

INSTRUCTION MANUAL

FOR THE

CHRONO-LOG® PLATELET AGGREGOMETER

MODEL 490 4+4

490 4+ Four Channel Optical Aggregometer
490 4+4 Eight Channel Optical Aggregometer

FOR IN-VITRO DIAGNOSTIC USE
For Measuring platelet aggregation in platelet rich plasma



- 2 West Park Road • Havertown PA 19083
- TEL 800-247-6665 • 610-853-1130



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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

UKRP: Obelis UK Ltd.
Sandford Gate,
East Point Business Park,
Oxford, OX4 6LB, UK

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

Fuse: For 115 VAC: 630 mA, 250V; Time lag, 5x20mm or equivalent;
For 230 VAC: 315 mA, 250V; Time Lag, 5x20mm or equivalent.



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.

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

NOTE: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off

and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

Caution: Federal law restricts this device to sale by or on the order of a physician for measuring platelet aggregation in platelet rich plasma.

VENTILATION

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

1. Do not place Aggregometer within a closed in wall or on top of cloth material which can act as insulation.
2. Do not place Aggregometer where it will receive direct sunlight.
3. Do not place Aggregometer next to a heat source of any kind including heating vents.
4. 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 Aggregometer.

However, normal precautions, which apply to the handling of blood, should be observed in handling the samples.

Non-factory authorized service personnel should not remove the instrument cover.

MISUSE

To prevent electrical shock, the Aggregometer and peripheral power cables 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 grounded 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

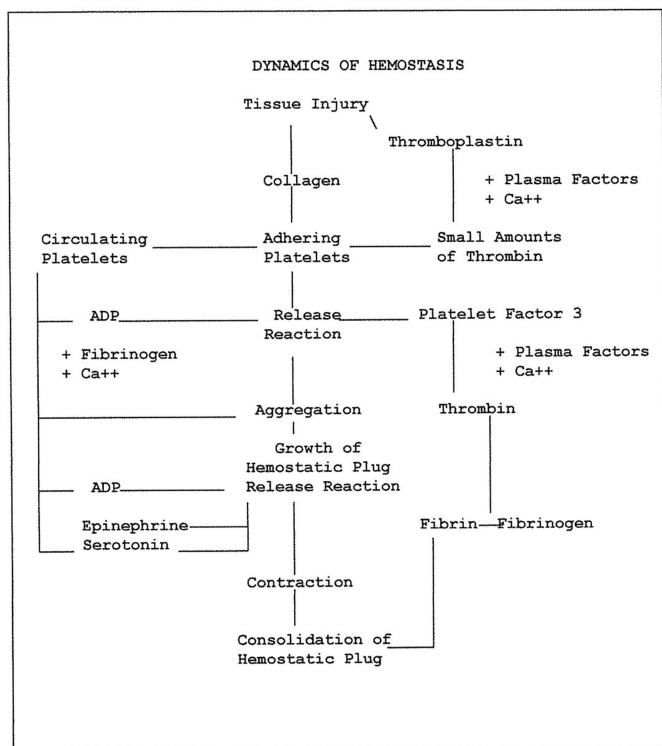
When a blood vessel is damaged, platelets adhere to the wound edges, aggregate, synthesize prostaglandins and release serotonin, ADP and ATP.⁸ Prostaglandin synthesis and release products cause further aggregation. The coagulation cascade is initiated, thrombin generated, fibrin formed and the platelet plug anchored to the damaged vessel.¹⁵

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.¹

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.⁹ 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.¹⁵

Formation of the primary hemostatic plug

Platelets adhere and aggregate at any site of sub endothelial exposure.¹⁵ 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.¹⁶

Von Willebrand factor (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.¹⁷

Qualitative platelet function disorders

- Defective adhesion
 - Von Willebrand Disease -quantitative or qualitative defect in plasma von Willebrand Factor (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 (the stable analog of thromboxane A2)
 - 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. 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 concentrations. For optical aggregometry, ADP, epinephrine, collagen and ristocetin are used extensively for screening purposes and provide the most immediate information for basic diagnostic considerations.¹⁸

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.³ 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.^{4,12}

Other reagents such as thrombin³, 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 dysfunctions⁹ 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

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 anti-coagulated blood sample by a relatively low centrifugal force centrifugation. This material is known as platelet rich plasma (PRP). Platelet poor plasma (PPP) is prepared by centrifuging the blood sample at a relatively high force.

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. 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. A SELECT/SET switch allows the use of either a separate or a common reference sample. 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 $75 \times 10^3/L$ 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 and a Range Error will be indicated on the front panel.

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 recorded as a signal on chart paper or digitized into a computer using AGGRO/LINK® Opti8™ 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 (percent aggregation)
- the slope, or rate of aggregation, reported in % of aggregation per minute.

Multiple aggregating agents and concentrations 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.

CHRONO-LOG® MODEL 490 4+, 490 4+4 AGGREGATION SYSTEM INSTRUMENT SPECIFICATIONS

Intended Use

The CHRONO-LOG® Model 490 4+4 Aggregometer is intended for use for in-vitro diagnostic use for measuring Platelet Aggregation in Platelet Rich Plasma. This device is intended to be used in a clinical laboratory environment by laboratory technicians. For use only with light transmission aggregometry assays cleared for use with the CHRONO-LOG® Platelet Aggregometry systems.

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, stirring in RPM's, PPP/reference Select, and calibration 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 the front panel. Stirrer speed accuracy is better than 0.01%. There is a feature that prevents operation if stirrer is not within ± 10 RPM of the selected setting.
- 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 Interface – USB
- Power requirements - 115 or 230VAC; 50 or 60 Hz 60 watts max. The main supply voltage fluctuations are not to exceed 10% of the nominal supply voltage.
- Fuse – For 115 VAC: 630 mA, 250V; Time lag, 5x20mm or equivalent; For 230 VAC: 315 mA 250V, Time Lag, 5x20mm or equivalent.
- For continued protection only the correct stated fuse values may be used. Failure to use the correct value may result in a hazard.
- DIN input connector for an eight (8) 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

- Four (4) Channels [One, 4-Channel Module] or Eight (8) Channels [Two, 4-Channel Modules] with connecting cable
- Sample Volume - 500 µL or 250 µL [spacer not required for micro-volume testing]
- Cuvettes - P/N 312
- Stir Bars - reusable teflon coated P/N 313, disposable P/N 311
- Incubation Wells – two (2) wells for each channel for P/N 312 cuvettes at 36.5° ± 1.0°C.
- Dimensions - 14" (35.5cm) wide, 8.5" (21.6cm) high, 15" (38cm) deep [per Module].
- Weight – 19.3 lbs. (8.75kg) [per Module]
- Output
 1. **Analog:** Connectors on rear chassis marked 1, 2, 3 and 4 [Module 1] and 5, 6, 7 and 8 [Module 2], for interface to a strip chart recorder. Normal operating voltages are in the Millivolt range.
 2. **USB:** Internal AGGRO/LINK® interface for use with a computer. This option includes AGGRO/LINK® Opti8™ and vW CoFactor Opti8™ software.
 3. **Interconnecting:** To connect two, 4-channel modules for eight (8) channel configuration.
- Pollution Degree 2
- Installation Category II
- Protection Class 1
- Equipment Mobility – Desktop
- Continuous Operation – Continuous

Recommended Optional Recorder(s) - CHRONO-LOG® Model 709 Dual Pen (Quantity 4 Recorders required). Other recorders must have 1 megohm minimum input impedance and 100 mV range.

The minimum computer requirements for the AGGRO/LINK® are as follows:

- 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.

Instrument Controls

- Power ON/OFF switch - powers instrument on and off.

- BASELINE Pushbutton(s), one per channel - zeros instrument output 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.
- SELECT Pushbutton(s) – allows for toggling between the Stirring Speed, Temperature and PPP/Reference Setting, using the SET pushbutton switch. One SELECT and SET pushbutton controls two channels. For example, Channel 1 and 2, Channels 3 and 4, Channels 5 and 6 and Channels 7 and 8 are set in pairs.
- Stirring Range: 400 to 1200 RPM in 100 RPM increments.
- Temperature Range: 35.0°C to 39.0°C in 0.1°C increments.
- PPP/Reference Setting ... each pair of channels can be set to reference a PPP sample in the well of that channel or can be set to reference the PPP sample in the well of Channel 1.
- SET Pushbutton(s) - sets the Stirring Speed, Temperature and PPP/Reference depending on which feature is selected.
- Each time the SET button is pushed, the selected function is incremented until it reaches the maximum setting.
- Calibration Switch – located on the front of the instrument and is key-activated.

INSTALLATION

NOTE: For a four-channel instrument, approximately 1.5 sq. ft. (0.139 sq. m.) 14 x 15" of bench space is required. For an eight-channel system, approximately 3 sq. ft. (0.278 sq. m.) of bench space is required.

NOTE: Software packages cannot be run simultaneously on one Aggregation system. Be sure to close the AGGRO/LINK® Opti8™ Software prior to opening the vW CoFactor Opti8™ Software and vice versa.

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. Place the Aggregometer and Computer in a clean dry area on a bench top at a height of 28-35".
6. 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.
7. Turn power ON for the Aggregometer(s).
8. Computer Connections: (See Connection Charts in Appendix B) If the computer was purchased through Chrono-log the software was pre-installed, proceed to step 9.
 - a. Turn power ON for the computer.
 - b. Place the Chrono-log supplied AGGRO/LINK® Opti8™ Software Installation CD/USB in the computer and run the setup file "CDM v*. *WHQL Certified" located in the folder named USB Drivers.
 - c. Connect the USB cable to the Aggregometer. Windows should complete setting up the USB drivers on the computer.

NOTE: If the steps above are not followed in sequence, the USB driver may not install completely. This will be indicated by FTDI or USB Serial port devices showing up in Device Manager under the Other Devices Category. Right Clicking these and selecting Update driver may complete the installation. Please consult

your IT department or Chrono-log service department for assistance.

- d. The AGGRO/LINK® Opti8™ and vW CoFactor Opti8™ Software packages are in separate folders on the CD/USB. Double click each Setup.exe file to start installation of each software and follow on-screen instructions to complete the installation.
 - e. It is recommended that the software be run with desktop composition disabled (a setting in the Windows Operating System) and 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.
9. Start the AGGRO/LINK® Opti8™ 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 IS NOT RESPONDING in the message box.
 - b. Check the cable and make sure the Aggregometer has power.
 - c. Select the RETRY button in the message box to establish connection.
 - d. Once AGGREGOMETER READY appears on the lower right side of the status bar, **close the AGGRO/LINK® Opti8™ Software.**
 10. Start the vW CoFactor Opti8™ 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 AGGREGOMETER / AGGRO/LINK NOT RESPONDING in the message box.
 - b. Check the cable and make sure the Aggregometer has power.
 - c. Select the RETRY button in the message box to establish connection.
 - d. Once AGGREGOMETER READY appears on the lower right side of the status bar, **close the vW CoFactor Opti8™ Software.**
 11. The LCD display on each channel will show a temperature reading. This reading will settle at the desired temperature in approximately 15 minutes.
 12. The System is now installed and ready for use.

NOTE: Extreme care should be taken when saving to an external drive to avoid potential accessibility issues.

INSTRUMENT FUNCTION VERIFICATION

1. Temperature Check – set to 37° in all channels.
2. Stirring - take a cuvette with stir bar and place in each reaction well to observe it spinning.
3. Optical Circuit Function - insert a BLACK cuvette from P/N 322 Calibration Kit into the reaction well for PRP. Insert a water cuvette from P/N 322 calibration kit into the reaction well for PPP. 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.

4. Replace the BLACK cuvette with the other water cuvette from P/N 322 calibration kit and observe the tracing near the 100% light transmission baseline.

OPERATING INSTRUCTIONS

For Model 490 4+/490 4+4, install the AGGRO/LINK® Opti8™ Software as described previously under INSTALLATION section of this manual.

For Model 490 4+DR/490 4+4DR the software for running the AGGRO/LINK® Opti8™ programs were installed at the factory. Before attempting to run aggregation testing, read the Software section on page 11 and PROCEDURE – STEPWISE on page 13 of this manual to learn the operation of AGGRO/LINK® Opti8™ software.

SPECIMEN COLLECTION:

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) FEDERAL REGISTER.

