LUMI PLATELET AGGREGATION

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Principle

This protocol is written for running optical method of turbidimetric aggregation using a Chrono-log® Aggregometer with Optical Mode Model 480-VS with AGGRO/LINK® for Windows Software version 5.1 or higher.

"Platelet aggregation" is the term used to describe the formation of platelet clumps or aggregates. In vivo, these aggregates are formed at the site of injury to the blood vessel wall and contribute to the initial step in primary hemostasis.

Aggregation can be measured with a platelet aggregometer either photometrically using platelet rich plasma (PRP) or by measuring change in electrical impedance in whole blood or PRP. The rate and the degree of aggregation are plotted using a recording device. Platelet aggregation in vitro is dependant on calcium ions. Although sodium citrate is the anticoagulant of choice, the concentration of calcium that is left behind is enough to support aggregation. Plasma factors must be present, an essential one being fibrinogen, and the stimulus is brought about using a platelet aggregating agent (agonist). Platelet aggregation studies must be completed within four hours of venipuncture.

During aggregation platelets release their granule contents. Measurement of released dense granule ATP during aggregation with weak (ADP) and strong (collagen and TRAP) agonists is performed in the aggregometer using an ATP dependent luciferin/luciferase reaction. In this reaction the ATP released from dense granules and the enzyme luciferase hydrolyze the substrate luciferin and releases photons which are detected by a separate photodetector in the instrument. Aggregation (ATP release) and photon production are recorded. An ATP standard is used to quantify the amount of released ATP by comparing photon peak heights for each agonist to the standard.

Specimen Collection and Handling/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. Outpatients scheduling an appointment for platelet aggregation studies through the Appointment Center are informed of these requirements. **Patients taking prescription medications are asked to consult with their physician before stopping their medications in preparation for this test.** Refer to Platelet Aggregation Controls binder at platelet aggregation bench for list of drugs that affect platelet aggregation.

Specimen Type:

Five (5) 2.7 mL blue top Hemogard® evacuated tubes per patient. Specimen should be drawn with a minimum of trauma or stasis at the venipuncture site and anticoagulated with 3.2% sodium citrate, in the ratio of one (1) part anticoagulant to nine (9) parts of blood.

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

Handling Conditions:

Outpatient aggregations are drawn by appointment in the Rose Cancer Center on Thursday. The specimens are transported by courier immediately to the Coagulation Lab. The outpatient aggregation specimens are **not** to be sent via the pneumatic tube system! Testing can start 30 minutes after PRP preparation and continue for about 4h after venipuncture. Repeat any suspicious findings and save results in LIS. Do not verify, the pathologist will verify after the interpretation. Specimen should be kept at room temperature (24° to 27°C). Do not put on ice or refrigerate.

Supplies/Equipment:

1. Chrono-log® Aggregometer with Optical Mode, Model 480-VS
2. Chrono-log® AGGRO/LINK® Interface, Windows®-Compatible Computer and AGGRO/LINK® for Windows® software installed.

MATERIALS:

1. Cuvettes, Chronolog®
2. 0.5-10 mcL Adjustable Pipette, Finnpipette digital (0.5-10.0mcL)
3. 10-100 mcL Adjustable Pipette, Finnpipette digital (10-100mcL)
4. 100-1000 mcL Adjustable Pipette, Finnpipette digital (100-1000mcL)
5. ADP (Bio Data®)
6. Collagen, Chronolog®, cat# 385
7. Arachidonic Acid (Bio Data®)
8. Chrono-Lume® with ATP standard, Chronolog®
9. ATP Standard, Chronolog®
10. Thrombin Receptor Activator Peptide 6 (TRAP), Peninsula Lab
11. Disposable stir bars
12. Physiological saline (0.85% w/v), Stores
13. Printer
14. Centrifuge, Megafuge 1.0 at 22^0C
15. 1% Phosphoric acid, Mallinckrodt®, 1, stock concentration 85%

> 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 saline are inappropriate because they contain benzyl alcohol or other preservatives. Such preservatives inhibit platelet function.

16. Water - Reagent grade, distilled, deionized water from US Filter Pure Lab Plus® source.

> Should be pyrogen free (ATP free) for reconstituting reagents. Avoid any sterile water for injection that contains benzyl alcohol because it inhibits platelet function.

17. Falcon Polypropylene 14ml 17 x 100 mm polypropylene tubes.
18. 12 x 75 mm polypropylene tubes, Dynamic Diagnostics.
19. Lint free wipes, such as Kimwipe®.

> Gauze squares are **NOT** suitable!!!

REAGENTS:

1. **ADP (Bio Data®) -** reconstitute with 0.5 mL of distilled water. Ready for use after 10 minutes. Reconstituted ADP is stable for 30 days when stored at 2º – 8ºC in the original, tightly sealed container. Stock concentration is 200 mcM (2 x 10^{-4}M).

