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

CHRONO-LOG CORPORATION

● 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

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.

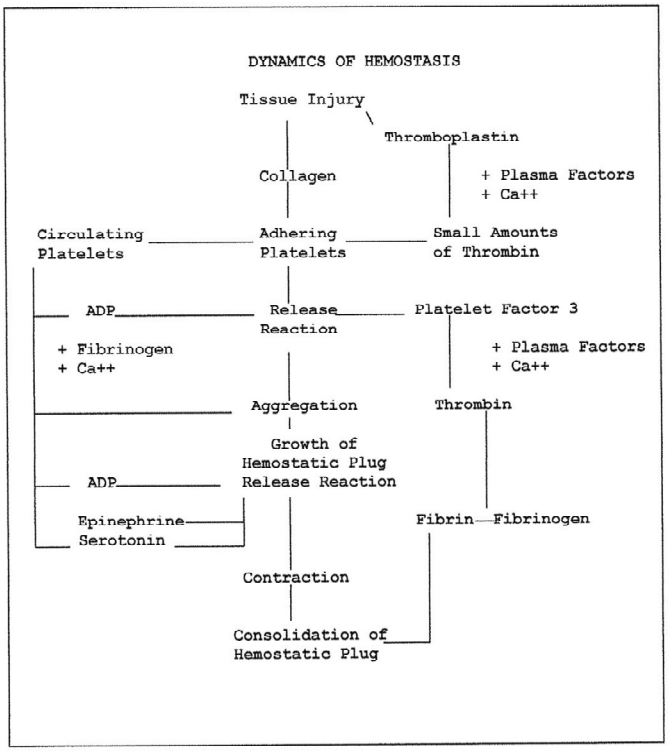
SUMMARY AND EXPLANATION

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 pseduopods 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 thrombin[3], the calcium ionophore A23187, arachidonic acid, ristocetin, Bovine Factor VIII, and serotonin have also been used to study platelet response for more specific purposes.

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

Platelet aggregation is clinically significant in the detection and diagnosis of acquired or congenital qualitative platelet defects. The platelet's ability or inability to respond to particular aggregating reagents is the basis for differentiating platelet dysfunctions[6] 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; O% 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 x 10^9/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 ±0.2°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 a Pentium/compatible processor running at 133 MHz or higher.
- 32 Meg 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 for installation: 3MB.
- Additional Hard Disk Space for storage of tests.
- 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.

Instrument Calibration

The optical technique is calibrated automatically during the testing procedure.

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 in the drive 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. 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 NOT CONNECTED on the lower right side of the status bar if connection cannot be established.

b. Check the cable and make sure the Aggregometer has power.
c. To retry, select "Aggregometer" from the ribbon, then "Connect" from the dropdown menu.
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 A/L NOT CONNECTED on the lower right side of the status bar if connection cannot be established.
    b. Check the cable and make sure the Aggregometer has power.
    c. To retry, select "Aggregometer" from the ribbon, then "Connect" from the dropdown menu.
    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.

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 10 and PROCEDURE – STEPWISE on page 11 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 ($P2Y_{12}$), Prasugral ($P2Y_{12}$)
- **RGD Peptomimetics**
  Eptifibatide, Tirofiban
- **Phosphodiesterase Inhibitors**
  Dipyridamole, Cilostazole
- **Anticoagulants**
  Heparin, Warfarin, Direct Thrombin Inhibitors (lepirudin, argatroban, bivalirudin)
- **Cardiovascular Agents**
  β-adrenergic blockers (propranolol), Vasodilators (nitroprusside, nitroclygerin), Diuretics (furosemide), Calcium channel blockers
- **Antimicrobials**
  β-lactams (penicillin, cephalosporins), Amphotericin (antifungal), Hydroxychloroquines (antimalarial), Nitrofurantoin
- **Chemotherapeutics Agents**
  Asparaginase, Plicamycin, Vincristine
- **Psychotropics and Anesthetics**
  Thricyclic antidepressents (imipramine), Phenothiazines (chlorpromazine), Local and general anesthesia (fluothane)
- **Miscellaneous Agents**
  Clofibrate, Dextrans, Guaifenesin (expectorant), Radiographic contrast
- **Foods/Herbals**
  Alcohol, Caffeine (methylxanthine), Garlic, Onion, Ginger, Fish Oil, Vitamins C and E.