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 Optical 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 follow: ¹⁹

- **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 (P2Y₁₂), Prasugral (P2Y₁₂)
- **RGD Peptomimetics**

- Eptifibatide, Tirofiban
- **Phosphodiesterase Inhibitors**
Dipyridamole, Cilostazole
- **Anticoagulants**
Heparin, Warfarin, Direct Thrombin Inhibitors (Ilepirudin, argatroban, bivalirudin)
- **Cardiovascular Agents**
β-adrenergic blockers (propranolol), Vasodilators (nitroprusside, nitroglycerin), Diuretics (furosemide), Calcium channel blockers
- **Antimicrobials**
β-lactams (penicillin, 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 anti-coagulated with 3.2% or 3.8% sodium citrate, in the ratio of one (1) part anticoagulant to nine (9) parts of blood.

Needle size should be between 19 and 21 gauge. NOTE: A needle with a diameter greater than 1mm (19 gauge) may cause tissue injury 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.

For testing with 500 µL PRP samples: Five (5) 4.5 mL blue top evacuated tubes per patient plus one (1) discard tube. For testing with 250 µL PRP Samples: Three (3) 4.5 mL blue top evacuated tubes per patient plus one (1) discard tube.

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.

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

Handling Conditions:

1. Testing can start 30 minutes after venipuncture and continue for about 3 hours after.
2. Specimen should be kept at room temperature (20° to 25°C).

EQUIPMENT AND MATERIALS:

Equipment:

1. CHRONO-LOG® 490 4+ or 490 4+4 Aggregation system with internal AGGRO/LINK® Interface installed on a bench top at a height of 28-35”.
2. Windows®-Compatible Computer and AGGRO/LINK® Opti8™ software installed.
3. 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 312 Cuvettes
2. P/N 311 Disposable Siliconized Stir bars
3. P/N 322 Calibration Kit containing 1 Black Cuvette and 2 Sealed Water Cuvettes.
4. P/N 331 .5-10 µL Adjustable Pipette
5. P/N 335 Tips for P/N 331
6. P/N 332 10-100 µL Adjustable Pipette
7. P/N 337 Tips for P/N 332
8. P/N 333 100-1000 µL Adjustable Pipette
9. P/N 339 Tips for P/N 333
10. P/N 384 CHRONO-PAR® ADP
11. P/N 385 CHRONO-PAR® Collagen
12. P/N 390 CHRONO-PAR® Arachidonic Acid
13. P/N 393 CHRONO-PAR® Epinephrine
14. P/N 396 CHRONO-PAR® Ristocetin
15. 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.

16. 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. DO NOT use water from the laboratory Millipore filtration system.

17. Polypropylene conical centrifuge tubes and caps (per test subject) for storage of PRP and PPP and for standardizing PRP.
18. Ice for maintaining Reconstituted Working Reagents at appropriate temperatures
19. Vortex-type mixer: for mixing the Arachidonic Acid
20. Long-stemmed plastic transfer pipettes to take off PRP and PPP for placing into 15 mL polypropylene centrifuge tubes.

21. 0.5 mL Freestanding polypropylene microcentrifuge tubes and twist caps with O-ring seals or 1.7 mL polypropylene microcentrifuge conical tubes with snap tops... for storage of reagent aliquots.
22. Syringe to extract Collagen from Vial
23. Parafilm for collagen vial and aliquot
24. Lintless wipes, such as KimWipes®.

Gauze squares are NOT suitable.

PREPARATION:

1. Aggregometer

- a. Turn on the Model 490 4+ or 4+4 and let it heat up for 10-15 minutes or until the heater block stabilizes at 37°C.
- b. Place P/N 311 Stir bars in P/N 312 Cuvettes.
- c. Put cuvettes containing stir bar in the incubation wells to warm up.
- d. When the Model 490 4+4 test channels are set to Reference Channel 1, a single sample containing 500 µL of PPP can be used as the reference sample for all tests run with the same patient's blood. (The Model 490 4+4 requires only one (1) 500 µL cuvette of PPP for testing up to 8 channels with the same patient's sample)

2. Preparation of Sample:

- a. Mix sample by gentle inversion; DO NOT SHAKE.
- b. To prepare the platelet rich plasma (PRP):
 - 1) Centrifuge sample at approximately 100-170g for 15 minutes. Be sure brake is set in the OFF position.
 - 2) Take off the PRP with a polypropylene transfer pipette and place into a polypropylene conical centrifuge tube and add cap. Keep at room temperature (20° to 25°C).
 - 3) Recap the blue top tubes.

NOTE: Per CLSI Document H58-A, the pH of the PRP sample can be preserved as follows:¹⁹

- **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 CO₂ 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.**

- 4) Label the tube containing PRP with the patient's name, sample type, etc
- 5) Although not an absolute requirement, it is common practice to adjust the platelet count in the PRP to be tested to a final count of approximately 250,000 per µL. Use the same donor's PPP to adjust the count.

NOTE – Platelet counts lower than 50,000/µL may present difficulties in setting optical baseline.

- c. To prepare the platelet poor plasma (PPP):
 - 1) Place blue top tubes into centrifuge.
 - 2) Centrifuge sample at approximately 1500-2400 g for 20 minutes.

- 3) Take off the PPP with a polypropylene transfer pipette and put it into a polypropylene conical centrifuge tube and add cap. Keep at room temperature (20° to 25°C).
- 4) Label the tube containing PPP with the patient's name and sample type. Parafilm or cap the top.
- 5) When ready to begin testing, dispense one aliquot per channel of adjusted or non-adjusted PRP of 500 µL or 250 µL volume into P/N 312 cuvettes with stir bars. Warm for a minimum of 3 minutes.

3. Reagent Preparation

The following reagents are sourced from:

Chrono-log Corp.
2 W. Park Road
Havertown, PA 19083 USA
Tel: 610-853-1130

a. ADP

Catalog No.: 384
Supplied As: 2.5 mg of lyophilized preparation of adenosine diphosphate.

Stock Conc.: 1 mM
Stock Storage: Store frozen at below 0°C
Stock Shelf life: Until expiration date.

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

Reconstitute with 5.0 mL of irrigation grade physiological saline.

Preparation: 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. Add 5 µL of reagent to 500 µL sample or 2.5 µL of reagent to 250 µL sample for a final concentration of 10 µM. Normal aggregation is seen in PRP with final concentrations of 5-10 µM.

Stability: The reconstituted ADP reagent can be stored frozen at -70°C in volumes suitable for a day's testing for one year or until expiration date, whichever comes first.

b. Arachidonic Acid

Catalog No.: 390
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.: 50 mM
Working Storage: 2-8°C [in the dark]
Working Shelf Life: 8 hours

Reconstitute with 0.7 mL of the saline-albumin solution.

Preparation: First tap contents gently to the bottom of the vial of albumin. Reconstitute the albumin with 1 mL of irrigation grade physiological saline. Allow to sit, then mix with occasional swirling. Allow 15 to 30 minutes for the albumin to go completely into solution (check visually) and gently invert to take up any albumin in the plug.

The Arachidonic Acid in the vial is an oily drop which must be shaken or tapped to the bottom of the vial. Break vial tip with Cap Cracker™ supplied. Pipette reconstituted albumin into both the tip and body of the vial in 100 µL aliquots to a combined total volume of 700 µL. Make sure any Arachidonic Acid remaining on the tip or body of the vial is mixed by rotating the vial as the albumin is added. Repeat a few times in each section of the vial then vigorously mix the Arachidonic Acid into the albumin using a 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 micro centrifuge tube and vortex at highest speed for 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.

Add 5 µL of reagent to 500 µL PRP sample or 2.5 µL of reagent to 250 µL sample for a concentration of 0.5 mM. Normal aggregation is seen with final concentrations of 0.5 -1.0 mM.

Stability: The reconstituted Arachidonic Acid can be stored frozen at -70°C in the dark in 100 µL volumes for 3 months or until expiration date, whichever comes first. When stored frozen at -20°C in the dark Arachidonic Acid is stable for 1 month or until expiration date, whichever comes first. Aliquots can be hand thawed and vigorously re-suspended with a vortex mixer for 2 minutes just before use.

c. **Collagen**

Catalog no.: 385

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.: 1 mg/mL

Stock Storage: Refrigerate at 2-8°C

Stock Shelf Life: Until expiration date.

Working Conc.: 1 mg/mL

Working Storage: 2-8°C

Working Shelf life: Until expiration date.

Preparation: Collagen can be used directly as supplied. Invert or swirl vial before use, as Collagen fibrils are in suspension. Do not freeze. If required, Collagen can be further diluted in isotonic glucose pH 2.7. Do not

dilute entire bottle, only enough for a day's testing.

Add 1 µL of reagent to 500 µL sample or 0.5 µL of reagent to 250 µL sample for a final concentration of 2 µg/mL. Normal aggregation is seen with final concentrations of 1-5 µg/mL.

Stability: Collagen does not contain any preservative, but because of its very low pH, organisms do not grow as readily. If aseptic techniques are used (sterile syringe and needle to remove one day's use), remaining reagent, if stored at 2 - 8°C, is stable until expiration date. Reagent removed from the vial is stable for one week at 2-8°C when parafilm. Parafilm both original vial and aliquot. Invert gently before use.

d. **Epinephrine**

Catalog No.: 393

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.: 1 mM for PRP testing

Working Storage: 2-8°C in dark container

Working Shelf life: 8 hours (in the dark)

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

Preparation: Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with 5.0 mL 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. Invert gently.

Adding 2.5 µL of 1:10 Diluted Solution to 500 µL sample of platelet rich plasma or 1.25 µL of reagent to 250 µL sample gives a final concentration of 5 µM. Normal aggregation is seen with final concentrations of 5-10 µM in platelet rich plasma.

Stability: The unused reconstituted Epinephrine can be stored frozen at -70°C in the dark and in 100 µL aliquots for 3 months or until the expiration date, whichever comes first.

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

e. **Ristocetin**

Catalog No.: 396

Supplied As: 62.5 mg of stabilized freeze dried Ristocetin.

Stock Conc.: 125 mg/mL

Stock Storage: Refrigerate at 2-8°C (in the dark)

Stock Shelf Life: Until expiration date

Working Conc.: 125 mg/mL
 Working Storage: 2-8°C
 Working Shelf Life: 8 hours (in the dark)

Reconstitute with 0.5 mL of sterile distilled water.

Preparation: Tap vial gently to get contents to the bottom. Remove stopper and reconstitute with 0.5 mL of sterile distilled water. Do not shake or invert vial. Re-stopper and allow to sit for 10-15 minutes. Visually inspect bottom of vial to confirm reagent is fully in suspension. Do not shake the reagent, invert gently, to take up any reagent remaining in stopper and allow vial to sit for another 10-15 minutes until all particulate matter is well dissolved. Reagent may have a clear-to-brownish color suspension after reconstitution. Never shake reagent. Swirl gently just before use.

Add 5 µL of reagent to 500 µL PRP sample or 2.5 µL of reagent to 250 µL sample for a concentration of 1.25 mg/mL. Normal aggregation is seen with final concentration of 0.63 - 1.5 mg/mL.

Stability: The unused reconstituted Ristocetin reagent can be stored frozen at -20°C in volumes suitable for a days testing for 3 months or until the expiration date, whichever comes first. DO NOT STORE AT -70°C.

4. AGGRO/LINK® Opti8™ Software [Version1.1.0]

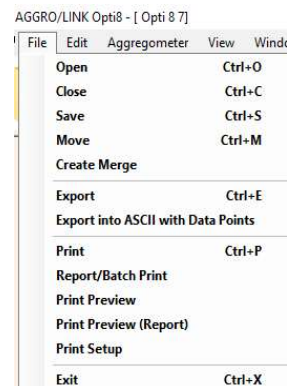
- Turn on Computer and Start AGGRO/LINK® Opti8™ for Windows® program. Be sure "Aggregometer Ready" appears in the bottom right-hand corner of the screen. If not, check USB Port, cables and connectors. If USB/Com Port is changed, go to AGGREGOMETER then CONNECT.
- Click on EDIT and CONFIGURATION. Type in your institution's information under Report Header. This will be printed out on top of Report Format when Report Batch Print is selected under File. Click on OK when completed.
- To have Area Under Curve and Lag Time Calculated and printed on test data, click on the box to select. To disable, leave this box unchecked.
- To display and print 2% lines on the graph, click on the box to select.
- To display and print Start Indicators, click on the box to select. To disable, leave this box unchecked.
- To utilize Legacy Report Print, click on the box to select. This option provides a slightly larger graph on the printout with layout similar to previous AGGRO/LINK software.
- To invert aggregation trace so it starts at 100% of graph and aggregates toward 0% of the graph, click on the box to select.
- Auto Activate – Removes the need to manually click on "activate" for each test channel. To select, click on Enabled Box and set time delay between channels from 5 seconds up to 3 minutes. "Activate All Channels" and "Start All Channels" under the AGGREGOMETER window will not be available due to interfering with the Auto Activate functionality.