2. **Collagen (Chrono-Log®) -** ready to use. Invert or swirl before use, as collagen fibrils are in suspension. Do not freeze. If required, collagen can be further diluted in isotonic glucose pH 2.7. If aseptic techniques are used (sterile syringe and needle to remove one day's use), remaining reagent, if stored at 2–8ºC, is stable until expiration date. Stock concentration is 1 mg/mL.

3 **Arachidonic Acid (Bio Data®)** - reconstitute with 0.5 mL of deionized water, pH 5.3 – 7.2. The reagent may appear cloudy, but will become clear and colorless within a few minutes. Arachidonic Acid must be kept stoppered at all times when not in use. Re-stopper the vial immediately after removing reagent. If reagent is yellow discard. Reconstituted Arachidonic Acid is stable for 24h at 2°– 8°C. For long-term storage, freeze at –20°C for up to 8 weeks.

4 **Chrono-Lume® (Chrono-Log®) -** reconstitute with 1.25 mL of sterile water. Allow to stand for 20 minutes prior to use. Working stock: Stable for 8h at 2–8ºC in the dark. Reconstituted reagent can be stored frozen at -20ºC. Stock concentration is 2 mcM /L.

NOTE: Keep Chrono-Lume® working solution on a cold block when in use.

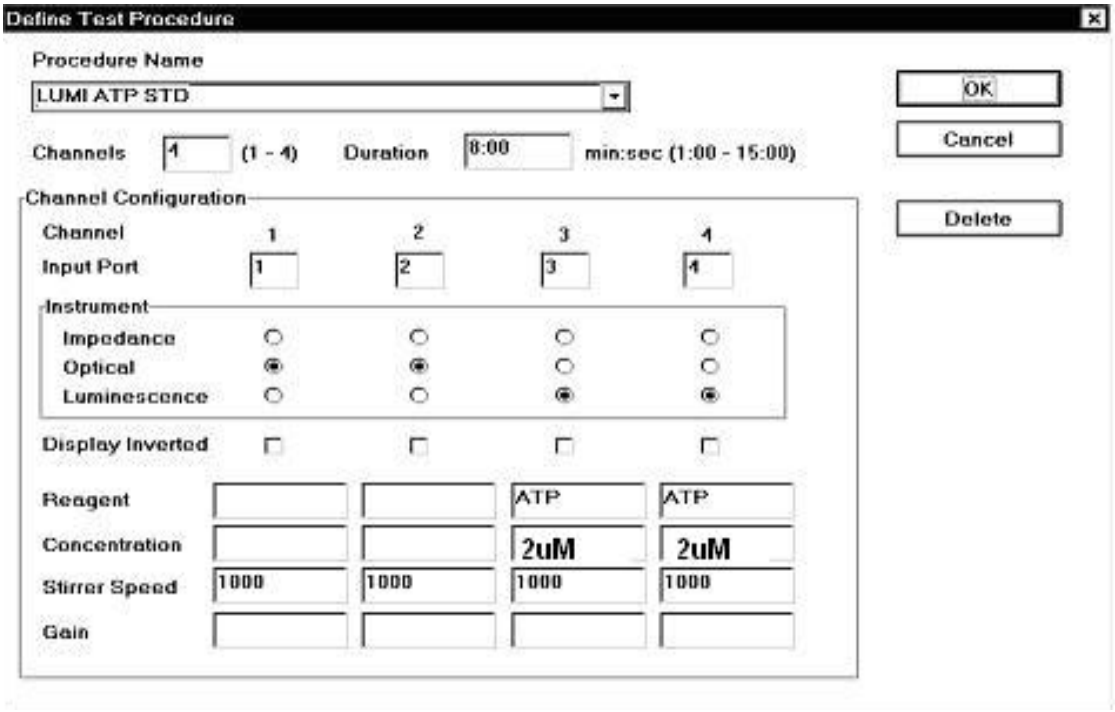
5 **ATP Standard (Chrono-Log®) -** reconstitute with 5.0 mL of 0.85% physiological saline. Reconstituted reagent can be stored frozen at –20ºC for 2 weeks. Stock concentration is 2 mcM.

6 **Thrombin Receptor Activator Peptide 6 (TRAP) -** store desiccated at –70ºC prior to use. Do not freeze/thaw repeatedly. See Attachment A for instructions on how to prepare TRAP. 20mcL aliquots should be prepared and stored in –70ºC freezer. Each aliquot needs to be diluted with saline. The amount of saline may vary with different lots. No manufacturer's expiration date. Per known stability, frequency of use and low risk of deterioration, reagent expiration is 12 months from opening, or until expected performance is not achieved (i.e. cloudiness, color change, inability to recover expected aggregation patterns, etc.). If performance not satisfactory, discard and open new vial of reagent.

