombosis Research* 55: 267-277, 1989.

Sebastiano C, Bromberg M, Breen K, Hurford MT: *Case Report* Glanzmann's thrombasthenia: report of a case and review of the literature. *Int J Clin Exp Pathol* 2010;3(4):443-447

Sieders FC, Houwelingen AC and Hornstra G: Measuring whole blood platelet aggregation and ATP-release with a Chrono-log whole blood lumi-aggregometer: Effect of storage time of blood samples. *Thromb Haemostas*, THHADQ 58-1 (1-676) July 6, 1987.

Sinusas AJ, Blyton JB, Tribble CG, Sullivan GW,, Bergin JD, Ross G, Greenwald TP, Mandell GL and Beller GA: Effects of intravenous pentoxifylline on ischemic injury in a canine occlusion/reperfusion model. Poster Session 4/29/89, *Clinical Research*, Vol. 37, No. 2, 1989.

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

Snook JT, Lee JS and Wardlaw GM: Effects of dietary fatty acids on platelet (PLAT) function, plasma thromboxane B2 (TXB2) levels, and PLAT phospholipid (PL) fatty acids. *The FASEB Journal*, Abstracts 1-3340, Volume 4, Number 3, February 26, 1990.

Sugiyama T, Okuma M, Ushikubi F, Sensaki S, Kanaji K and Uchino H: A novel platelet aggregating factor found in a patient with defective collagen-induced platelet aggregation and autoimmune thrombocytopenia. *BLOOD*, 69-6 (1712-1720) June, 1987.

Swart SS, Pearson D, Wood JK and Barnett DB: Effects of adrenaline and alpha adrenergic antagonists on platelet aggregation in whole blood: evaluation of electrical impedance aggregometry. *Thromb. Res.* 36, (411- 418) 1984.

Sweeney JD, Hoernig L, Masferrer E and Fitzpatrick J: A study of the relationship between platelet function in the donor and platelets pheresis harvest. *BLOOD*, Vol. 70, No. 5, Suppl. 1, Abstract 1192, November 1987.

Sweeney J, Labusetta J and Fitzpatrick: Comparison of aggregation responses of stored platelets using impedance and optical

techniques. *BLOOD*, VOL 72, No. 5, Suppl 1, Abstract, November 1988.

Sweeney JD: Platelet aggregation in whole blood. Banff 1989, Laboratory Hematology & Platelet & Phagocyte Activation. Abstract, April 16-20, 1989.

Sweeney JD, Labuzetta JW, Bernstein ZP, Bielat KL and Fitzpatrick JE: Ristocetin-induced platelet aggregate formation and adherence to the probe of an impedance aggregometer. *Amer. Jnl of Clin. Path.*, Vol. 93, No. 4, pp. 548-551, April 1990.

Sweeney JD, Piedmont MP, Hoernig LA, Fitzpatrick JE (Intr. by M.A. Stein): The effect of salsalate on bleeding time, platelet aggregation in whole blood and the release reaction. *BLOOD*, Vol. 72, No. 5, Suppl 1, Abstract, November 1988.

Sweeney JD, Labuzetta JW and Fitzpatrick JE: The effect of the platelet count on the aggregation response and adenosine triphosphate release in an impedance lumi-aggregometer. *Amer. Jrl. of Clin. Pathology*, Vol. 89, No. 5, (655-659) May 1988.

Sweeney JD, Hoernig LA, Behrens AN, Novak E and Swank RT: Von Willebrand's Variant (type II Buffalo). Thrombocytopenia after desmopressin but absence of in vitro hypersensitivity to Ristocetin. *Amer. Jnl. of Clin. Path.*, Vol. 93, No. 4, pp. 522-525, April 1990.

Sweeney JD, Hoernig, Fitzpatrick JE, (Intr. by M.A. Stein) Whole Blood Aggregation in von Willebrand's Disease. *BLOOD*. Vol. 72, No. 5, Supp 1, Abstract, November 1988.

Sweeney JD. Hoernig LA, Fitzpatrick JE: Whole Blood Aggregation in Von Willebrand Disease. *American Journal of Hematology* 32:190-193 (1989)

Sweeney JD, Hoernig LA, Michnik A and Fitzpatrick JE: Whole Blood Aggregometry. *A.J.C.P.*, pp. 676-679, November 1989,

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.