NOTE: Computers with CPU speed of lower than 3.0 GHz may cause this function to be disabled

- Under the AGGREGOMETER window, select or set-up a test Procedure page. Click on box under each Channel to select for testing. Use arrows to select Reagent, Concentration and Stirrer speed for each channel or a special Reagent and Concentration can be typed into the appropriate fields. To save the procedure for future use, change the name in the Procedure Name field. Click on OK and select Run New Patient under AGGREGOMETER.

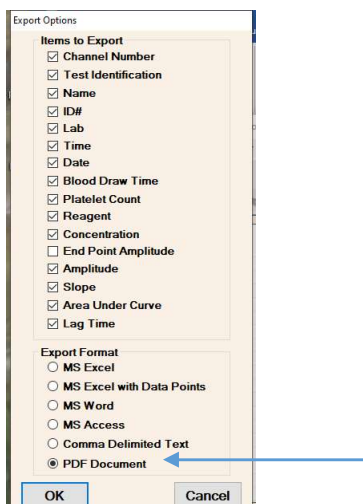
NOTE – AGGRO/LINK® Opti8™ Software features:

FILE



- **Open – Test Directory of Previously run tests**
- **Close – Closes Test Currently Active on Screen**
- **Save – Selected after completing test**
- **Move – Test Directory of Previously run tests can be selected and moved to a USB device**
- **Create Merge – Creates a blank document for merging curves and test data**
- **Export - Export test data for use with another program -**

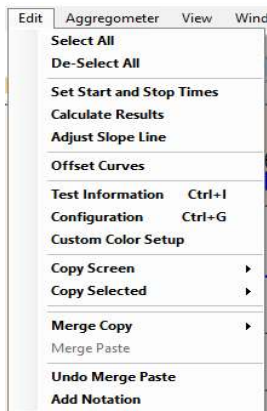
NOTE: Tests can now be Exported into a PDF Document



- **Export into ASCII with Data Points**

- Report /Batch Print
- Print Preview
- Print Preview (Report)
- Print Setup
- Exit

EDIT



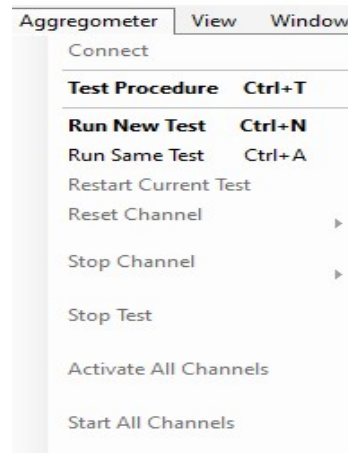
- Select All – Highlights all tracings on graph for copy and paste
- De-Select All – Removes highlights from tracings
- Set Start and Stop Times – Allows Start and Stop times to be changed by moving dotted line indicators to preferred locations
- Calculate Results – Recalculates test results after editing start and stop times
- Adjust Slope Line - Change slope calculation time
- Offset Curves – Change the physical start time of a tracing on the graph
- Test Information– Enter Test Identification, Patient Data including Platelet Count and add Notes in Comments box.
- Configuration - Settings options include displaying of Area Under the Curve and Lag Time calculations with test, 2% grid lines on screen and printout, display start indicators on screen and printout, enable Legacy Report Print [similar to previous AGGRO/LINK programs] and Invert Aggregation Tracings. Auto Activate with Time Delay can be enabled with a time delay selected between test channels from 5 seconds to 3 minutes.

NOTE: Each channel can also be activated separately on the test graph by clicking on the “Activate” bar.

- Custom Color Setup – Use Default tracing colors or select custom colors
- Copy Screen – Copies the test grid and all tracings to the system clipboard
- Copy Selected – Copies the test grid and selected tracings to the system Clipboard
- Merge Copy [ALL or SELECTED] - To copy tracings and test data from a file to a Merge document.
- Merge Paste – To paste selected tracings and test data to a Merge document.

- Add Notation – Attaches “Reagent Field” information to identify each tracing.

AGGREGOMETER



- Test Procedure – Set Up and Save Test Procedures with specific Reagents and Concentrations

Test Procedure Settings

Procedure Name:

Channel Configuration:

Channel	1	2	3	4	5	6	7	8
Reagent	Collagen	Collagen	Collagen	Collagen	Collagen			
Concentration	2ug/mL	2ug/mL	2ug/mL	2ug/mL	2ug/mL			
Stimulus	1200	1200	1200	1200	1200			

Slope Length (secs):

Duration:

Buttons: Cancel, Delete, OK, Save

- Run New Test – Enter Patient Data, Select Test Procedure, Enable/Disable Optical Amplitude Offset

Run Test

Test Identification:

Test Information:

Copy Patient Data:

Channel:

Front:

Copy:

Ed:

Buttons: OK, Cancel

Optical Amplitude Offset: ☐ Enabled ☒ Disabled

Channel	Last Name	First Name	ID	Lab	Blood Draw Time (pts/min)	Platelet Count
1						
2						
3						
4						
5						
6						
7						
8						

Test Procedures:

Buttons: Edit

Channel	1	2	3	4	5	6	7	8
Reagent	Collagen	Collagen	Collagen	Collagen				
Concentration	2ug/mL	2ug/mL	2ug/mL	2ug/mL				
Stimulus	1200	1200	1200	1200				

NOTE: OPTICAL AMPLITUDE OFFSET – drops the 0% baseline to around 30% of the graph. This will allow the Shape Change portion of the tracing to remain on the graph. [Select Enabled or Disabled Under RUN TEST]

- **Run Same Test** - for testing using same patient data and test procedure as previous test
- **Restart Current Test** – Used to obtain a clean test screen
- **Reset Channel** – Resets individual channels currently running. Channel must be “Activated” to enable a Restart.
- **Stop Channel** – Stops individual channels currently running
- **Stop Test** – Stops all channels as well as the test itself
- **Activate All Channels** - Activates all channels at once. (Only available when running a test with Auto Activate disabled.)
- **Start All Channels** - Starts all available channels that have been activated at once. (Only available when running a test with Auto Activate disabled via unchecking its box in Configuration.) **NOTE:** It is recommended to click on the “Start Bar” for each channel separately, just before adding the reagent.

5. Micro-Pipettes with 2-Stop Control Button

a. Description of 2 Stops

- 1) First Stop – The measuring stroke for aspirating and dispensing the selected volume
- 2) 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

- 1) Press the control button down to the first stop
- 2) Immerse the pipette tip vertically into the liquid
- 3) Aspirate and dispense the liquid three times by using the first stop only.
- 4) Remove the tip slowly from the liquid. For large volumes, wait approximately 3 seconds before removing the tip from the liquid.
- 5) 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.

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

d. Dispensing

- 1) Place the pipette tip into the cuvette, so that the end of the tip is immersed in the sample.
- 2) Slightly angle the pipette so that the tip is angled to touch the wall of the cuvette.
- 3) 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.
- 4) Hold the control button down at the second stop and, while keeping the pipette tip at a slight angle, pull the pipette tip out of the sample.
- 5) Once the pipette tip is completely outside of the cuvette, release the control button.

CALIBRATION:

Baseline is set from 0% to 100% when the Set Baseline button is pressed and held. Refer to “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.

If desired, positive controls can be provided by collecting samples from aspirin volunteers or subjects previously diagnosed with a platelet disorder.

PROCEDURE - STEPWISE:

1. Check Aggregometer to be sure heater block has stabilized to 37°C.
2. Place P/N 311 Stir bars in P/N 312 Cuvettes. Put cuvettes containing stir bar in the incubation wells to warm up.
3. When testing the same donor in all channels, only one 500 μ L PPP is required, as all channels can reference Channel #1 PPP. [For testing multiple donors, a cuvette with 500 μ L of PPP may be required for each channel.]

SELECT Pushbutton(s) – allows for toggling between the Stirring Speed, Temperature and PPP/Reference Settings.

SET Pushbutton(s) - sets the Stirring Speed, Temperature and PPP/Reference, depending on which feature is selected. Each time the SET button is pushed, the selected function is incremented until it reaches the maximum setting.

NOTE: One SELECT and SET pushbutton controls two channels in tandem. For example, Channels 1 & 2, Channels 3 & 4, Channels 5 & 6 and Channels 7 & 8 are set in pairs.

4. Place the cuvettes containing 500 μ L PPP in the reference well(s) ...stir bar is not required. Check for bubbles and be sure to wipe cuvette with a clean KimWipe®.
5. Gently mix the adjusted PRP by end over end inversion 3-4 times before pipetting.
6. Place 500 μ L or 250 μ L Platelet-RICH-Plasma (PRP) into pre-warmed cuvettes with stir bars. [Prepare (1) for each test channel.]

NOTE: Dispense the PRP directly into the test cuvette and do not allow it to flow down the inside wall of the cuvette.

7. **Incubate for three (3) minutes in incubation wells.**

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

8. Start AGGRO/LINK® Opti8™ for Windows® program and select TEST PROCEDURE under the AGGREGOMETER window. Under PROCEDURE NAME, set-up or load the procedure that corresponds to the reagent and method being used. **Optical tests with PRP should run for a minimum of 5 minutes.** Slope length can be set from 16 to 99 seconds. Chrono-log standard setting is 16 seconds. Click OK.

9. Select "RUN NEW TEST" under AGGREGOMETER window. Patient information page will appear. Enter patient information. **When "Run Same Test" is selected, patient data from previous test will be used.**

NOTE – Patient info can be copied from one channel to all or select channels. Click on Select All if testing same donor in all channels or Click on appropriate Trace Nos. and then Click on COPY.

NOTE – The Test Identification field at the top of the page is used to identify and select test(s) in the Test Directory. For Clinical testing, place patient identifier in this field.

10. Place the cuvettes containing PRP in the PRP wells (One for each Optical Channel). Check for bubbles and be sure to wipe cuvette with a clean lintless wipe.
11. Click OK to begin test. Click on color-coded "Activate" bar for each channel.
12. Push the SET BASELINE buttons for each channel. The tracing should move to 100% when the button is depressed and to 0% when the button is released. Be sure to press and hold the Baseline button until the tracing reaches 100% of the graph and then release.
13. Monitor tracings for stability.
14. When tracing(s) have reached stability, click on the STOP and RERUN Icons at the top of the screen and Click on OK to obtain a clean screen.
15. Click on the "Activate" bar for each channel.
16. Push the SET BASELINE button for each channel. The tracing should move to 100% when the button is depressed and to 0% when the button is released. Be sure to press and hold the Baseline button until the tracing reaches 100% of the graph and then release.
17. Take up the appropriate reagent and click on the color-coded "Start" bar for Channel 1.
18. After wiping the outside of the pipette tip with a clean KimWipe®, add reagent to test cuvette. Be sure tip is immersed in sample when reagent is added 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.

- a. Use 1 µL of Collagen with 500 µL sample volume or 0.5 µL with 250 µL sample for 2 µg/mL final concentration. For a 5 µg/mL final concentration use 2.5 µL with 500 µL sample volume or 1.25 µL with 250 µL sample.

NOTE: As shown above, reagent volumes are cut in half when testing with 250 µL PRP samples.

- b. Use 5 µL of ADP with 500 µL sample volume for a final concentration of 10 µM...or 2.5 µL for 5 µM.
- c. Use 5 µL of Arachidonic Acid with 500 µL sample volume for a final concentration of 0.5 mM...or 2.5 µL for 0.25mM.
- d. Use 5 µL of Ristocetin with 500 µL sample volume for a final concentration of 1.25 mg/mL.

- e. Use 2 µL of Ristocetin with 500 µL sample volume for a final concentration of 0.5 mg/mL (Type 2B vW).
- f. Use 2.5 µL of Epinephrine with 500 µL sample volume for a final concentration of 5 µM.

NOTE – CHRONO-PAR® Reagents do not require preparation of multiple stock solutions. To change final concentration, adjust pipette volumes as shown above.