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 stir bars in cuvettes.
   c. Put cuvettes containing stir bar in the incubation wells to warm up.
2. **AGGRO/LINK® and Software**
   a. Turn on computer and start AGGRO/LINK® for Windows® program. Click on "WINDOW" and then "STATUS BAR" to obtain AGGRO/LINK status. Be sure "A/L Ready" appears in the bottom right-hand corner of the screen. If not, check com port, cables and connectors.
   b. Click on "EDIT" and "CONFIGURATION".

c. Under the AGGREGOMETER window, select or set-up a test procedure page for Optical-Luminescence mode. (See attached for examples.) Click on "OK", then select "Run Test" under AGGREGOMETER.



3. Sample and Control

a. Platelet Rich Plasma (PRP)

1. Centrifuge sample immediately at 92 x g (700 RPM) for 10 minutes at room temperature (22°C) in Megafuge 1.0R.
2. Remove the upper one-third platelet rich plasma (PRP) with a polypropylene transfer pipette and put into a polypropylene plastic tube.
3. Properly label the tube, include the patient's name and sample type. Parafilm or cap the top. Keep at room temperature (24° to 27°C).
4. Perform a platelet count on the PRP sample.
5. If the platelet count on the PRP sample is less than 100,000/mcL, cancel the specimen as "Unable to perform due to low platelet count".
6. If the platelet count on the PRP sample is 100,000-150,000/mcL, call the pathologist on service to see if the platelet aggregation should be performed. If unable to reach pathologist on service, perform the aggregation and hold the results. Platelet counts lower than 150,000/mcL may present difficulties in setting optical baselines.
7. If the platelet count is greater than 150,000/mcL, the sample can be used.
8. When resulting in the LIS, a comment must be added to indicate the low platelet count.
9. It is necessary to adjust platelet count on PRP to 200,000 (± 20,000) platelets/mcL by using the patient's platelet poor plasma (PPP). After adjustment, perform platelet count. See below for PPP preparation. Use the following formula to adjust PRP:
   a) desired final platelet count X needed final volume of PRP/ beginning PRP count = beginning volume PRP to use
   b) Final volume PRP needed – beginning volume PRP to use = volume of PPP to use

   Example:

   Desired final PRP count = 200,000 platelets/mcL and need a final volume of 4.5mL PRP. This allows for 10 aggregations using 450 mcL each. The PRP platelet count is 495,000 plts/mcL for this example.

   $$\frac{200{,}000 \text{ plts/mcL} \times 4.5 \text{ mL}}{495{,}000 \text{ platelets/mL}} = 1.8\text{mL of PRP to be used}$$

   4.5 mL of PRP – 1.8 mL of PRP = 2.7mL of PPP to be used

   Therefore, add 2.7 mL of PPP to 1.8ML of PRP

b. Prepare the Platelet Poor Plasma (PPP)

1. Centrifuge tubes from which PRP has been removed at 2312 x g (3500 RPM) for 15 minutes at room temperature (22°C) in Megafuge 1.0R centrifuge.
2. Take off the PPP with a polypropylene transfer pipette and put into a polypropylene plastic tube.
3. Properly label the tube, include the patient's name and sample type. Parafilm or cap the top. Keep at room temperature (24 to 27°C).
4. A single sample of PPP per channel can be used as the reference sample for all tests run with the same patient's blood so the amount of PPP required is only 450 mcL.

c. Dispensing

When ready to begin testing, dispense one aliquot of adjusted PRP (450 mcL) per channel into cuvettes with stir bars. Warm for a minimum of 3 minutes. It is not recommended to incubate a sample beyond 30 minutes.

Calibration

Chrono-log® recommends that the Chrono-log® aggregometer must have an annual preventive maintenance and calibration. This includes cleaning out the heater blocks/optical paths, inspection of stirring function and replacement of any defective parts. This service can be performed by the biomedical engineering department, by Chrono-log® as an onsite service call, or at the factory.

Maintenance

MONTHLY:

1. Place a cuvette containing 450 mcL of water in Channel 1 **PRP** well and leave the Channel 1 PPP well empty. Press and hold the Set Baseline Button for a few seconds and check that the tracing moves to 100% Aggregation Baseline. Release the Set Baseline Button and check that the tracing moves to 0% Aggregation Baseline. Allow to run for a few seconds.

2. Place a cuvette containing 450 mcL of water marked PPP in Sample 1 **PPP** well. Check the tracing on the computer screen (or chart recorder). The tracing should move near the 100% baseline. Allow to run for a few seconds.
3. Repeat steps 1 & 2 for each channel.

> **NOTE**: If the tracings are greater than ± 5% from the 0% or 100% baselines, this indicates that Auto-Calibration was not set properly. Repeat the entire calibration procedure using new cuvettes of water and 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 greater ± 5%, the system needs service. Contact the Chrono-log® Service Department at 1-800-247-6665 for further assistance.

4. After completion, print out the graphs, mark the date and mark the water tests for pre-calibration and post calibration. Document on baseline aggregation form. Store the graph for reference and store the key in a safe location.
5. Save and print test for files.