Sylvester DM and Craig, Johnston A: Effects of estrogen modulation on platelet aggregation. *The FASEB Journal*, Abstracts 3341-5632, Volume 4, Number 4, February 28, 1990.

Tahamont MV, Gee MH and Flynn JT: Aggregation and thromboxane synthesis and release in isolated sheep neutrophils and lymphocytes in response to complement stimulation. *Prostaglandins Leukotrienes & Med.* 16 (181- 190) 1984.

Takimoto G, Glang J, Lee GK and Bradlow BA: Plasma fibrinolytic activity after ingestion of omega-3 fatty acids in human subjects. *Thrombotic Research* 54: 573-582, 1989.

Teng CM, Ko FN and Yu SM: Action mechanisms of antiplatelet agents isolated from chinese herbs. *Thromb Haemostas THHADQ* 62 (1) 560 (1989) August 19, 1989.

Thomas JS, McConnell MF and Bell TG: Abnormal platelet Ca Mobilization and cytosolic pH changes in basset hound hereditary thrombopathy. *The FASEB JOURNAL*, Abstracts 3341-5632, Volume 4, Number 4, February 28, 1990.

Tschope D, Kaufmann L, Roesen P, Ohlrogge R and Gries FA: Influence of a single dose of netrendipine on whole blood. Platelet activity in health subjects. *Jnl. of Cardiovascular Pharmacology*, 12 (Suppl. 4): S167-S169 1988.

Ueshima R, Suehiro A, Tanaka H, Kakishita E, K. Nagai and Hatake K: Evaluation of antiplatelet effect of a low dose of aspirin with trapidil or dipyridamole. *Thromb Haemostas THHADQ 62 (1) 559 (1989) August 19, 1989.*

UMDNJ, Spec. Hemostasis Lab, New Brunswick, NJ.

van Houwelingen AC, Hornstra G, Kromhout D and de Lezenne Coulander C: Habitual fish consumption, fatty acids of serum phospholipids and platelet function. *Atherosclerosis*, 75 (1989) 157-165.

Vanrenterghem Y, Lerut T, Roels L, Gruwez J, Michielsens P, Gesele P, Colucci M, Deckmyn H, Arnout J and Vermynen J: Thromboembolic complications and haemostatic changes in cyclosporin-treated cadaveric kidney allograft recipients. *Lancet*, Sat. 4 May (999-1002) 1985.

Vericel E, Crosset M, Courpron Ph, Dechavanne M and Lagarde M: Blood platelet functions: Influence of aging. *Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.*

Vickers JD and Mustard JF: The phosphoinositides exist in multiple metabolic pools in rabbit platelets. *Biochem. J.* 238, (411-417) 1986.

Vreeken J and Hardeman MR: Fish and platelets. *Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.*

Ward M, Pavlina T, Butchin R, Johnson R and Cotter R: Rapid onset inhibition of african green monkey platelets after intravenous administration of a marine oil lipid emulsion. *Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.*

Ware JA, Clark BA, Smith M and Salzman EW: Abnormalities of cytoplasmic [Ca<sup>++</sup>] in platelets from uremic patients studied with aequorin and indo-1. *Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.*

Ware JA, Clark BA, Smith M and Salzman EW: Abnormalities of cytoplasmic Ca<sup>2+</sup> in platelets from patients with uremia. *Blood*, Vol 73, No. (January), 1989: pp 172-176.

Ware JA, Saitoh M, Smith M, DeCenzo M and Salzman EW: Activation of platelets by a synthetic polymer surface. *Thromb Haemostas THHADQ 62 (1) 104 (1989) August 19, 1989.*

Ware JA, Johnson PC, Smith M and Salzman EW: Aequorin detects increased cytoplasmic calcium in platelets stimulated with phorbol ester or diacylglycerol. *Biochemical & Biophysical Research Comm.*, Vol. 133, No. 1, (pps 98-104) Nov. 27, 1985.

Ware JA, Smith M and Salzman EW: Diacylglycerol and phorbol ester reduce aequorin-indicated [Ca<sup>++</sup>] elevations induced by thrombin or ADP. *Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.*

Ware JA, Johnson PC, Smith M and Salzman EW: Inhibition of human platelet aggregation and cytoplasmic calcium response by calcium antagonists: Studies with Aequorin and Quin 2. *Circ. Res.* 59-1 (39-42) July 1986.