19. Repeat steps 16 – 18 for each test channel.
20. Allow all tests to run for a minimum of five (5) minutes.

NOTE: The Graph Range time can be adjusted under VIEW and then Set Graph Range. When the test tracings have reached the end of the Graph Range, additional time is added automatically in one-minute increments, up to a total of 60 minutes.

21. While tests are running, gently mix PRP by end over end inversion 3-4 times. Prepare one test cuvette for each channel (Cuvette, stir bar and 500 µL or 250 µL PRP). Dispense the PRP directly into the test cuvette and do not allow it to flow down the inside wall of the cuvette. Begin three (3) minute incubation.
22. There are a number of Options to Stop Tests as each test can be stopped individually or all tests stopped at the same time, as follows:
 - a. To Stop Tests individually, click on Aggregometer, click on Stop Test and select the appropriate Channel.
 - b. STOP Icon at top of screen stops all tests at the same time.
23. Clicking on the "Start" bar prior to adding the reagent sets the Start and Stop time for each channel. If there is a baseline shift or some other artifact that should not be included in the final calculation, Click on EDIT and SET START & STOP TIMES (or use ICONS at top of screen).
 - a. If only calculating Slope and Amplitude:
 - 1) A small box will appear with Trace 1 selected.
 - 2) If the Automatic Start Time Feature was used before the addition of the reagent, 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).
 - 3) If required, Start & Stop times can be moved:
 - a) Individually – Left Click, Hold and Drag to new position. (Be sure Start Line is placed on stable baseline just before or just after adding the reagent. Stop Line should be placed five (5) minutes after the Start Time.)
 - b) Simultaneously – Place cursor near Start Line. Right click, Hold & Drag Start Line to new position. Stop Line will move in tandem.
 - 4) Click on other Tracings and repeat, if needed.
 - b. If calculating Slope, Amplitude, Lag Time and Area Under the Curve at the same settings:
 - 1) Click on Trace 1
 - 2) Click on "Set All" to calculate all parameters with one setting.
 - 3) Follow Steps 2) through 4) above.

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

24. After setting the Start and Stop Times, Click on DONE, then select Calculate Results under the EDIT window (or use ICON). This command will calculate aggregation percentage, slope and if selected, lag time and area under the curve. Check Duration times to be sure Start & Stop Lines were set correctly. Click on OK and calculations will appear in Data Box.
25. After the calculations have been completed, SAVE the test, using the SAVE command under FILE. Tests can be printed using the PRINT command in the FILE WINDOW. PRINT is for printing one test per page.
26. To print multiple test graphs separately at one time or in a Report Format, click on File and Report Batch Print, followed by:
 - a. Select the tests to be printed by placing (v) in boxes to left of tests listed.
 - b. Click on the "Insert Selected" button to move tests to lower panel.
 - c. The order the tests are printed out can be arranged by using UP or DOWN buttons.
 - d. Click on "Report Print" to include all selected tests in a single report ... or,
 - e. Click on "Batch Print" to print each test separately.
27. Remove the samples from the reaction well and discard.

CALCULATIONS:

1. Amplitude – Optical aggregation results are expressed as a percentage of aggregation at a given time interval from reagent addition; 100% 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® Opti8™ Software can be set from 16 to 99 seconds (32 point to 198 point) sliding curve. To change the length of a slope line, click on **EDIT**, then **ADJUST SLOPE LINE**. Click on Trace number and use arrows to adjust slope length up to 99 seconds. Chrono-log standard setting is 16 seconds (32 points).

REPORTING RESULTS:

1. Report Hematocrit.

2. Report Platelet Count.
3. Report Platelet Aggregation Maximum Amplitude (MaxA).

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 in each and every laboratory.

Normal Ranges in Platelet Rich Plasma (Mean \pm 1 SD)		
Reagent	Conc.	Agg. (%) ¹⁸
Collagen	2 μ g/mL	70 – 94
Arachidonic Acid	0.5 mM	74 – 99* ¹³
ADP	10 μ M	71 – 88
Epinephrine	5 μ M	78 – 88
Ristocetin	1.25mg/mL	87 – 102* ¹³

(* \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 is clinically significant in the detection and diagnosis of acquired¹⁴ 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 on the following page.

AGGREGATION RESPONSE WITH SELECTED ABNORMALITIES					
Reagent	Final Concentration	Aspirin Effect		Von Willebrand & Bernard Soulier	Storage Pool/ Secretion Defect
ADP	5 – 20 μ M	N, R *		N	N, R *
Arachidonic Acid	0.5 mM	A		N	N
Collagen	1 – 5 μ g/mL	1 or 2 μ g/mL	5 μ g/mL	N	N
		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. In addition, when cryoprecipitate is added to test sample from patient with Platelet-Type [pseudo] VWD, enhanced response to low concentration Ristocetin will continue, a Type 2B patient will show no response.⁷

*** To distinguish between von Willebrand & Bernard Soulier, add normal plasma or cryoprecipitate to patient sample, vW patient will respond, Bernard Soulier will not.¹⁹

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 TEST RESULTS**

<u>AGONIST</u>	<u>NORMAL RANGES</u> (Mean \pm 1 SD)	<u>CONTROL</u> <u>VALUE</u>	<u>PATIENT</u> <u>VALUE</u>	<u>TEST</u> <u>RESULTS</u> (Normal, Reduced, Increased)
ADP, 5 μ M				
ADP, 10 μ M				
ADP, 20 μ M				
COLLAGEN, 2 μ g/mL				
COLLAGEN, 5 μ g/mL				
ARACHIDONIC ACID * 0.5 mM				
EPINEPHRINE, 5 μ M				
RISTOCETIN, 1.25mg/mL*				
RISTOCETIN, 0.5 mg/mL				

(* +/- 2 SD)

INTERPRETATION:

TEST PERFORMED BY: _____ INTERPRETED BY: _____

INTERPRETATION:

Aggregation curves in PRP can be interpreted as follows:

- By direct comparison to a normal drug free control which also provides real time quality control.
- Comparison to published normal values that can be verified and reproduced by any laboratory.

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.

With Arachidonic Acid: Arachidonic Acid is converted to thromboxane A₂ 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. Aspirin effect may be seen with mid-range concentrations such as 5 µM.² 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 A₂ 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."¹⁸

With Ristocetin: This antibiotic is 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 0.9 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).⁷ 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 subjects taking aspirin.

PROCEDURE NOTES:

1. With the Model 490 4+4, spacers are not required when testing micro-volume samples (250 µL of PRP) and all

CHRONO-PAR® reagent volumes are reduced by half. For example: 5 µL of ADP = a final concentration of 10 µM with a 500 µL PRP sample... use only 2.5 µL with a 250 µL PRP sample.

2. It is important that the pipette tip be pushed to the bottom of the cuvette & the reagent forcefully 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. (DO NOT forcefully inject reagent if testing with smaller volume of 250 µL PRP.)

LIMITATIONS OF THE PROCEDURE:

- In a study of one hundred and six patients with storage pool deficiency (SPD), 23% had normal optical (PRP) aggregation responses to ADP, Epinephrine and Collagen; and 44% had miscellaneous aggregation abnormalities. The authors concluded that SPD is common, heterogeneous and not necessarily associated with optical (PRP) aggregation abnormalities.¹⁰
- Tests should be performed within 3 hours of venipuncture.
- Many drugs inhibit platelet function.^{5,11,19} Unless the aim of testing is to demonstrate drug-induced inhibition, patients should be free of drugs known to affect platelets for two (2) 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.¹⁹
- Hemolysis results in release of nucleotides from the red cells which may cause activation or desensitization of platelets, especially to ADP.¹⁹
- Lipids in PRP can interfere with light transmission readings & prevent recording of aggregation.
- Icteric samples containing bilirubin of greater than 34.2 µmol/L decrease aggregation by ~12.5%.¹⁹
- Platelet counts below 50,000/µL may cause problems with the setting of optical baseline, preventing the recording of aggregation
- This device has not been evaluated for pediatric use.

vW COFACTOR ASSAY

1. PRINCIPLE:

This procedure is for running the Ristocetin Cofactor Assay using the vW CoFactor Opti8™ software, CHRONO-LOG® Ristocetin Cofactor Assay Kit, and the CHRONO-LOG® Model 490 4+/490 4+4 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:

- a. Patient Preparation: No special Requirements
- b. Specimen Preparation:
 - 1) Anticoagulant: Buffered sodium citrate, 3.2%.
 - 2) Type: One (1) Blue Top Tube is sufficient.
 - 3) Specimen Collection:
 - a) Obtain venous blood by clean venipuncture.
 - b) Immediately mix 9 parts blood with 1 part anticoagulant,
 - c) mix well by inversion of tube against the stopper.
 - d) Centrifuge the specimen at 1500 – 2400 g's for 15 minutes.
 - e) Remove plasma from the tube within 60 min. using a plastic pipette and store in a plastic tube.
- c. **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:

- a. Equipment: CHRONO-LOG® Model 490 4+/490 4+4 Aggregometer with four or eight channels of Optical capability, vW CoFactor Opti8™ Software and Computer
- b. Materials:
 - 1) P/N 312 Cuvettes
 - 2) P/N 311 Disposable, Siliconized Stir Bars **or**
 - 3) P/N 313 teflon coated Stir Bars
 - 4) Precision Pipettors: 1 mL, 500 µL, 400 µL and 50 µL - - calibrated to deliver ±3% of the selected volume.
 - 5) Plastic transfer pipettes
 - 6) P/N 299 CHRONO-LOG® vW Cofactor Assay Kit
 - 7) P/N 299-6 Hemostasis Reference Plasma (Normal), 1 mL (Control)
 - 8) P/N 299-7 Hemostasis Reference Plasma (Abnormal), 1 mL (Control)
 - 9) P/N 398 Purified Water 15mL

NOTE: 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.

- 10) Lintless wipes, such as KimWipes® - **Gauze squares are NOT suitable**
- 11) Glass tubes with caps (10x75 mm).
- 12) Plastic conical test tubes and caps (for plasma storage).

c. Preparation:

- 1) Aggregometer
 - a) Turn the CHRONO-LOG® Model 490 4+/490 4+4 Optical Aggregometer ON.
 - b) Allow the unit to heat up for 10-15 minutes or until the heater block stabilizes at 37°C.
 - c) Check to ensure the stirring speed is set to 1200 rpm.
 - d) Place P/N 311 or 313 Stir bars in P/N 312 Cuvettes.
 - e) Put cuvettes containing stir bar in the incubation wells to warm up.
- 2) Reagents
 - a) **Ristocetin (7.5 mg/vial):** Store lyophilized reagent at 2°- 8° C before reconstitution. Reconstitute with 0.75 ml (750 µL) 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 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.

NOTE: 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.
 - f) Hemostasis Reference Plasma, Normal, 1 mL. Store at 2 - 8° C before reconstitution. Reconstitute vial 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.
 - e) Hemostasis Reference Plasma, Abnormal, 1mL. Store at 2 - 8° C before reconstitution. Reconstitute vial 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 Assay Software Setup
- a) Turn on Aggregometer.
 - b) Turn on Computer and Start vW CoFactor Opti8™ software
 - c) Click on **EDIT** then **CONFIGURATION**
 - d) Check that Data Directory reads – C:\Chrono-Log_Corporation\vW_CoFactor_Opti8\Data
 - 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.
 - f) Click 'OK' to save these settings.
- 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 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
 - 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.
 - 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.
5. **STORAGE REQUIREMENTS:**
- a. Ristocetin (7.5 mg/vial): Store at 2°- 8° C before reconstitution. After reconstitution, any unused portion may be stored frozen at -20° C or below.
 - b. Lyophilized Human Platelets, 6 ml. Store at 2°- 8° C before reconstitution. After reconstitution, stable for 30 days at 2°- 8° C, or may be frozen up to 30 days.
 - c. von Willebrand Reference Plasma, Normal. Store at 2° - 8°C before reconstitution. After reconstitution stable for 4 hours at room temperature. May be stored frozen at -20° C for up to 30 days.
 - d. von Willebrand Reference Plasma, Deficient. Store at 2- 8°C before reconstitution. After reconstitution, stable for 4 hours at room temperature. May be stored frozen at -20° C for up to 30 days.
 - e. Tris Buffered Saline (TBS), 12 ml. Store at 2°- 8° C. Do not use after expiration date on label.
 - f. P/N 299-6: Hemostasis Reference Plasma, Normal, 1 mL. Store at 2 - 8° C before reconstitution. It is advisable to use this product within 1 hour of reconstitution.
 - g. P/N 299-7: Hemostasis Reference Plasma, Abnormal. Store at 2 - 8°C before reconstitution. It is advisable to use this product within 1 hour of reconstitution.