DISCUSSION:

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 the 100% aggregation baseline. This tracing is used to check that the calibration is set properly.

If, after running a second time, the tracing is still out of tolerance, this is an indication of an imbalance in the system's optics. This could be caused by a number of factors such as: dirt or film in the optical path, an obstruction in the optical path or drift in the optical circuit. An instrument cleaning and calibration may be required to correct the problem. It is recommended that you contact the Chrono-log® Service Department at 1-800-247-6665 to inquire about having the instrument serviced.

Quality Control

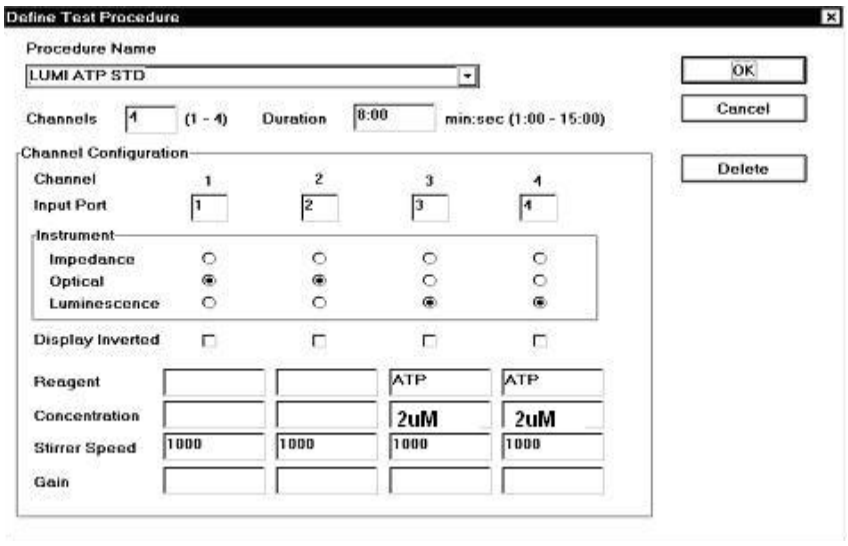
Normal donor must be a non-smoker, not on aspirin, ibuprofen, alcohol or antihistamines for 10 days. Refer any questions to hematopathologist. A control needs to be run with every patient.

PROCEDURE FOR STANDARD:

1. Toggle switches on switch box must be toward the right for Luminescence.
2. Turn on the aggregometer and let it heat up for 10-15 minutes or until the heater blocks stabilize at 37°C.
3. Toggle switch on Channel 2 of aggregometer must be set on 2. This will allow you to run the control in channel 1 and the patient in channel 2.

> **NOTE:** Control is run in Channel 1 and patient is run in Channel 2. Channel 3 and Channel 4 are not used for luminescence.

4. Under Test Procedure name, select "LUMI ATP STD". See below for example. Now select "Run Test" under AGGREGOMETER window, complete patient information and click "OK". Patient information can also be entered while test is running. Click on "EDIT" and "TEST Information".



5. Pipette 450 mcL of control PPP into channel 1 and pipette 450 mcL of patient PPP into channel 2.
6. Pipette 450 mcL control PRP into testing well into channel 1 and 450 mcL of patient PRP into testing well into channel 2. Make sure there is a stir bar in each PRP cuvette. Incubate for 5 minutes.
7. Check GAIN knobs. The black one is for channel 1 and the red one is for channel 2. Turn them both all the way clockwise to the right, then turn each one separately 2 clicks to the left (counterclockwise). The starting point will be at 0.02
8. Set baseline and "STOP TEST".
9. Select RESTART TEST. Add 50 mcL Chrono-lume, wipe pipette tip each time, and just open door enough to insert pipette into cuvette (tip should be submerged). Quickly close door. The graph will show a small spike. This represents the opening/closing of the door. Monitor baseline for contamination. If there is a large spike, this means that your sample is contaminated.
10. Incubate chrono-lume for about 1 minute and 45 seconds (approximately 2 minutes). **NOTE: Chrono-lume® loses one-half of its activity in the first 2 minutes; therefore, don't over-incubate.** Press "RESTART TEST" to clear the screen. This will eliminate any artifacts from the screen.

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

> **NOTE:** Chrono-lume® is time and temperature dependent. To obtain accurate results, be sure to incubate each sample for 2 minutes prior to adding reagent, ATP.

11. At 2 minutes, add 5 mcL ATP (be sure to wipe tip) to cuvette in channel 1. Quickly close the door and place your hand on the black GAIN knob. If the tracing is <40%, then turn knob one click up (counterclockwise), before the reaction deteriorates. The peak must be between 35% and 80% (refer to the scale on the right of the graph).