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

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:
Testing can start 30 minutes after venipuncture and continue for about 2.5 hours after. Specimen should be kept at room temperature (24° to 27°C).

EQUIPMENT AND MATERIALS:

Equipment:
1. CHRONO-LOG® 490 4+ or 490 4+4 Aggregation system with internal AGGGRO/LINK® Interface.
2. Windows®-Compatible Computer and AGGRO/LINK® Opti8™ software installed.

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 osmolality. 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.

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.

17. 15 mL conical test tube and cap, or similar (per test subject): for storing the PRP and PPP specimens.
18. Ice bucket: for maintenance of the reagents during the course of the working day
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 test tubes.
21. Lintless wipes, such as KimWipes® .

Gauze squares are NOT suitable.

PREPARATION:
1. **Aggregometer**
   a. Turn on the unit 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 of PPP can be used as the reference sample for all tests run with the same patient's blood, so the amount of PPP required is only enough for one sample, about 500 µL.
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.
      2) Take off the PRP with a polypropylene transfer pipette and place into a polypropylene plastic tube and add cap.
      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 CO_2 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) Properly label the tube, include the patient's name and sample type. Parafilm or cap the top. Keep at room temperature (24°C to 27°C).
   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 PRP with a polypropylene transfer pipette and put it into a polypropylene plastic tube.
      4) Properly label the tube, include the patient's name and sample type. Parafilm or cap the top. Keep at room temperature (24°C to 27°C).

When ready to begin testing, dispense one aliquot per channel of adjusted or non-adjusted PRP of 500 µL volume into P/N 312 cuvettes with stir bars. Warm for a minimum of 3 minutes. It is not recommended to incubate a sample beyond 30 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. Water
Catalog No.: 398
Supplied As: Purified Water 15mL

Sterile distilled, bottled water suitable for CHRONO-PAR® Reagent preparation.

Pyrogen free (ATP free) for reconstituting reagents and not containing preservatives/additives such as benzyl alcohol which inhibits platelet function. Do not use water from the lab purification system.

b. **Saline**
Catalog No.: 397
Supplied As: Saline (0.9%) 15mL

Sterile, physiological, saline for CHRONO-PAR® Reagent preparation.

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

c. **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 volumes suitable for a days testing for one year or until expiration date, whichever comes first.

d. **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 fully absorb the saline (check visually). 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 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 for 5 minutes. The reconstituted Arachidonic Acid suspension should appear very milky with numerous small bubbles.

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 just before use.

e. **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 asceptic 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.

f. **Epinephrine**
Catalog No.: 393
Supplied As: Lyophilized preparation of l-Epinephrine bitartarate 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. Dilute the stock 1:10 with physiological saline for PRP testing. Allow to sit for ten minutes with occasional inversion. Adding 5µL of 1:10 Diluted Solution to 500 µL sample of platelet rich plasma or 2.5 µL of reagent to 250 µL sample gives a final concentration of 10 µM. Normal aggregation is seen with final concentrations of 5-10 µM in platelet rich plasma.

Stability: Epinephrine is a comparatively unstable reagent. 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.

g. **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

a. 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.
b. Click on EDIT and CONFIGURATION. Type in your institutions 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.
c. 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.
d. To display and print Start Indicators, click on the box to select. To disable, leave this box unchecked.
e. Under the AGGREGOMETER window, select or set-up a test Procedure page for Optical mode. 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:

- **CREATE MERGE – Creates a blank document for pasting curves**
- **EXPORT - Export test data for use with another program**
- **ADJUST SLOPE LINE – Change slope calculation time**
- **OFFSET CURVES – Change the physical start time of a test Channel**
- **COPY SCREEN – Copies the test grid and curves to the system Clipboard**
- **MERGE COPY ALL or SELECTED - To copy all the curves from a file or to copy only selected curves from a file to a merge document.**
- **START & STOP TIMES – Automatic marking at addition of reagent.**
- **CUSTOM COLOR SETUP – Use Default tracing colors or customize**