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

vW CoFACTOR SOFTWARE [V1.0.4]

Introduction

The CHRONO-LOG® AGGRO/LINK® Opti8™ program is designed to operate with Models 490 4+/490 4+4, a four (4) or eight (8) channel Aggregometer, and a Windows Compatible computer. The 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 Software. For information on Windows and mouse operation, see the Windows Instruction Manual.

The software calculates a best fit standard curve for three (3) 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 Batch Print command allows for batch printing of reports. The reports are printed in standard size (8.5" X 11").

General Description

The vW CoFactor Opti8™ Software 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 Opti8™ Software. 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.

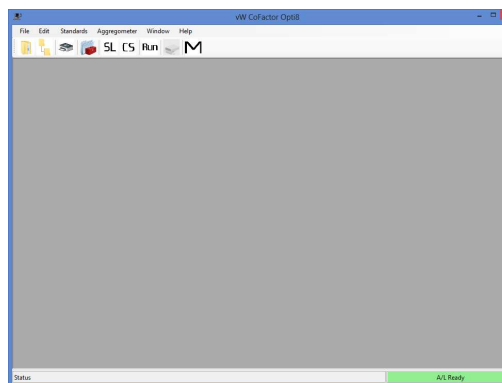
A vW CoFactor Icon can be placed on the desktop using the following procedure:

1. Choose "My Computer"
2. Choose "C:" Drive
3. Choose "Chrono-Log Corporation"
4. Choose "vW_CoFactor_Opti8"
5. Highlight "vW CoFactor Opti8" Icon
6. Right click the mouse on the highlighted icon.
7. Select "Create Shortcut". This will create an icon titled "vW CoFactor Opti8 - Shortcut".
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

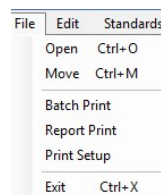
Double click the vW CoFactor Opti8™ Assay Icon to start the program. The following Document box will be displayed:



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 drop-down menu listing all the options available under that category will be displayed.

File

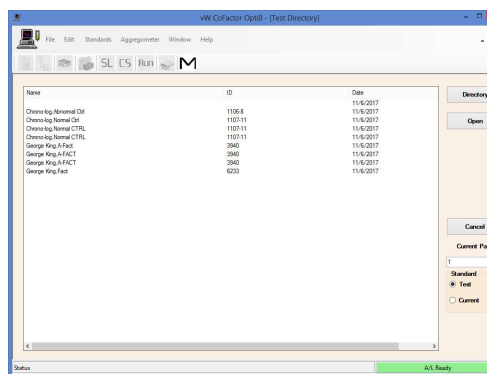
The File command group allows the operator to perform basic file functions. When the File group is selected, the submenu shown below is displayed. To select an option in the File group, click the mouse over the desired command.



1. OPEN (View Test)

This command is used to open a file that has been saved in the test directory. 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 box labeled "Standard" 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 a list of saved 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. 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:

- 1) 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.
- 2) 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 directory dialog that will allow the directory path to be changed.

The default directory for vW CoFactor Opti8™ Software is:

C:\Chrono-Log_Corporation\vW_CoFactor_Opti8\Data

Open:

Left clicking the mouse on the OPEN button will open all selected tests in the file list box.

Cancel:

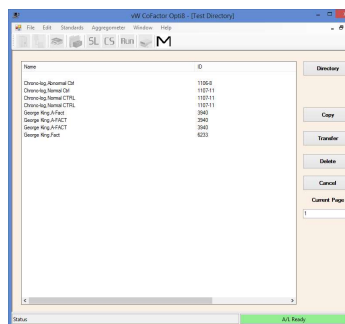
Left clicking the mouse on the Cancel button will close the Test Directory dialog box.

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 per page will be shown at one time in the file list box.

2. MOVE

This selection is used to copy previously saved tests to a CD or USB; to transfer saved tests to a CD or USB or from one CD or USB to another; or to delete a saved test from the Hard drive, CD or USB. When selected, the dialog box below will be displayed:



The Move 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:

- 1) 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.
- 2) 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 another source, set the source directory to "E:_____\data" [the location of the files in the computer] and set the destination directory to:

C:\Chrono-Log_Corporation\vW_CoFactor_Opti8\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:

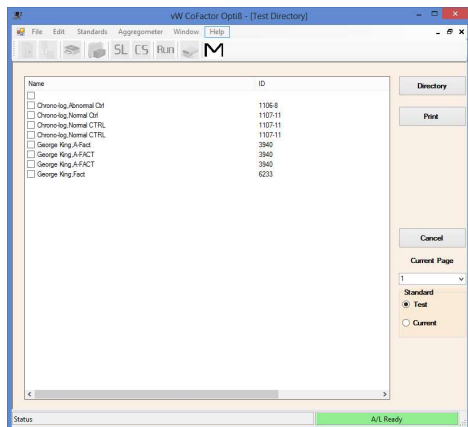
Left clicking the mouse on the Cancel button will close the Test Directory dialog box.

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 per page will be shown in the file list 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.



NOTE: 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:

- 1) 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.
- 2) 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:

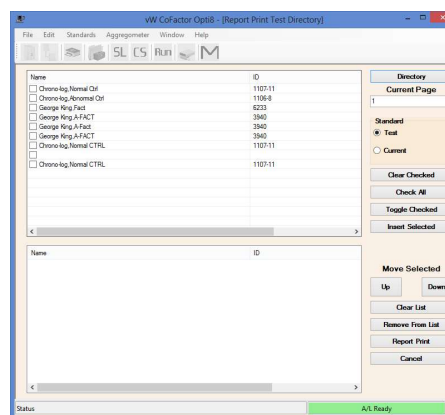
Left clicking the mouse on the Cancel button will close the Test Directory dialog box.

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 per page will be shown in the file list 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 clicking on the accompanying check-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 selected 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 between all checked vs. all unchecked 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 tests to the desired order on the report. Click on the test to be moved and then click on the up or down commands to change the 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. Click on the test to be removed and then click on the Remove from List command.

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

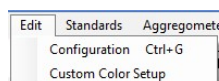
Use this command to select paper size, orientation and to change margin settings.

6. EXIT

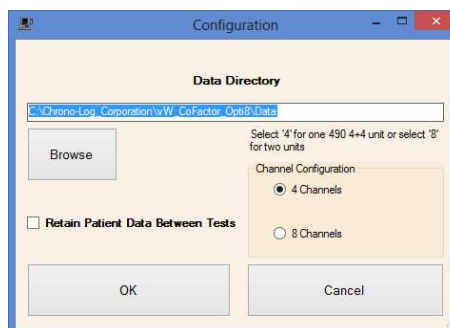
This command closes the vW CoFactor Opti8 Software.

EDIT

The Edit function is used to change the Data Directory; to instruct the software to Retain Patient Data Between Tests and to setup Custom Colors. When the edit group is selected from the menu bar, the menu shown below is displayed.

**1. CONFIGURATION**

When the Configuration command is selected, the Configuration dialog box is displayed:



This dialog has the following controls:

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.

Retain Patient Data Between Tests:

Select this checkbox if you would like the entered patient information to remain after saving or abandoning a test.

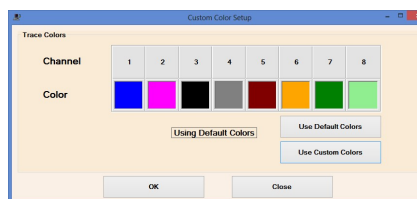
Channel Configuration:

Select a 4-channel [Model 490 4+] or an 8-channel System [Model 490 4+4]

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.

2. CUSTOM COLOR SETUP

When Custom Color Setup is selected, the Custom Color Setup box is displayed:

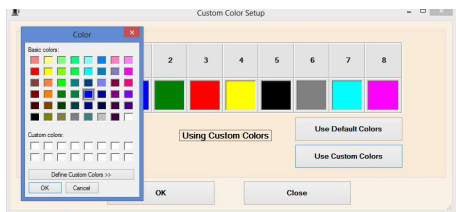


To "Use Default Colors" as shown above, Click on the "Use Default Colors" command and then Click on OK.

To customize the tracing colors, click on "Use Custom Colors".

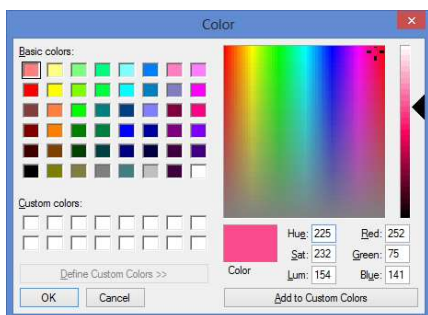
To change colors, click on the **colored block for Channel 1**. A new color can be selected from:

- 1) The "Basic Colors" section shown below. Click on the preferred color then click on OK. Repeat for Channels 2 through 8, if applicable. Click on OK or Close to save selected colors and to end color selection.



SELECT BASIC COLORS SCREEN

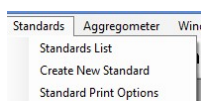
- The “Define Custom Colors” command to obtain the Color Chart shown below. Slide through the Color Chart until an acceptable color appears in the “Color” field. Click on “Add to Custom Colors” then Click OK to change the selected channel to the custom color. Click on colored block for each channel and repeat above. Click on “OK” to save selected colors and to end color selection.



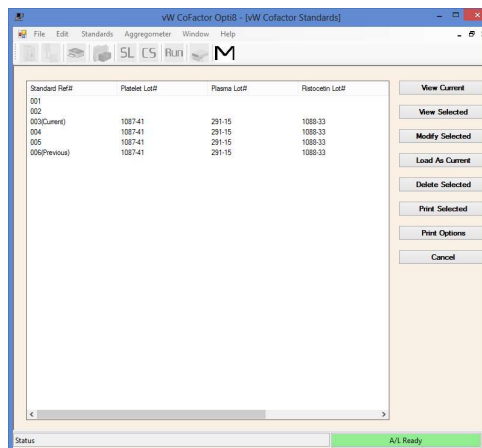
DEFINE CUSTOM COLORS SCREEN

STANDARDS

Without a standard curve, you cannot run a test sample. When the Standards command is selected from the menu bar, the sub-menu shown below is displayed. To select an option in the Standards group, click the mouse on the desired command.



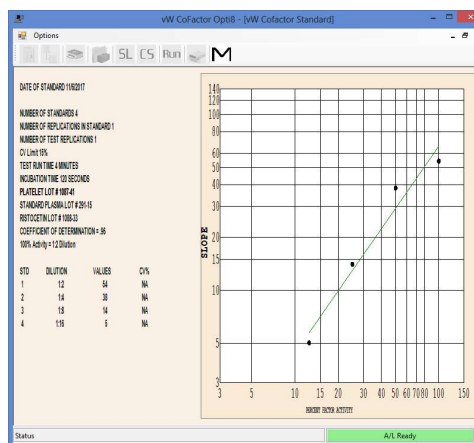
- Standards List** – This selection displays the Standards List dialog. When this selection is chosen the following dialog is displayed:



This dialog allows you to view, modify, delete and print Saved standards.

View Current:

Displays the currently loaded standard with Standard Points plotted on Graph and calculation of Coefficient of Determination, as shown below:



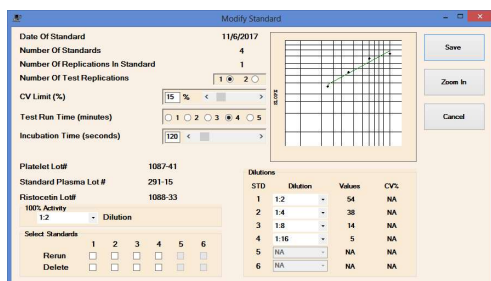
View Selected:

Views the selected standard. Only one standard may be viewed at a time. Left click the mouse on the standard to select and then click the “View Standard” command.