Ware JA, Johnson PC, Smith M and Salzman EW: Inhibition of platelet cytoplasmic calcium responses and aggregation by calcium antagonists. *Thromb. & Haemos.*, No. 1, Vol. 54, July 14, 1985

Ware JA: Interrelationship of Ca<sup>2+</sup> rises, protein kinase, and fibrinogen binding in activated platelets. *Thromb Haemostas THHADQ 62 (1) 461 (1989) August 19 1989.*

Ware JA, Johnson PC, Smith M and Salzman EW: Platelet cytoplasmic [Ca<sup>++</sup>] indicated by Quin -2 and aequorin: Is there a "Ca<sup>++</sup>-independent" pathway to platelet activation? *Thromb. & Haemos.*, No. 1, Vol. 54, July 14, 1987.

Ware A, Saitoh M, Smith M, Johnson PC and Salzman EW: Response of aequorin loaded platelets to activators of protein kinase C. *American Physiological Society*, C35-C43, 1989.

Ware JA, Smith M and Salzman EW: Synergism of platelet-aggregating agents: Role of elevation of cytoplasmic calcium. *J. Clin. Invest.*, Vol. 80 (267-271) July 1987.

Wehmeier A, Menzel C and Schneider W: Platelet aggregation in myeloproliferative disorders in whole blood and platelet rich plasma: evaluation of platelet volume subpopulations. *Thromb Haemostas, THHADQ 62 (1) 108 (1989) August 19, 1989.*

Weiss HJ, Hoyer LW, Rickles FR, Varma A and Rogers J.: *J. Clin. Invest.* 52:2708. 1973

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

Yamaguchi A, Yamamoto N, Kitagawa H, Tanoue K and Yamazaki H: Ca<sup>2+</sup> influx mediated through the GPIIb/IIIa complex during platelet activation. *FEBS Letters*, Vol. 225, No. 1, 2 (228-232) December 1987.

Yamaguchi A, Tanoue K and Yamazaki H: Roles of GPIIb/IIIa complex in Secondary Ca<sup>2+</sup> influx in thrombin-aggregated platelets. *Thromb Haemostas THHADQ 62 (1) 98 (1989) August 19, 1989.*

Yamaguchi A, Suzuke H, Tanoue K and Yamazaki H: Simple method of aequorin loading into platelets using dimethyl sulfoxide. *Thromb. Res.* 44 (165-174) 1986.

Yatomi Y, Higashihara M, Ozaki Y, Kume S and Kurokawa K: Intracellular ionized calcium mobilization of cd 9 monoclonal antibody-activated human platelets. *Biochem. & Biophys. Res. Comm.* Vol. 171, No. 1, Pages 109-115, August 31, 1990

Yatomi Y, Higashihara M, Tanabe A, Ohashi T, Oka H and Kume S: Synergism between subthreshold concentrations of thrombin and 12-O-tetradecanoylphorbol 13-acetate in platelet activation. *Biochem. & Biophys. Res. Comm.* Vol. 148, No. 3 (1025-1029) November 13, 1987.

Yatomi Y, Higashihara M, Tanabe A, Ohashi T, Kariya T and Kume S: The synergism between protein kinase C activation and calcium mobilization in platelet activation. *Thromb Haemostas, THHADQ 58-1 (1-676) July 6, 1987.*

Yunfei J: Mode of action of anisodamine, an anti-platelet agent. Thromb Haemostas, THHADQ 62 (1) 556 (1989) August 19, 1989.

Zwierzina WD and Kunz F: A method of testing platelet aggregation in native whole blood. Thromb. Res. 38 (91-100) 1985.