- **ACTIVATE Bar – provides the ability to control each test individually or in sets of 2 or 4 channels, eliminating the risk of some samples sitting in test well for extended period of time before tests are started.**
  1. To run up to Eight (8) tests at the same time:
     a. After a 3-minute incubation, place test cuvettes in PRP test wells – from 1 to 8 channels
     b. Click on "Activate" Bar and press the Set Baseline button for each channel.
     c. Monitor all tracings for stability.
     d. Once tracings are stable and, if a clean test screen is needed, Click on "Aggregometer" and "Reset" for all channels.
     e. If required that the Baseline setting appear on test printout, press Set Baseline for all channels.
     f. Click on "Start" Bar for Channel 1 and add reagent.
     g. If Baseline setting not required on test printout, Click on the "Start" Bar for Channel 1 and add reagent.
     h. Repeat "e and f" or "g" for remaining channels.
  2. To run tests in 4 channel sets:
     a. After a 3-minute incubation, place 4 test cuvette(s) in PRP test well(s) in Channels 1 thru 4.
     b. Click on "Activate" Bar and press the Set Baseline for Channels 1 thru 4.
     c. Monitor tracings for stability. [**NOTE:** At this point, place 4 additional test cuvettes in Incubation wells for Channels 5 thru 8 for a 3-minute incubation.]
     d. Once tracings are stable and, if a clean test screen is needed, Click on "Aggregometer" and "Reset" for Channels 1 thru 4.
     e. If required that the Baseline setting appear on test printout, press Set Baseline for Channels 1 thru 4.
     f. Click on "Start" Bar for Channel 1 and add reagent.
     g. If Baseline setting not required on test printout, Click on the "Start" bar for Channel 1 and add reagent.
     h. Repeat "e and f" or "g" for remaining channels.
     i. After 3-incubation, place 4 test cuvette(s) in PRP test well(s) in Channels 5 thru 8.
     j. Repeat "b" thru "h" for Channels 5 thru 8.

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 Platelets do NOT aspirate/dispense 3 times. Only take up the sample one time.

d. Dispensing

Place the pipette tip into the cuvette, so that the end of the tip is immersed in the sample.

1) Slightly angle the pipette so that the tip is angled to touch the wall of the cuvette.
2) 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.
3) 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.
4) Once the pipette tip is completely outside of the cuvette, release the control button.

CALIBRATION:

For testing platelets, the Optical mode is calibrated automatically during the testing procedure by the setting of 0% (PRP) and 100% (PPP) baselines.

QUALITY CONTROL:

It is good laboratory practice to run a drug free normal control whenever reagents are reconstituted or thawed. 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.

Positive controls can also be made in-vitro by the addition of aspirin or the depletion of plasma. A final concentration of 1 mM aspirin in citrated blood will inhibit the response to arachidonic acid. Centrifugation, removal of platelet poor plasma and replacement with an equal volume of saline, while leaving the buffy coat in place will inhibit response to Ristocetin.

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. For testing the same donor in all channels, set all channels to reference channel #1 PPP. [If each channel is set to its own PPP, a cuvette with 500 µL PPP is 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 µL **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. Place 500 µL or 250 µL Platelet-RICH-Plasma (**PRP)** into pre-warmed cuvettes with stir bars. [Prepare (1) for each test channel.] Incubate for three (3) minutes in incubation wells
6. 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.
7. Select "RUN NEW TEST" under AGGREGOMETER window. Patient information page will appear. Patient information can be completed at this time or can be entered during or after test is completed. [Click on EDIT then TEST INFORMATION] ***When "Run Same Test" is selected, patient data from previous test will be used.***

NOTE – Patient data from Trace 1 can be copied to other tracings. 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.

8. 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.
9. Click OK to begin test. Click on color-coded "Activate" bar for each channel.
10. 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, using the numbers on the left side of the graph. Be sure to press and hold the Baseline button until the tracing reaches 100% of the graph and then release.

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.

11. Monitor tracings for stability.
12. When tracing(s) have reached stability, take up the appropriate reagent and click on the color-coded "Start" bar for Channel 1. [**Or** … See **NOTE 2** below].
13. Add reagent
    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…or 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.