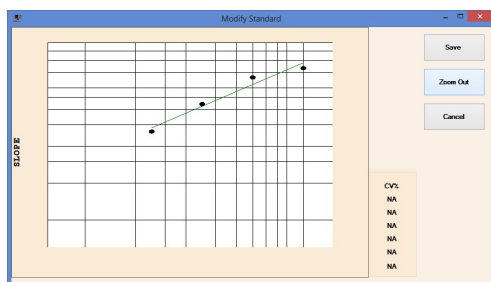
Modify Selected:

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 the 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.



The Modify Standard dialog box 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:

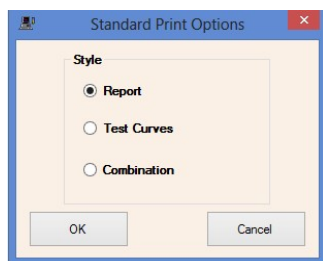
Deletes selected standards from the Standards List.

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 above.

- 1) Report: Prints standard only.
- 2) Test Curves: Prints associated curves only.
- 3) Combination: Prints standard plus associated curves.

Cancel:

Closes the Standard List dialog box

2. **Create New Standard** – This Command 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.



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 as 'Previous' Standard. Now you are set up to run the new standard dilutions.

NOTE: If you do not want to run the new Standard and want to Test with the now 'Previous' 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.

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 procedure 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 three.
- b. Number of Standard Replications – If a Replication is desired for each Standard Dilution, select 2. If you do not want to run a replication test for Standard Dilution, select 1.
- c. Number of Test Replications – If a replication is desired for each Test Sample, select 2. If you do not want to run a replication test for Test Sample, select 1.
- 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. You may enter from one to five minutes in whole minute increments. Once the test reaches the entered time the test will stop automatically.
- f. Incubation Time, In Seconds – Select or Enter the desired Incubation Time of the sample. You can set the incubation time from zero to nine hundred and 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 – Under the dilution column, enter or select the Dilutions from the drop-down menus. These dilutions will be used to calculate the standard curve. 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. Select Standards 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. Click on the appropriate Standard # [1 thru 6] to select for Rerun. Following retesting, the standard curve will then be recalculated with the new standard dilution value combined with the previous standard dilution values.

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".

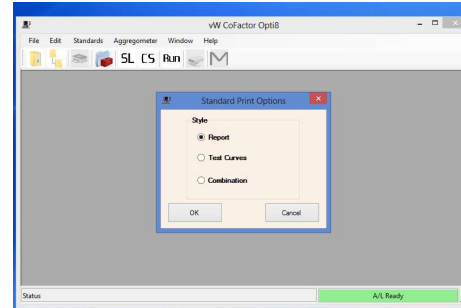
- m. Select Standards to Delete – This selection will be displayed only when in the Modify Standard screen. If a standard dilution is abnormal or when running replicates or the CV is over the limit, you can select to delete that dilution. The standard curve will then be recalculated with the remaining 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 only.
- 2) Test Curves: Prints associated curves only.
- 3) Combination: Prints standard 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 a Valid standard curve has been obtained or has been 'Loaded as Current'.

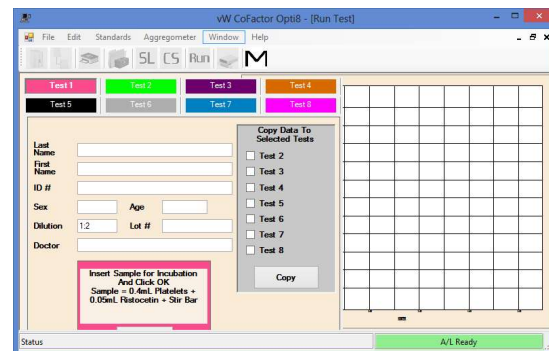
In Run Standard, the program enters a routine for running the Standard Dilutions to calculate the Standard Curve.

When the Aggregometer group is selected, the menu below is displayed.



1. Run Test

The Run Test command will open the screen shown below. The following screen allows up to eight (8) tests.



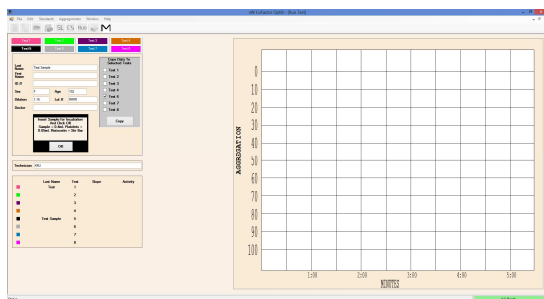
When not running test replications, there will be one (1) control box and one information tab box for each channel.

If the system is set to run duplicate tests [replicates], the screen will display two test channels for Test 1 but only one information tab box for each set of replicates, as shown below.



The control boxes are shown to the left of the graph and are accessible for data entry by clicking on the color-coded TAB as shown in the example below. When software is not set up to test replicates, to perform multiple tests from a single donor, enter data in one channel [ex. 5] and click on Test [ex. 6] to copy data.

Also notice that the Dilution of the test plasma can be changed as needed [ex. 1:16].



These control boxes also provide instructions for running the test. The first instruction given is “Insert Sample for Incubation and Click OK, Sample = 0.4 mL Platelets + 0.05 mL Ristocetin + Stir Bar”. Insert the test sample that is prepared 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 [ex. 2:00] and count down to zero.

The next message will be:

- “1) Set Baseline”
- “2) Add Plasma”
- “3) Click OK”

- 1) “Set Baseline” – Press the Aggregometer’s Set Baseline button 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 “Max Slope = #” with OK and RECALC button options.

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 with following message: “Reposition calculation start line then press RECALC when done.” Click on the Start Line, HOLD and DRAG the 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.

To accept the slope calculation, click on the OK button. The Cofactor Activity will be calculated and the program will respond with “Press OK to Save or Abandon”.

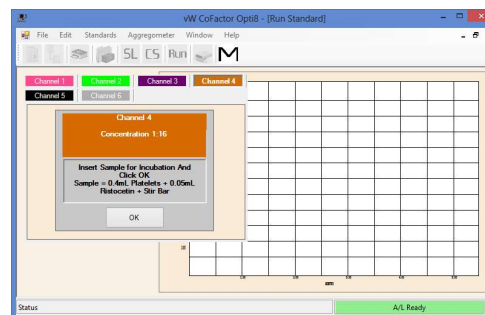
If the test is not satisfactory and you want to rerun it, press ABANDON. The program will loop back to the beginning of the procedure and the instruction box will display the first step.

If you want to save this test, click on the OK button. All information will be saved in the test directory.

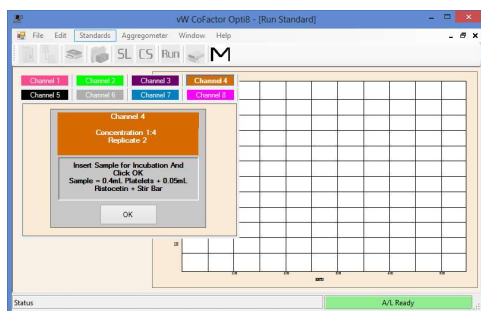
You can print a hard copy of the test by going to File, Open and selecting the appropriate test. Use the print command on the main Menu to print the test.

2. Run Standard

The Run Standard selection will display the following screen. Clicking on each color-coded tab shows a Control Box with the test Channel # [Channel 4 shown] and the plasma dilution value [Concentration 1:16 shown] to be tested in the Channel. One Control Box is displayed for each channel available.



If the procedure is set to run duplicate Standard Samples, each plasma dilution will be shown in two channels, referring to one sample as Replication 1 and the other sample as Replication 2. ODD numbered channels will display the dilution [Concentration 1:4] with Replicate 1. EVEN numbered channels will display Replicate 2.



The first message in the instruction box is "Insert Sample for Incubation and Click OK" with "Sample = 0.4 mL Platelets + 0.05 mL Ristocetin + Stir Bar". Insert the Standard Sample that is prepared 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:

- "1) Set Baseline"
- "2) Add Plasma"
- "3) Click 'OK'"

- a. "Set Baseline" - Press the Aggregometer's Set Baseline button 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, Click on OK to continue or RECALC to Recalculate. If you want to save this test select OK. If the slope calculation is incorrect due to an artifact in the curve, click on 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 "Press OK to save or ABANDON". If you want to save this test, select OK. If the test is not satisfactory and you want to rerun it, click on ABANDON. The program will loop back to the beginning of the procedure, assigning that test to the next available channel, and the instruction box will display the first step.

After all Standard curve tests have been saved, go to the Main "M" Screen and select the Standards List command "SL" to view the Current Standard. The Coefficient of Determination "CD" calculation will also be shown. A CD of $\geq .95$ is acceptable to use for running tests.

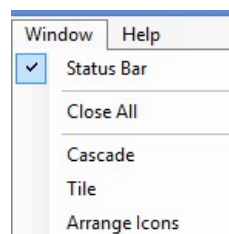
The Standard can be printed by clicking on 'Option' at the top of the screen and then on 'Print'.

3. Connect

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

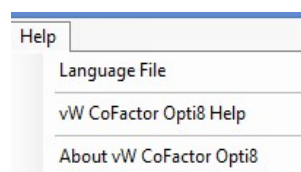
When the Windows menu is selected from the menu bar while tests are open, the sub-menu shown below is displayed.



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 menu is selected from the menu bar, the sub-menu shown below is displayed.



Language File – Contact Chrono-log Distributor for assistance.

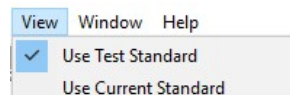
vW Cofactor Opti8 Help - Displays the vW Cofactor Opti8 program's Help File.

About vW Cofactor Opti8 - Displays Version Information for the vW Cofactor Opti8 program

ADDITIONAL OPTIONS WHEN A TEST IS OPEN

View

The View Menu is only available when a test is open. Previously run and saved tests can be viewed using the OPEN command from the FILE Menu.



The options in the View menu allow for calculating the opened tests against two standard options, the Standard it was originally run against or the Current Standard, as shown below:

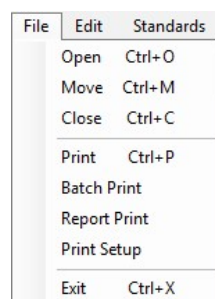
- 1) Use Test Standard – Calculates the test against the standard that was loaded when the test was run.

- 2) Use Current Standard – Calculates the test against the currently loaded standard.

NOTE: See the FILE menu description shown on page 21 for details on opening tests and the use of the Test List directory.

File

Also, when a test is opened for viewing there are several additional menu options available under FILE, as shown below:



The additions to the normal FILE menu options are:

Close:

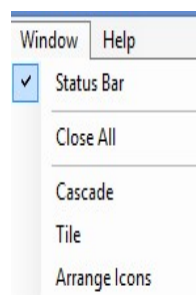
Closes the currently active test document.

Print:

Prints the currently active test document.

Window

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 cascade form.

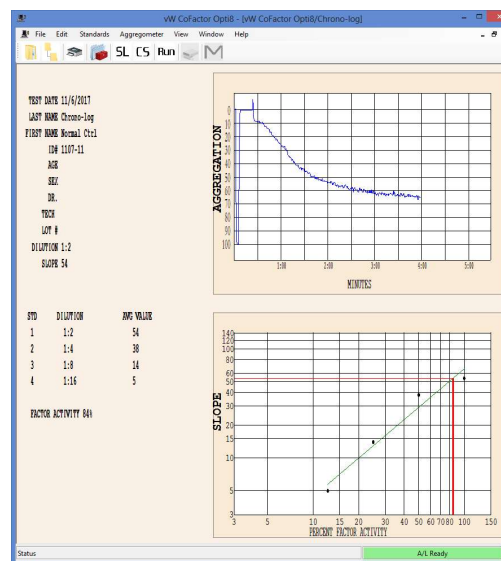
Tile - Arranges all open test documents in tiles form.

Arrange Icons - Arranges all minimized test documents.