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

12. If the gain had to be adjusted, repeat the procedure to verify the results.
13. Repeat step 11 for the patient in channel 2. Once both the control and patient signal decreases, you may stop the test, by clicking on "AGGREGOMETER" and "STOP TEST". You only need to see the peaks. The values of the signals are always the same. The GAIN allows it to be seen better on the graph.
14. Click on "EDIT" and "SET START and STOP TIMES". Set start and stop lines for channel 3 and channel 4 so that the peak is between the lines and that the left start line is on stable tracing (just before the "door jiggles". The stop line is at the point it starts to deteriorate.

LUMI PLATELET AGGREGATION

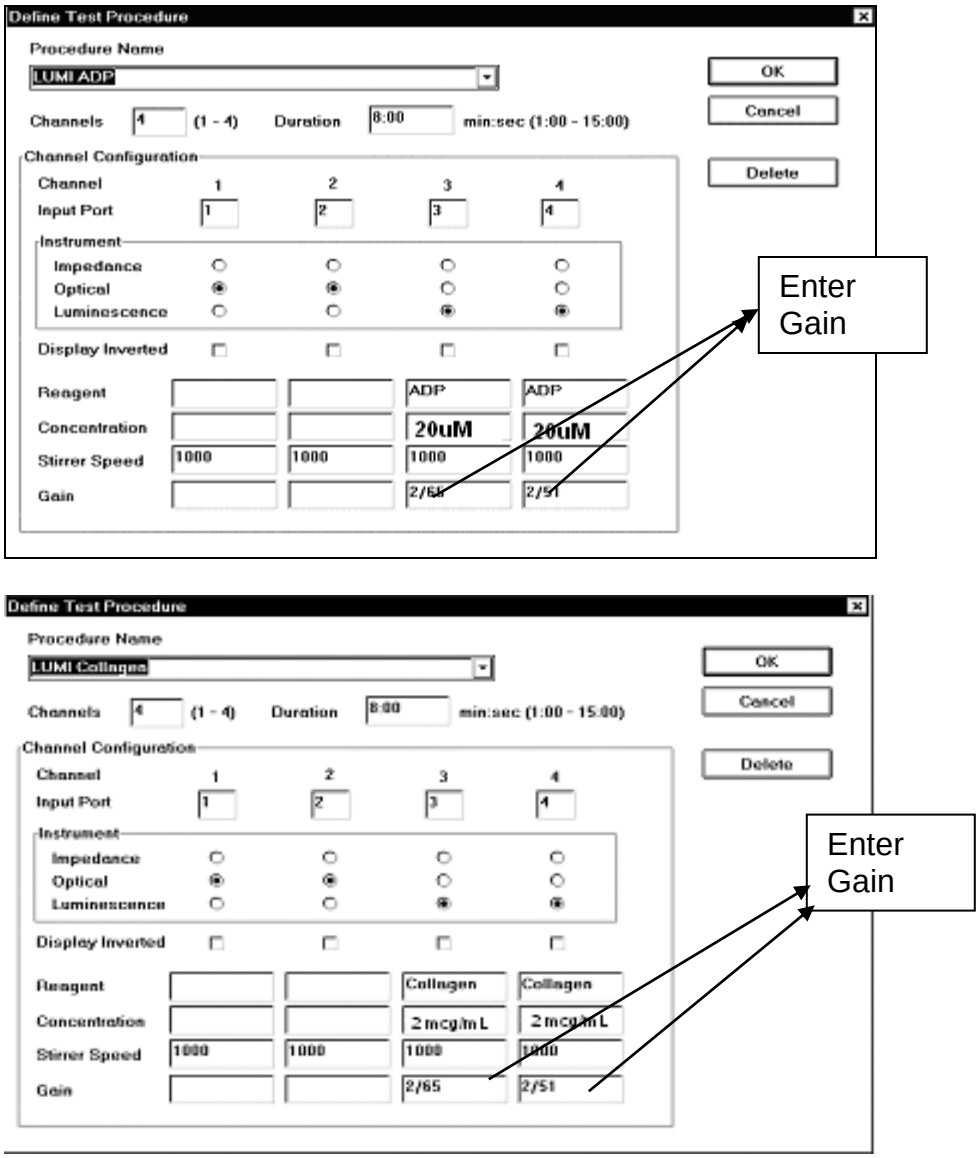
> **NOTE:** On the printout, Channels 1 and 2 are optical (Opt). Channels 1 and 2 are used for determining the aggregation of the agonist. Channels 3 and 4 are luminescence (Lum). Channels 3 and 4 are used to determine the release of each agonist. Therefore, Channels 1 and 3 are linked to the control which is run in channel 1 of the aggregometer. And Channels 2 and 4 are linked to the patient which is run in channel 2. See example of graph at the end of this procedure.