14. Repeat steps 12 and 13 for each test channel.
15. Allow all tests to run for a minimum of five (5) minutes.

NOTE: The "Activate" bar in Step 9 provides the ability to control each channel individually and to stagger the start of each test.

NOTE: To have the baseline setting visible on the final printout, when a tracing has reached stability, Click on Aggregometer … Reset Channel … and select appropriate Channel #. Click on the "Activate" bar and reset the baseline. Then, click on the "Start" bar and add the appropriate reagent for that Channel.

16. While tests are running, prepare one test cuvette for each channel (Cuvette, stir bar and 500 µL or 250 µL PRP) & begin three (3) minute incubation.
17. 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.
18. 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.

19. 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.
20. 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.
21. 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 (√) 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.
22. 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 percent and slope.

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 ± 1 SD)		
Reagent	**Conc.**	**Agg. (%) [18]**
Collagen	**2 µg/mL**	**70 – 94**
Arachidonic Acid	**0.5 mM**	**74 – 99* [13]**
ADP	**10 µM**	**71 – 88**
Epinephrine	**5 µM**	**78 – 88**
Ristocetin	**1.25mg/mL**	**87 – 102 [13]**

(*± 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 acquired[14] 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**	**Glanzmann's Thrombasthenia**
ADP	**5 – 10 µM**	**N, R ***		**N**	**N, R ***	**A**
Arachidonic Acid	**0.5 mM**	**A**		**N**	**N**	**A**
Collagen	**1 – 5 µg/mL**	**1 or 2 µg/mL**	**5 µg/mL**	**N**	**N**	**A**
		R	**N**			
Epinephrine	**10 - 50 µM**	**R***		**N**	**R ***	**A**
Ristocetin	**0.5 – 1.5 mg/mL**	**Qualitative Defect**		**** A,R,H *****	**N**	**N**

* 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

AGONIST	NORMAL RANGES (Mean ±1 SD)	CONTROL VALUE	PATIENT VALUE	TEST RESULTS (Normal, Reduced, Increased)
ADP, 5 µM	69 - 88%			
ADP, 10 µM	71 - 88%			
COLLAGEN, 2 µg/mL	70 - 94%			
COLLAGEN, 5µg/mL				
ARACHIDONIC ACID, 0.5 mM	74 - 99%*			
EPINEPHRINE, 5 µM	78 - 88%			
RISTOCETIN, 1.25 mg/mL	87 - 102%			
RISTOCETIN, 0.5 mg/mL	0%			

*** ±2SD**

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. A lag phase of up to a minute is typically seen with this agonist.

With Arachidonic Acid: Arachidonic Acid is converted to thromboxane A_2 in the presence of cyclooxygenase. Aspirin inhibits the cyclooxygenase pathway, causing a significant reduction in aggregation with this agonist. Normal aggregation is seen with concentrations of 0.5 mM to 1.0 mM.

With ADP: ADP exposes the fibrinogen binding site on the membrane glycoprotein GPIIb/IIIa complex. 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]

With Epinephrine: Shape change is not seen with this agonist. Higher concentrations (≥ 5 µM) produce a biphasic curve with second-wave aggregation dependent on thromboxane A_2 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 0.75 to 1.5 mg/mL. To detect Type 2B or Platelet-Type vW, test for a Hyper-response at low concentrations (0.2 – 0.6 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 drug free for ten (10) days to 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.
- Platelet counts below 100,000/µL may cause problems with the setting of optical baseline, preventing the recording of aggregation
- This device has not been evaluated for pediatric use.

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 <u>not</u> 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. [See "Additional Post Calibration test for Instruments with PPP Select Switches" on Page 19]***

4. Place the (2) water cuvettes from P/N 322 in the incubation wells and pre-warm for a minimum of five (5) 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. Under the AGGREGOMETER window, select or set-up a Test Procedure page for Optical mode. Click on OK.
7. Select RUN NEW PATIENT under AGGREGOMETER and Click on OK.
8. Type, "Optical Calibration Pre", in Test Identification box at top of screen. Click on OK and test will begin running.
9. After the five (5) 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. Click on Aggregometer, select Reset Channel and select ALL Channels. Click on OK.
2. 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 <u>left center.</u>
3. Click on the Channel 1 "Activate" bar. [NOTE: If this pre-test is to be SAVED, Click on the "Start" Bar for Channel 1. If not saving, the following steps can be performed after clicking on the "Activated" button.]
4. 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.
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 <u>left center</u>. 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 5 above for Channel 2, and then continue to repeat for Channels 3, 4, 5, 6, 7 and 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 <u>left center</u> 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.