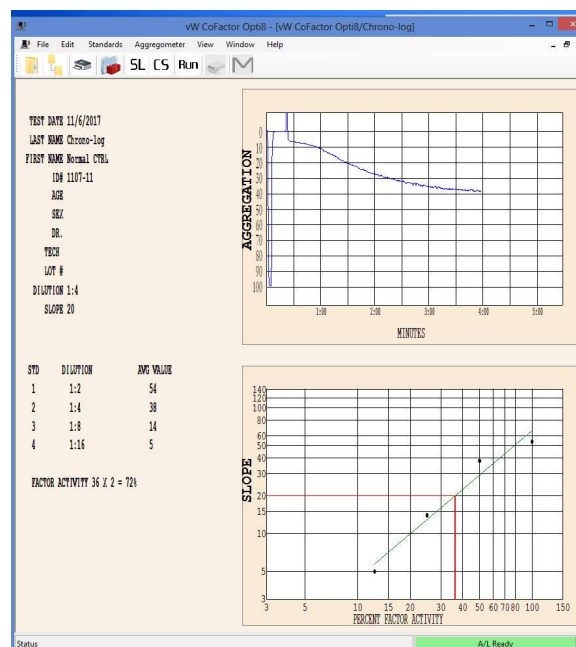
Results

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.

In this section there are three examples for reporting results. In the first example, the factor activity is 84%. This is determined by taking the slope of the test, which is 54, and intersecting the best fit line of the standard curve, as shown by the red lines on the graph. In this example, the test sample was run at the same 1:2 dilution as the 100% standard dilution.

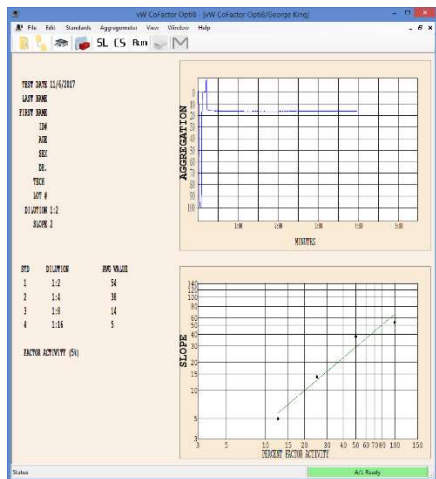


Sometimes, there is a need to run the test sample at a different dilution than the 100% standard dilution. In the second example, the test sample was diluted 1:4 and the 100% standard was diluted 1:2. The slope of the test, which is 20, intersects the best fit line of the standard curve at 36% factor activity. This is shown on the graph of the best fit line. 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 of 72% at the bottom of the dialog box. This calculation is done automatically by the software.



The third example shows a test with a slope that is lower than the slope of the 12.5% [1:16] 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.

Parenthesis are placed around the calculated FACTOR ACTIVITY to indicate that the results are outside the limits of the best fit line. Test results outside the limits of the best fit line may be inaccurate.



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.

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:

- Standard Curve Preparation (must run for each day of testing). 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 Opti8™ Software 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.

- 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.

- 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.

- Use SELECT and SET pushbuttons to set PPP selection for each channel. The SET pushbutton toggles each channel to reference either Channel 1 PPP or to reference each channel to its own reference cuvette. Each set of buttons controls two (2) channels. When all channels are set to reference Channel 1, only one Blank is required.

e. Start vW Cofactor Assay Program

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

- 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 with no replications):
 - a) NUMBER OF STANDARDS **3** (minimum 3)
 - b) NUMBER OF REPLICATIONS IN STANDARD **1**
 - c) NUMBER OF TEST REPLICATIONS **1**
 - d) CV LIMIT **15%**, **NOTE: CV Limit is only relevant when testing two replications.**
 - 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**

NOTE: 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. Click on color-coded Tab to review instructions for each channel.

- a) For testing with up to six (6) 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, etc.

NOTE: If a point is Abandoned and needs to be repeated, the software will automatically select “the next available channel” to perform the repeat test and not necessarily the original test channel.

- 7) Prewarm cuvettes (P/N 312) with Stir Bar (P/N 311 or 313) in incubation wells.
- 8) For each point on the Standard Curve (3 to 6), take one prewarmed cuvette with stir bar and

pipette 400 µL 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 µL Ristocetin, wipe tip with a 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 for Incubation and Click OK Sample = 0.4 mL Platelets + 0.05 mL 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) While first sample is incubating, add 50 µL Ristocetin into a second pre-warmed 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 Channel 3 and for Channel 4, if appropriate.
- 12) After the Incubation period is completed in Channel 1, the next instruction reads: “1)SET BASELINE, 2)ADD PLASMA, 3)CLICK OK” - **NOTE: When these instructions appear, the color-coded 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 µL 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) **Channel 2** Incubation period should be completed or near completion. Follow Steps 13 thru 16 above using the (1:4) Reference plasma tube.

- 18) **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 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 μ 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.
- 24) Repeat Steps 20 to 23 above for remaining test channels.

25) 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.

- a) After viewing the standard, click the mouse on "M" on the toolbar.
- b) If the standard is acceptable, go to section "g" - **RUNNING TESTS** below and continue with running control or patient tests.
- c) If the standard needs to be modified, click on **STANDARDS** and **STANDARD LIST**. Click the mouse on the standard listed as "Current" to highlight. Then click on

MODIFY SELECTED. The standard will appear on the screen with options to RERUN or DELETE selected dilutions.

- d) 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 and click on SAVE.
- e) Click on AGGREGOMETER and select RUN STANDARD. Software will be set automatically for the selected dilution(s) to be rerun.
- f) Repeat the assay for the dilution(s) to be rerun. Be sure to use the appropriate Plasma Dilution(s).
- g) Repeat Step 25 to view the newly created Current Standard to determine if acceptable for running tests.

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

- 1) To **Run Tests**, select **AGGREGOMETER** from the Menu bar.
- 2) Select **Run Test**. One instruction box for each channel will be displayed on screen by clicking on the color-coded Tab provided for up to eight (8) tests. 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.
- 3) For each channel, take a prewarmed cuvette with stir bar and pipette 400 μ L of reconstituted platelets in each. Incubate for a minimum of 4 minutes in the incubation well.
- 4) After incubation, swirl to mix and add 50 μ 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.

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.

- 5) Repeat Step "4" for each channel. During the 2-minute incubation, mix well the appropriate test plasma dilutions.
- 6) After the Incubation period is completed in Channel 1, the next instruction will read:

1) SET BASELINE 2) ADD PLASMA 3) PRESS OK.

- 7) 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.

- 8) Mix well by hand the (1:2) Patient Test or Control Reference plasma tube...then draw 50 µL into the pipette.
- 9) Wipe the pipette tip with a clean Kim Wipe and add plasma to test cuvette. Be sure the pipette tip goes into the sample.
- 10) **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.

Note: 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 start 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) Click on OK to save each test. Tests 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 Opti8™ software 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, other plasma dilutions should be tested 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, reduce test material volumes to 200 µL Platelets + 25 µL Ristocetin + 25 µL Plasma. Pay careful attention to pipetting to avoid the creation of bubbles which will affect the quality of the tracing and the results.
- 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, by hand, 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 20 for proper pipetting techniques.
- f) If testing for an extended period of time, mix blank periodically by hand.

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.

SERVICE/PREVENTATIVE MAINTENANCE

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

In the 48 continental United States a Factory Service and Loaner Contract program is available. On-site field service is also available in some areas. Contact the factory for details.

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

Replacement of Parts: Critical components should only be replaced with manufacturer approved parts. Maintenance should be performed according to service manuals and PM checklists.

Protective Earth Ground: The instrument is connected to ground at the instrument chassis. This sole purpose connection point is made between the power entry module and the chassis with green/yellow wire and is marked with the IEC 60417-5019 symbol. This connection must be reestablished if it is removed for any purpose during servicing.



CLEANING SYSTEM COMPONENTS

Recommended Tools and Accessories

A Liquid Dishwashing Detergent -- Use a mixture of one part liquid dishwashing detergent and three parts water to clean the exterior of the Aggregometer.

A Soft, Lint-Free Cleaning Cloth -- Moisten the cleaning cloth with the dishwashing detergent solution to clean the exterior of the Aggregometer.

A Small Vacuum Cleaner with a Brush attachment -- Use the vacuum cleaner to remove dust and dirt from the exterior of the Aggregometer.

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 holes in 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

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. Materials Required
 - a. P/N 322. Calibration Kit with (1) black cuvette and (2) sealed water cuvettes.
 - b. Lint-Free Wipe
 - c. Calibration Key
2. Turn on the unit and let it heat up until the heater blocks stabilize at 37°C.
3. **Since each Channel is calibrated independently, confirm that that PPP/Reference Select Switches are set to reference each Channel's PPP sample, and are not referencing 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 39]

4. Place the (2) water cuvettes from P/N 322 in the incubation wells and pre-warm for a minimum of three (3) minutes. Be sure to always wipe cuvettes with Kim Wipe® before placing in test or reference wells.
5. Turn on computer and Start AGGRO/LINK® Opti8™ for Windows® program.
6. In the Run Test window, select the previously created Calibration test procedure from the menu, OR, click on EDIT to create a new Test Procedure.
7. Create or change Procedure Name to Optical Calibration.
8. SAVE and Click on OK to return to Run Test.
9. Type, "Optical Cal Pre", in Test Identification box at top of screen
10. Click on OK and graph will appear.
11. After the three (3) minute incubation, check cuvettes for air bubbles. Eliminate bubbles by tapping the cuvette.

Pre-Calibration Test

NOTE: Throughout the entire procedure, the Water Cuvette used in the PRP well should only be used in PRP test wells and the PPP Water Cuvette should only be used in PPP reference wells. Be sure to use the Alignment Mark on side of each cuvette to ensure consistent placement of cuvettes into test and reference wells.

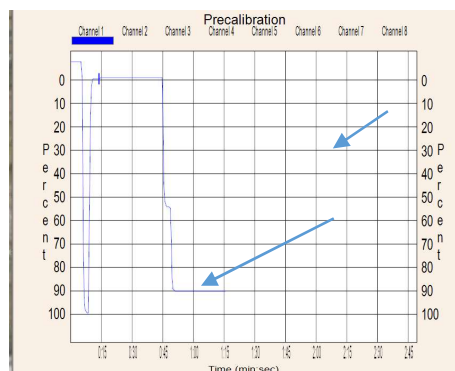
1. After wiping the outside with a Kim Wipe®, place BLACK cuvette in Channel 1 PRP well and a water cuvette in the PPP well of Channel 1, with the alignment mark placed left center.
2. Click on the Channel 1 "Activate" bar.
3. Click on the "Start" Bar for Channels 1 through 8.
4. Push and Hold the Set Baseline button for Channel 1 until the tracing reaches 100% of graph before releasing. Tracing will then return to 0% of graph.
5. Replace the BLACK cuvette in the PRP well with the other water cuvette, after wiping the outside with a Kim Wipe®. Be sure alignment mark is placed left center. The tracing should move near 100% on the graph. Allow to run for a few seconds and note the position where the tracing stabilizes. (Ex: 97%)
6. Repeat Steps 2 through 6 above for Channels 2 thru 8. Be sure to use the water cuvette in the PPP well in all PPP wells and the water cuvette in the PRP well in all PRP wells. Also

be sure to wipe the outside with a Kim Wipe® and to place the Alignment Mark to the left center of the test well.

7. If saving the test ... Once all channels are completed, click on the STOP icon followed by the SAVE icon and the PRINT icon to save and print the test.
8. If any tracing is > 4% from 0% or 100% on the graph, proceed with calibration steps below, calibrating all eight (8) channels.

NOTE: If the tracings are consistently > 4% from 0% or 100%, it is recommended to check that the two water cuvettes supplied in the Calibration Kit are still matched [$\leq 4\%$] by interchanging them between the PRP and PPP wells. See Appendix A for instructions.

Tracing below shows that calibration is out of specification or technical error exists.

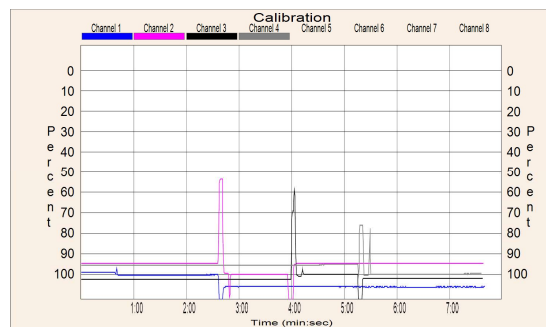


Calibration

NOTE: Calibrate ALL test channels, even if only one channel is out of tolerance. This will ensure that all LEDs are set to the same intensity.