15. Click on "DONE", and then select "COMPUTE SLOPE and AMPLITUDE". Check duration time to be sure Start and Stop Lines were set correctly. Click on "OK" and calculations will appear in Data Box. This command will calculate ATP peak as a percentage.
16. Record ATP (%) calculations for channel 3 and 4. These values will be used in Luminescence Gain Boxes for testing (2/##). The “2” is for nano-molars and the “##” is for the amplitude slope in %. For example, if the peak amplitude slope is 66% for the control. For each agonist used for the control, you must input the Gain as 2/66.
17. SAVE the test, using the SAVE command under FILE. Tests can be printed using the PRINT.
18. Remove samples from well and discard the cuvettes. Discard the stir bars.
19. Keep Luminescence Gain Knobs at final setting for all tests run with that subject’s blood.

PROCEDURE FOR AGONIST:

1. Select TEST PROCEDURE. There are four test procedures to be used: Collagen, ADP, Arachidonic and TRAP. See below for example. **Enter the Gain from the ATP standard readings**. See number 16 from above.

> **WARNING:** Do not forget to enter the Gain for both Control and Patient for each agonist. If this is omitted, you must repeat the agonist to get accurate results.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Procedure Name
LUMI ADP
OK
Cancel
Channels 4 (1 - 4) Duration 8:00 min:sec (1:00 - 15:00)
Delete
Channel Configuration
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Input Port 1 2 3 4
Instrument
Impedance
Optical
Luminescence
Display Inverted
Reagent ADP ADP
Concentration 20uM 20uM
Stirrer Speed 1000 1000 1000 1000
Gain 2/65 2/51
Enter Gain
Define Test Procedure
Procedure Name
LUMI Collagen
OK
Cancel
Channels 4 (1 - 4) Duration 8:00 min:sec (1:00 - 15:00)
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Input Port 1 2 3 4
Instrument
Impedance
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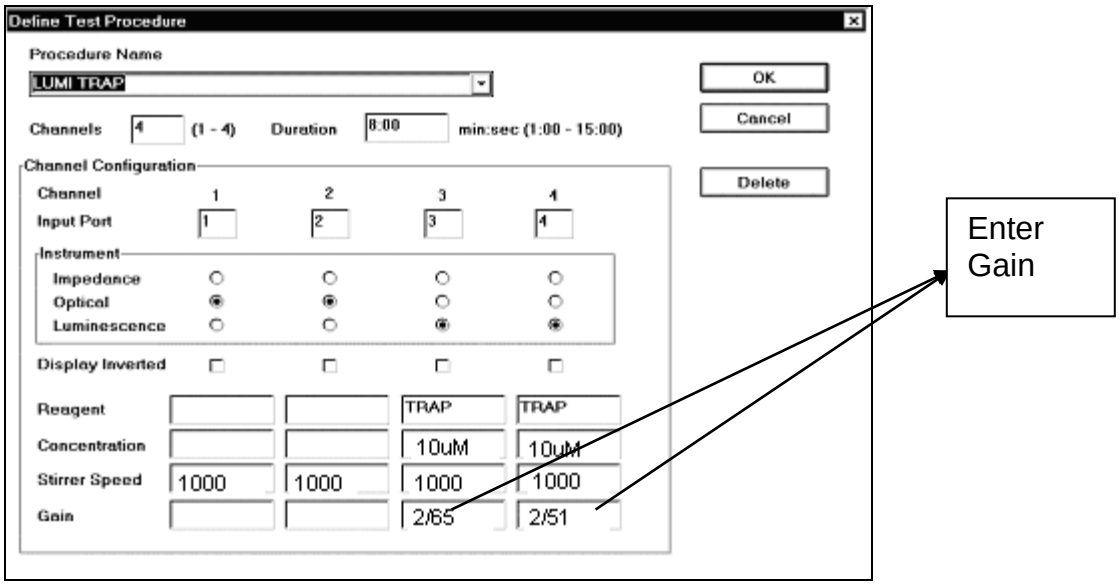
Stirrer Speed 1000 1000 1000 1000
Gain 2/65 2/51
Enter Gain



2. Using warmed cuvettes with 450 mcL PRP, place cuvettes in test wells. Place 450 mcL PPP cuvette in reference well. Check for bubbles and be sure to wipe cuvette with clean Kimwipe.
3. Set baselines (to appear on test for Pathologist). Let run for 30 seconds.
4. Add 50 mc of Chrono-Lume® to cuvette. Wipe tip before adding. Close door. Incubate 2 minutes.
5. Draw up appropriate agonist (see below), wipe tip, and insert into cuvette. Close door.

Collagen:	add 1 mcL
ADP:	add 50 mcL
TRAP:	add 50 mcL
Arachidonic	add 50 mcL

6. Since you are running both Luminescence and aggregation test, STOP TEST 6 minutes after the patient sample test was started.
7. Click on “EDIT” and “SET START and STOP TIMES” for all channels 1-4. For all agonist except ADP, set start and stop lines for Channel 3 and Channel 4 so that the peak is between the lines and that the left (start) line is on stable tracing (just before the “door jiggles”). Set the start line for the ADP agonist luminescence on the stable line after the residual ATP spike. For Channel 1 and Channel 2, set start line just before the agonist is added and stop line at maximum point of aggregation. Click on “DONE”, and then select COMPUTE SLOPE and AMPLITUDE. Check Duration time to be sure Start & Stop Lines were set correctly. Click on OK and calculations will appear in Data Box.
8. SAVE the test, using the SAVE command under FILE. Print the graphs.
9. Remove the samples from the reaction wells. Discard the cuvette and the disposable stir bar.
10. Repeat any suspicious findings for example one abnormality or a curve that is irregular. When finished with lumi-aggregation, switch toggle switches on aggregometer back to 1 on channel 2 and toggle switches on switch box must be towards the left for optical.