### **Assistance and Distributor Information**

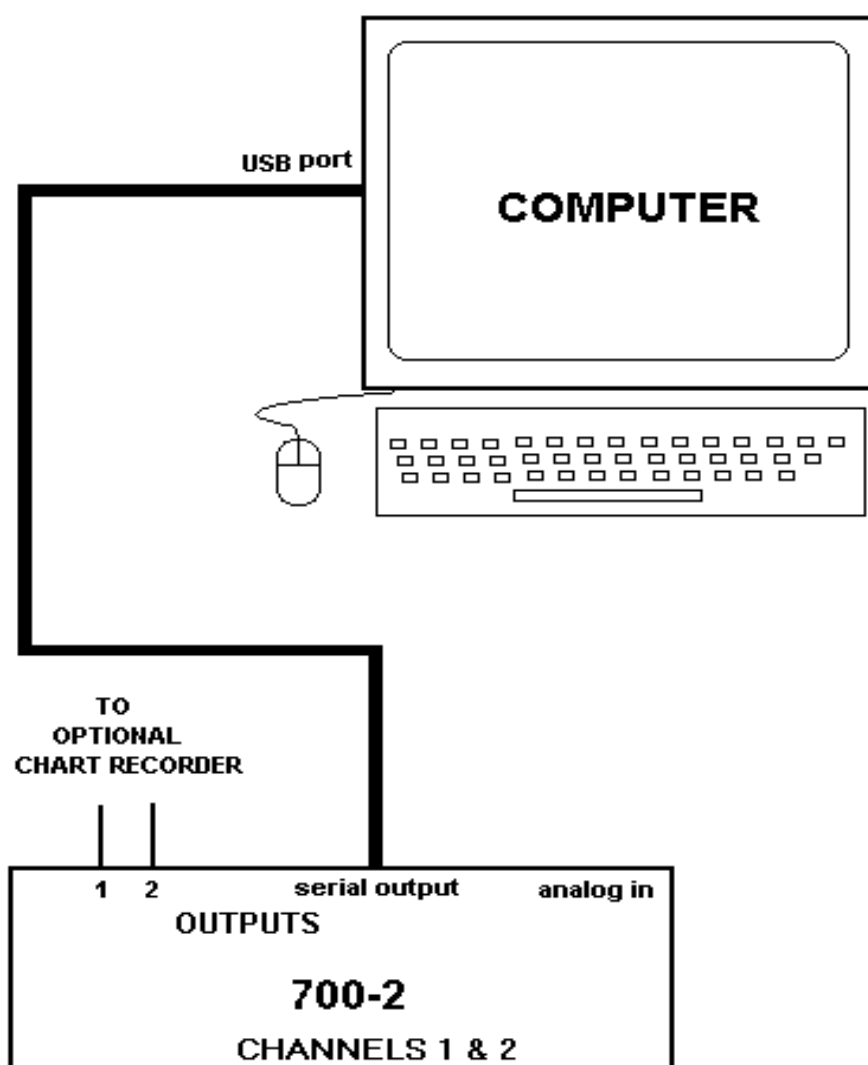
For Assistance on training, service, software updates and other information, contact your local distributor. Information on Chrono-log Distributors can be found on the Chrono-log website at [www.chronolog.com](http://www.chronolog.com).