Calibration

NOTE: Calibrate <u>ALL</u> 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 Reset Channel and then select ALL Channels. Click on OK.
2. After wiping the outside with a Kim Wipe®, place BLACK cuvette in PRP well of Channel 1 and place PPP water cuvette in PPP well.
3. Click on the ***"Activate"*** bar for Channels 1 through 8.
4. Press the Baseline button for Channel 1 and hold until the tracing reaches a full 100% of graph before releasing. Tracing will then return to 0% of graph.
5. Transfer the BLACK cuvette in PRP well and water cuvette in PPP well to Channel 2 and repeat Step 4.
6. Repeat Step 5 for Channels 3 through 8.
7. Remove Black cuvette from test well.
8. Click on the "Start" Bar for Channels 1 through 4.
9. After wiping the outside with a Kim Wipe® and keeping the Alignment Marks <u>left center</u>, place the PRP water cuvette in Channel 1 PRP well and the "P" water cuvette in Channel 1 PPP well.
10. 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.
11. Set the timer for one minute **<u>but do not start</u>**. Press the Set Baseline button for Channel 1, hold for a second, then release. **<u>Start the timer</u>** 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.

12. After waiting one minute, or when tracing stabilizes in Channel 1 … Repeat steps 9 through 11 for Channel 2, then 3, then 4. Calibration key remains ON throughout this process.
13. Once step 12 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.
14. ***To calibrate Channels 5, 6, 7 and 8 in Module 2, beginning with Channel 5, repeat steps 8 through 13above, using the calibrate key in that module.***
15. If required, SAVE and PRINT the test.

Post Calibration test

[Confirm the Calibration Key has been <u>removed</u> from lock]

1. Under the AGGREGOMETER window, select or set-up a Test Procedure page for Optical mode. Click on OK.
2. Select RUN NEW PATIENT under AGGREGOMETER and Click on OK.
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 <u>left center</u>.
4. Click on the Channel 1 "Activate" bar. [NOTE: If this test is to be SAVED, Click on the "Start" Bar for Channel 1. If not saving, the following steps can be performed after clicking on the "Activate" button.]
5. 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.
6. 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 <u>left center</u>. 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%)
7. Repeat Steps 3 through 6 for Channel 2, then repeat for Channels 3, 4, 5, 6, 7 and 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 <u>left center</u> of each test well and to wipe the outside of the cuvettes with a Kim Wipe® when placing into the test well.
8. 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.
9. 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 > 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 <u>against the Channel 1 PPP Reference</u>.**

[Confirm the Calibration Key has been <u>removed</u> 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.

<u>NOTE</u>: 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. Under the AGGREGOMETER window, select or set-up a Test Procedure page for Optical mode. Click on OK.
3. Select RUN NEW PATIENT under AGGREGOMETER and Click on OK.
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. [NOTE: If this test is to be SAVED, Click on the "Start" Bar for Channel 1. If not saving, the following steps can be performed after clicking on the "Activate" button.]
6. 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.
7. 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%).
8. Repeat Steps 4 through 7 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.
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.

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 75K, 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.

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.