Calculations and Interpretations

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® Software uses a 32-point sliding curve to calculate slope.

REPORTING RESULTS:

1. Report platelet aggregation percent and slope for both patient and donor control.
2. Report PFA for patient.
3. Save results in the LIS. Do not verify. The pathologist will verify the test after the interpretation.
4. Attach collection label to the aggregation printout.
5. Under patient information in the test menu enter PRP count for both prior to adjustment and after adjustment.
6. Attach script/requisition and control results.
7. Results require Pathologist interpretation.

REFERENCE RANGES:

Refer to Coagulation tests: Reportable limits and Normal Therapeutic Values procedure.

Interpretation

1. Optical Aggregation

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

With Collagen: Collagen is useful for checking the platelet's general ability to aggregate. The aspirin effect is reduced aggregation at a 2.2 mcg/mL concentration. A lag phase of up to a minute is typically seen with this agonist.

With ADP: ADP exposes the fibrinogen binding site on the membrane GPIIb/IIIa complex. Aggregation testing is typically performed in PRP with concentrations ranging from 1 mcM to 10 mcM. At the lower concentrations up to 3 mcM, 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 bi-phasic wave. It is often necessary to perform dose-response testing with multiple concentrations to obtain a bi-phasic response. Aspirin effect may be seen with mid-range concentrations such as 5 mcM.

With Arachidonic: Arachidonic is use in routine aggregation studies for the differential diagnosis of aspirin-like release defects and Storage Pool Disease and also to evaluate the inhibitory effect of aspirin on platelet aggregation.

2. ATP Release with Luminescence

Thrombin – is useful for determining the **maximum** amount of ATP secretable from the dense granules. Secretion is independent of thromboxane synthesis. If ATP Release is <0.5 nM, run a second test with a higher amount of Thrombin (up to 5 Units). If ATP Release increases, consider a Secretion Defect. If ATP Release does not increase, consider Storage Pool Disease.[2]

Collagen – is useful for checking the general aggregability of the platelets. Aggregation and secretion are partially dependent on thromboxane synthesis. Secretion to collagen of less than half the secretion to thrombin may be indicative of impaired thromboxane synthesis. Aspirin, and similar drugs, inhibit the production of thromboxane A_2 which, in turn, inhibits aggregation and ATP secretion.

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

PLATELET ABNORMALITIES:

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

AGGREGATION RESPONSE WITH SELECTED ABNORMALITIES						
Reagent	**Final Concentration**	**Aspirin Effect**		**Von Willebrand & Bernard Soulier**	**Storage Pool/ Secretion Defect**	**Glanzmann's Thrombasthenia**
ADP	5 - 20 mcM	**N,R***		**N**	**N,R***	**A**
Collagen	2 – 5 mcg/mL	2 mcg/mL	5 mcg/mL			
		R	**N**	**N**	**N**	**A**
Ristocetin	0.25 – 1.0 mg/mL	Qualitative[8] Defect		** **A,R,H** ***	**N**	**N**
Arachidonic Acid	500 mcg/mL	**A**		**N**	**N**	**A**

* Second-wave Inhibited

** Type 2B and Platelet-type von Willebrand increased at low concentration 0.2 -0.6 mg/mL [9, 10]

*** To distinguish between von Willebrand & Bernard Soulier, add normal plasma or cyoprecipitate to patient sample, vW patient will respond, Bernard Soulier will not.[10]

ATP SECRETION WITH SELECTED ABNORMALITIES					
Reagent	**Final Concentration**	**Aspirin Effect**	**Von Willebrand & Bernard Soulier**	**Storage Pool/ Secretion Defect***	**Glanzmann's Thrombasthenia**
ADP	5 - 20 mcM	**A, R**	**N**	**A,R**	**A**
Collagen	2 – 5 mcg/mL	**R**	**N**	**A,R**	**R**
Ristocetin	0.25 – 1.0 mg/mL	**--**	**--**	**--**	**--**
Thrombin	1 Unit	**N**	**N**	**A,R**	**R**
Arachidonic Acid	50 mcg/mL	**A**	**N**	**A,R**	**R**

* Higher concentrations of any agonist including Thrombin up to 5 Units will induce ATP secretion with a Secretion disorder but **will not** with a Storage Pool Defect.[2]