## INDEX

- abnormal, 2, 4, 19, 29, 34, 38, 39, 62, 65
- abnormalities, 1, 2, 3, 20, 30
- absent, 3, 39
- ADP, 1, 3, 8, 9, 11, 15, 16, 17, 18, 20, 21, 22, 23, 26, 27, 28, 29, 30, 31, 32, 33
- AGGRO/LINK, 4, 6, 10, 17, 35, 38, 39, 42, 45, 47, 48, 49, 50, 51
- amplitude, 14, 17, 22, 26, 28, 42, 46, 48, 49, 53
- Arachidonic Acid, 3, 8, 9, 15, 16, 17, 18, 20, 22, 26, 27, 28, 29, 30, 32
- Area Under Curve, 10, 44, 48, 50
- Aspirin, 5, 7, 22, 32
- Assistance, 74
- ATP Release, 15, 17, 18, 22, 25, 26, 28, 30, 32, 42
- ATP Standard, 8, 9, 11, 12, 13, 15, 23, 24, 25, 27, 53
- Baseline, 11, 13, 14, 15, 16, 22, 24, 26, 27, 32, 35, 36, 37, 40, 41, 42, 63
- Browse, 44, 45, 60
- calculate, 13, 15, 17, 18, 25, 26, 28, 36, 37, 38, 49, 53, 62, 63, 64, 65
- Calculate Results, 49, 55
- Calibration, 5, 39, 40, 41, 42
- Cascade, 65
- Chrono-lume*, 17
- Cleaning, 35
- Close, 6, 12, 14, 16, 24, 25, 27, 46, 54, 64, 65
- Collagen, 2, 3, 8, 10, 14, 15, 18, 20, 22, 25, 26, 27, 29, 30, 32
- computer, 4, 6, 7, 8, 40, 41, 43, 55, 56, 63, 64
- concentration, 3, 10, 11, 13, 16, 17, 18, 20, 22, 25, 27, 28, 30, 32, 33, 46, 53
- configuration, 5, 48, 50, 52, 53, 54, 55, 59, 60
- Connect, 6, 7, 11, 62, 64
- control, 11, 12, 14, 16, 18, 22, 23, 24, 25, 27, 28, 29, 32, 34, 35, 36, 37, 38, 59, 62, 63
- cuvettes, 1, 4, 5, 11, 12, 15, 16, 17, 23, 25, 26, 27, 28, 33, 36, 40, 41, 42
- Dipyridamole, 5, 7
- Directory, 12, 14, 15, 24, 25, 27, 44, 45, 57, 58, 59, 60
- Distributors, 73
- duration, 48, 49, 53
- EDTA, 8
- electrode, 4, 7, 8, 11, 14, 15, 16, 17, 18, 22
- equipment, 8
- Exit, 39, 59
- Export, 45, 46
- gain, 12, 24, 48
- handling, 23
- hematocrit, 8
- ice, 33
- impedance, 2, 4, 5, 6, 7, 15, 22, 39, 49
- incubate, 12, 14, 17, 23, 24, 25, 26, 28
- incubation, 1, 5, 11, 12, 14, 15, 17, 23, 26, 27, 28, 33, 36, 37, 38, 39, 40, 42, 62, 63
- key, 19
- Lag Time, 10, 13, 15, 17, 25, 26, 28, 48, 49, 50
- Luminescence, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 31, 32, 42, 49, 53 m
- maximum, 13, 21
- Menu, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50
- Merge Copy, 50
- Merge Paste, 50
- Move, 10, 38, 40, 50
- normal, 11, 17, 18, 19, 20, 21, 35
- Offset Curves, 51
- ohms, 4, 5, 16, 17, 18, 21
- Open, 10, 12, 14, 38, 39, 40, 50
- optical, 6, 37
- photomultiplier, 6
- pipette, 8
- platelet, 7, 17, 19, 37
- platelet aggregation, 19
- platelet count, 37
- PPP, 6, 37
- Preview Report, 10
- Print, 10, 16, 42, 43
- Print Preview, 10
- Print Report, 16
- Print Setup, 43
- procedure, 7, 11, 12, 15, 23, 47, 48, 49
- protocol, 7
- PRP, 6, 37
- pushbutton, 5, 6
- qualitative, 19, 21
- ranges, 11, 17, 18, 19
- reagents, 8, 9, 21
- reduced, 19, 20
- ReoPro, 5
- Restart Current Test, 12, 15, 50
- Ristocetin, 8, 10, 11, 17, 18, 19, 21
- RPM, 5, 37, 38
- Run New Patient, 10, 12, 23
- Run Same Patient, 13, 15, 49, 50
- Run Test, 15
- saline, 38
- Save, 10, 15, 16, 35
- screening, 7
- secretion, 11, 19, 20, 21
- Set Graph Range, 12, 15, 23, 49
- Set Start and Stop Times, 50
- slope, 10, 13, 14, 15, 16, 17, 44, 45, 48
- software, 4, 6, 7, 10, 17, 38, 45
- specimen, 8
- stir bar, 8
- Stirrer, 48
- Stop, 10, 12, 13, 14, 15, 16, 26, 43, 44, 49, 50
- store, 6
- switches, 35
- temperature, 5
- Test Information, 12, 13, 15, 23, 25
- Test Procedure, 12, 15, 23, 44, 49, 50
- Thrombasthenia, 19, 21
- Thrombin, 8, 10, 13, 14, 17, 19, 21, 25
- Tile, 16
- time, 10, 13, 14, 15, 16, 45, 49
- Turbidimetric, 7
- Type 2B, 19, 21
- von Willebrand, 19
- windows, 6, 12, 23, 38, 42, 43, 46, 51

## APPENDIX A

### Model 700-2 Connection Chart



## Model 700-4 Connection Chart