BIBLIOGRAPHY

1. Born, GVR: Quantitative investigation into the aggregation of blood platelets. J. Physiol. London 162, 67, 1962.
2. Genton E, Gent M, Hirsh J, and Harker LA: Platelet- Inhibiting drugs in the prevention of clinical thrombotic disease. New Eng. J. Med. 293: 1174-1178, 1236-1240, 1291-1300 (4, 11 and 18 Dec.) 1975.
3. Grette, K: Studies on the mechanism of Thrombin-catalyzed hemostatic reactions in blood platelets. Acto Physiologica Scandinavia 56, suppl. 195, 1962.
4. Holmsen H: The platelet: its membrane, physiology and biochemistry. In: Clinics in Hematology, W.B. Saunders Co. London, Philadelphia, Toronto, Vol. 1, No. 2 p.235.
5. Kinlough - Rathbone RL: The effects of some other drugs on platelet function. Platelets, Drugs and Thrombosis. Edited by J. Hirsh, J.F. Cade, A. S. Gallus, et al. Basel, S. Karger, 1975, pp 124-131.
6. Marcus AJ, Zucker MS: Physiology of blood platelets, Grune and Stratton, N.Y., NY 1965.
7. Miller JL: Platelet-Type von Willebrand's Disease. Thrombosis and Hemostasis Vol. 7, No. 4, 1985.
8. Mustard JF, Packham MA: Factors influencing platelet function: adhesion, release, and aggregation. Pharmacol Rev. 22:97-187, 1970.
9. Mustard JF, Packham MA: Platelets, drugs and thrombosis. Drugs 9:19-76, 1975.
10. Nieuwenhuis HK, Akkerman J-W N and Sixma JJ: Patients With a Prolonged Bleeding Time and Normal Aggregation Tests May Have Storage Pool Deficiency: Studies on One Hundred Six Patients. Blood 70-3 (620-623) 1987.
11. Packham MA, Mustard JF: Non-steroidal anti-inflammatory drugs, pyrimido-pyrimidine compounds and tricyclic compounds: effects on platelet function. Platelets, Drugs and Thrombosis. Edited by J. Hirsh, J.F. Cade, A.S. Gallus. et al. Basel, S. Karger, 1975, pp 124-131.
12. Sirridge, M: Laboratory Evaluation of the Bleeding Patient. Clinics in Laboratory Medicine, Vol. 4, No. 2, June 1984.
13. UMDNJ, Spec. Hemostasis Lab, New Brunswick, NJ.
14. Weiss HJ: Acquired qualitative platelet disorders, Hematology, Edited by W. J. Williams, E. Bentler, A.J. Erslev, et al. New York, McGraw-Hill Book Company, 1972.
15. Weiss HJ: Platelet physiology and abnormalities of platelet function. New Eng. J. Med. 293:531-541, 580- 588, ([11] and 18 Sept.) 1975.
16. Weiss HJ, Rogers J: Thrombocytopathia due to abnormalities in platelet release reaction - studies on six unrelated patients. Blood 39: 187-196, 1972.
17. Weiss HJ, Hoyer LW, Rickles FR, et al: Quantitative assay of a plasma factor deficient in von-Willebrand's disease that is necessary for platelet aggregates: relationship to factor VIII procoagulant activity and antigen content. J. Clin Invest 52:2708-2716, 1973.
18. White M MC, Jennings LK: Platelet Protocols: Research and Clinical Laboratory Procedures. Academic Press, 1999.
19. CLSI (Clinical Laboratory Standards Institute), Publicaton H58-A, Platelet Function Testing by Aggregometry; Approved Guideline; November 2008

APPENDIX A

1. Ensure the Two Water Cuvettes Match

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

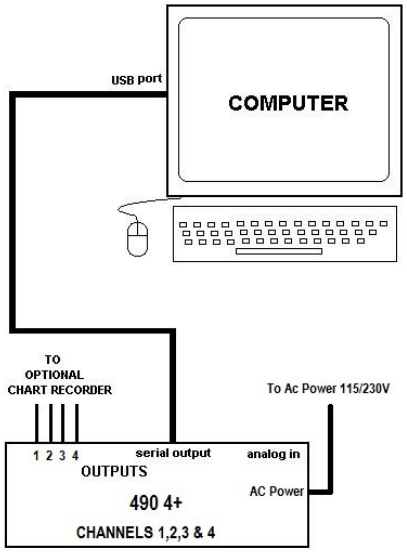
   a. After wiping the outside with a Kim Wipe®, place BLACK cuvette in the PRP well of Channel 1 and a Water cuvette in the PPP well with the alignment mark placed left center.
   b. Click on the Channel 1 "Activate" bar.
   c. Push and Hold the Set Baseline Button until the tracing reaches a full 100% 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