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

Limitations

1. Tests should be performed within 4 hours of venipuncture.
2. Many drugs inhibit platelet function. 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.
3. Further clinical and laboratory evaluation may be required to confirm diagnosis.
4. 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.
5. Hemolyzed samples are not acceptable. Hemolysis releases erythrocetin, which is a platelet activator.
6. Clotted or quantity insufficient samples are not acceptable.
7. Lipids in PRP can interfere with light transmission readings & prevent recording of aggregation.
8. Platelet counts below 150,000/mcL may cause problems with the setting of optical baseline, preventing the recording of aggregation.
9. Only polypropylene transfer pipettes and polypropylene tubes can be used when working with platelets to avoid activation prior to testing. DO NOT use polystyrene (clear plastic).
10. Numerous drugs can affect platelet function. Refer to Platelet Aggregation Controls binder at platelet aggregation bench for list of drugs that affect platelet aggregation.

References

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Authorized Reviewers

Chair, Pathology and Laboratory Medicine
Medical Director, Coagulation

Attachments

Attachment A - HOW TO PREPARE TRAP

TRAP (Thrombin Receptor Agonist Peptide)

Molecular Weight: 747.42

Make 6.69 mM stock solution:

Dissolve 0.5 mg (.0005 g) with 100 mcL distilled water.

This will give you approximately 6.69 mM TRAP (for stock solution). Depending on the % peptide content for each lot, you will have to multiply 6.69 mM by % peptide. Then you will have to calculate ratio from 6.69 mM (or slightly less) to 0.1 mM (or 100 mcM) for working solution.

Example:

Lot# 036015; 78.8% peptide: 0.5 mg powder dissolved 0.5 mg with 100 mcL distilled water; this will give approximately 6.69 mM solution TRAP (stock solution). Aliquot into 20 mcL aliquots and freeze at -70^0C.

Take 6.69 mM (approximate) x .788 (78.8% peptide-actual) = 5.27 mM.

5.27 mM is actual concentration for this batch

5.27 mM diluted to 0.1 mM (100 mcM=working stock) = 1:52.7 ratio

Take 10 mcL of 5.27 mM and dilute with 517 mcL normal saline
(10 mcL + 517 mcL)

You now have 527 mcL of a [0.1 mM (100 mcM)] TRAP working stock.

LUMI PLATELET AGGREGATION

Document Control

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Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Maria Luhring, MT(ASCP)	04/24/2003			
Approved by: Joan C. Mattson, MD	05/07/2003			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Joan C. Mattson, MD	05/07/2003		New protocol	
Joan C. Mattson, MD	01/20/2004		New principle, new handling conditions. Outpatient aggregations are sent by courier not by the pneumatic system.	
Noelle Procopio, MT (ASCP), SH	01/04/2005		No change.	
Joan C. Mattson, MD	11/18/2005	r00	Standardized procedure format; updated reference range table, pg. 10; eliminated section on aspirin containing drugs.	
Joan C. Mattson, MD	12/22/2006	r01	Added working chronolume stability, pg. 3.	
Marc Smith, MD	01/11/2007	r02	Pg. 2, 12. changed from teflon reusable to disposable stir bars. Pg. 3, 18 added falcon polypropylene 14ml, cardinal health. Pg. 3 changed Appendix to attachment. Pg 5 a) changed PRP calculation. Pg. 11 deluxe sheet. Pg. 7 lumi trap reference range correct from 1.40 to 0.40. Pg. 15 changed Appendix to Attachment.	

Marc Smith, MD	03/12/2008	r03	Pg. 6, 10. Added eliminate artifacts from screen. Pg. 12, Changed final agonist ADP Max and Lumi ADP to 20 uM. Pg 12, Updated Lumi ADP normal range.	
Marc Smith, MD	02/11/2009	r04	Pg. 12, added start line for ADP agonist and discard disposable stir bar. Pg. 13, referred reference ranges to Coag test reportable ranges procedure.	
Marc Smith, MD	05/05/2010	r05	Deleted materials catalog numbers. Added criteria for accepting or canceling a specimen with a low platelet count.	
Marc Smith, MD	08/12/2011	r06	RC.HM added to SOP#; new format. Under handling deleted Tuesday and changed vein puncture to PRP preparation. Added repeat any suspicious findings. Added save results in LIS. Do not verify. The pathologist will verify after the interpretation.	
Marc Smith, MD	09/03/2013	r07	Pg 7 # 7 changed 3 clicks to 2 clicks;starting point 0.05 to 0.02	NA
Marc Smith, MD	03/03/2015	r08	Deleted the use of Arachidonic agonist.	NA
Marc Smith, MD	09/09/2015	r09	Added TRAP stability information.	NA
Marc Smith, MD	08/04/2016	r10	Added Arachidonic Acid agonist	NA
Elizabeth Sykes, MD	02/22/2018			
Peter Millward, MD	3/13/2019			
Marc Smith, MD	11/18/2019	r11	Changed logo. Remove calibration every six months to be a part of preventative maintenance.	N/A