1. Click on Aggregometer, select Restart Current Test.
2. Click on EDIT, Test Information and Type in Calibration in Test Identification field. Click on OK to return to graph.
3. After wiping the outside with a Kim Wipe®, place the PRP water cuvette in Channel 1 PRP well and the “PPP” water cuvette in Channel 1 PPP well, keeping the Alignment Marks left center.
2. Click on the “**Activate**” bar for Channels 1 through 8.
4. Click on the “Start” Bar for Channels 1 through 4.
5. Place the key in the calibrate switch on the module containing Channels 1 through 4 and turn the key clockwise 90° and keep it in this position. Since calibration does not start until the baseline is set, all four (4) channels can be calibrated.
6. Set the timer for one minute but do not start. Press the Set Baseline button for Channel 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 one minute [*or when tracing runs stable for around 20 seconds*] before proceeding to the next step.

NOTE: Disregard traces during calibration, as shown below.



7. After waiting one minute, or when tracing stabilizes in Channel 1 ... Repeat steps 2, 5 and 6 for Channels 2 thru 4. Calibration key remains ON throughout this process.
8. Once step 7 is completed for all four (4) channels, turn OFF the calibration mode by turning calibrate key counter-clockwise 90° and **remove the key from the lock**. Calibration of Module 1 is now completed.
9. **To calibrate Channels 5, 6, 7 and 8 in Module 2, beginning with Channel 5, repeat steps 3 through 8 above, using the calibrate key in that module.**
10. If required, SAVE and PRINT the test.

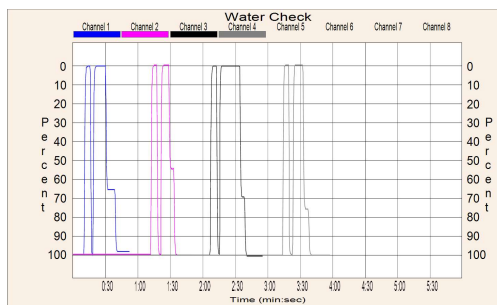
Post Calibration test

[Confirm the Calibration Key has been removed from lock]

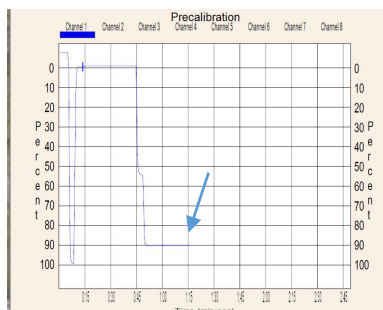
1. Select RUN SAME TEST under AGGREGOMETER and Click on OK.
2. Click on EDIT, Test Information and Type, “Optical Cal Post” in Test Identification field. Click on OK to return to graph.
3. After wiping the outside with a Kim Wipe®, place BLACK cuvette in Channel 1 PRP well and the PPP water cuvette in the PPP well of Channel 1, with the alignment mark placed left center.
4. Click on the Channel 1 “Activate” bar.
5. Click on the “Start” Bar for Channels 1 through 8.
6. Push and Hold the Set Baseline button for Channel 1 until the tracing reaches a full 100% of graph before releasing. Tracing will then return to 0% of graph.
7. After wiping the outside with a Kim Wipe®, replace the BLACK cuvette in the PRP well with the PRP water cuvette with the alignment mark placed left center. The tracing should move near 100% on the graph. Allow to run for a few seconds and note the position where the tracing stabilizes. (Ex: 97%)
8. Repeat Steps 3 through 7 for Channels 2 thru 8. Be sure to use the PPP water cuvette in the PPP wells and the PRP water cuvette in the PRP wells. Also be sure to place the Alignment Mark to the left center of each test well and to wipe the outside of the cuvettes with a Kim Wipe® when placing into the test well.

9. If any tracing is > 4% from 0% or 100% on the graph, calibration of that Channel will need to be repeated, following the previous calibration steps.
10. If saving the test ... Once all channels are completed, Click on the STOP icon followed by the SAVE icon and the PRINT icon to save and print this test.

Tracings below show that calibration is within specifications for all 4 Channels.



NOTE: If any of 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. See Appendix A for further instructions on matching water cuvettes, 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.



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.

[Confirm the Calibration Key has been removed from lock]

SELECT Pushbutton(s) – allows for toggling between the *Stirring Speed, Temperature and PPP/Reference Settings*.

SET Pushbutton(s) - sets the *Stirring Speed, Temperature and PPP/Reference, depending on which feature is selected. Each time the SET button is pushed, the selected function is incremented until it reaches the maximum setting.*

NOTE: One **SELECT** and **SET** pushbutton controls two channels in tandem. For example, Channels 1 & 2, Channels 3 & 4, Channels 5 & 6 and Channels 7 & 8 are set in pairs.

1. Set all Channels to Reference Channel 1 PPP
 - a. Push SELECT pushbutton for Channels 1 and 2 until PPP/Reference setting is displayed on the LCD screen.
 - b. Push SET pushbutton for Channels 1 and 2 until the reference to Channel 1 PPP is displayed.
 - c. Push SELECT pushbutton for Channels 1 and 2 to return to Default screen ... which will inactivate the SET pushbutton for those two channels.
 - d. Repeat steps a through c for remaining channels.
2. Select RUN SAME TEST under AGGREGOMETER and Click on OK.
3. Click on EDIT, Test Information and Type, "Optical Cal PPP 1" in Test Identification field. Click on OK to return to graph
4. After wiping the outside with a Kim Wipe®, place BLACK cuvette in Channel 1 PRP well and the PPP water cuvette in the PPP well of Channel 1, with the alignment mark placed left center.
5. Click on the Channel 1 "Activate" bar.
6. Click on the "Start" Bar for Channels 1 through 8.
7. Push and Hold the Set Baseline button until the tracing reaches a full 100% of graph before releasing. Tracing will then return to 0% of graph.
8. After wiping the outside with a Kim Wipe®, replace the BLACK cuvette in the PRP well with the PRP water cuvette. The tracing should move near 100% on the graph. Allow to run for a few seconds and note the position where the tracing stabilizes. (Ex: 97%).
9. Repeat Steps 5 through 9 for remaining channels, except ... **leave the PPP cuvette in the Channel 1 PPP well.** Also be sure to place the Alignment Mark to the left center in each test well and to wipe the outside of the cuvettes with a Kim Wipe® when placing into the test well.
10. If any tracing is > 4% from 0% or 100% on the graph, calibration of that Channel will need to be repeated, following the previous calibration steps.
11. If saving the test ... Once all channels are completed, Click on the STOP icon followed by the SAVE icon and the PRINT icon to save and print this test.

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. See Appendix A for further instructions on matching water cuvettes, 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.

Discussion

The Black cuvette blocks all light from passing through. 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, the P/N 322 Kit may need to be replaced.

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.

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

Troubleshooting Guide

Many factors will affect the performance and reproducibility of test runs 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.

- ☐ Missing stir bar
- ☐ Bubbles in sample blocking optical path
- ☐ 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.

ROUTINE QC CHECKS should be performed on a quarterly basis, or if any of the following conditions should exist during testing. Optical calibration should be checked with Water Samples as described under the Pre-Calibration procedure. If required, Calibration of the Optical Circuits can be performed using the same Water Samples as described under Calibration.

- ☐ Test Results > 100%
- ☐ Test Results on all channels do not fall within +/- 10% of the average

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

Data Back-up

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.

The CHRONO-LOG® AGGRO/LINK® Opti8™ and vW CoFactor Opti8™ Software packages install automatically as Administrator for all users on Windows 7 or later operating systems. This eliminates permission issues with operating the software.

It is also possible to use Microsoft Windows to back up the data. It is useful to search for the files by partial name such as OPTI8*.*, opti8dirV2.dat, opti8dataV2.dat, or the data file names appropriate for the software as listed below. 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 Opti8™ data files are saved in the install folder:

- a. vwstd.dat - for saved standards
- b. vwdir.dat – for listing saved tests to open
- c. vwtestD.dat – data file for tests run as duplicate
- d. vwtestS.dat – data file for tests run as non-duplicate

Data files associated with various CHRONO-LOG® programs:

AGGRO/LINK® Opti8™ – opti8dataV2.dat and opti8dirV2.dat
vW CoFactor Opti8™ - vwstd.dat, vwdir.dat, vwtestd.dat and vwtests.dat
AGGRO/LINK® 8 – datafile8.dat, dirfile8.dat, notefile8.dat (not present on older versions)
AGGRO/LINK® – datafile.dat, dirfile.dat
AGGRO/LINK® WBA – WBAdata.dat, WBAdir.dat
vW CoFactor Assay – vwstd.dat, vwdir.dat, vwtestS.dat, vwtestD.dat

Assistance and Distributor Information

For Assistance on training, service, software updates and other information, contact your Chrono-log representative or your local distributor. Information on Chrono-log Distributors can be found on the Chrono-log website at www.chronolog.com.

Incident Reporting

Serious incidents related to the device shall be reported to Chrono-log Corp. at regulatory@chronolog.com as well as to the competent authority of the Member State/Country in which the user and/or patient is established as per the requirements of local regulations.

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APPENDIX A

1. Ensure the Two Water Cuvettes Match

Follow this procedure if the tracings are > 4% from 0% or 100% on the graph.

- a. After wiping the outside with a Kim Wipe®, place BLACK cuvette in the PRP well of Channel 1 and a Water cuvette in the PPP well with the alignment mark placed left center.
- b. Click on the Channel 1 “Activate” bar.
- c. Push and Hold the Set Baseline Button until the tracing reaches a full 100% of graph before releasing. Tracing will then return to 0% of graph.
- d. After wiping the outside with a Kim Wipe®, replace the BLACK cuvette with the 2nd Water cuvette from the kit, with the alignment mark placed left center. The tracings should move near 100% on the graph. Allow to run for a few seconds and note the position where the tracing stabilizes. (Ex: 98%)
- e. Keeping the alignment marks in the left center position, 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%)**
- f. If the difference between the two numbers (98% and 97%), is < 4%, the two Water cuvettes are matched and can be used to check the calibration and/or to perform Calibration.
- g. If the difference between the two numbers is > 4%, the two Water cuvettes are not matched and should be replaced.
- h. Contact Chrono-log to order P/N 322 Calibration Kit containing (1) BLACK Cuvette and (2) sealed Water Cuvettes.

NOTE: If it is determined that Calibration cannot be delayed to wait for delivery of a new Kit, standard P/N 312 cuvettes can be substituted, as described in Step 2.

2. Ensure P/N 312 Water Cuvettes Do Not Contain any Major Scratches/Flaws

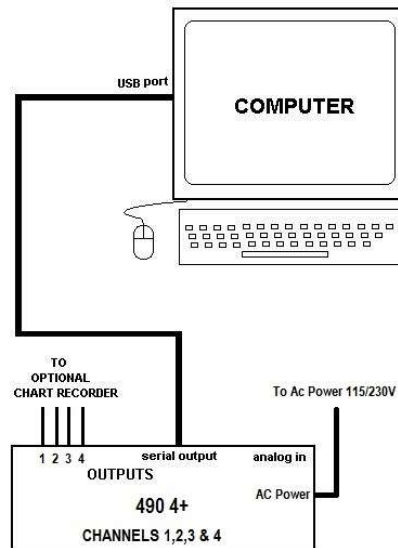
- a. Make sure that the cuvettes are clean and there are no fingerprints on the bottom half. Different cuvettes will give slightly different outputs, so visually inspect several cuvettes and pick three (3) cuvettes that appear to be most free from blemishes. The fewer blemishes in the cuvette the more accurate the calibration.
- b. Take the three (3) new cuvettes and pipette 500 µL of water into each. Incubate the cuvettes for a minimum of three (3) minutes in the incubation wells.
- c. After the three (3) minute incubation, check cuvettes for air bubbles. Eliminate bubbles by tapping the cuvette.
- d. After wiping the outside with a Kim Wipe®, place BLACK cuvette in the PRP well of Channel 1.
- e. Using a Kim Wipe®, wipe the outside of (1) prewarmed P/N 312 cuvette containing water and place in the PPP well of Channel 1.
- f. Press Set Baseline. Be sure tracing reaches a full 100% of graph before releasing.
- g. With tracing stable at 0%, slowly turn the Water cuvette in the PPP well and 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 left center of the cuvette with a dark Alignment Mark.

- h. Repeat steps 5 thru 7 above until there are 3 marked cuvettes.
- i. Follow Instructions in 1. above to select (2) matched Water cuvettes.

APPENDIX B

Model 490 4+ Connection Chart



Model 490 4+4 Connection Chart

