Whole Blood Platelet Aggregation

1. Prepare Chrono-Lume (1.25ml) and any needed agonists. Turn on Agg, 25 min.
2. Add a large stir bar to Whole Blood cuvettes (1mL plastic)
   8 patient, 8 control
3. Perform a CBC on the patient and control samples to obtain PLT count
4. Obtain 2 square bottom flasks from Set up. Fill one with dH2O and one with saline, for cleaning probes between tests.
5. Add Blood to **6** cuvettes in proper dilution:
   To avoid creating bubbles in pipette, do not "blow out" tip when adding blood.
   PLT >100: 450µl Blood + 450µl Saline
   PLT = 50-100: 900µl Blood
   PLT<50: Below instrument range. See Dr Refaai on how to proceed.
6. For last 2 cuvettes (RIPA) use 500µl Blood: 500 saline, final vol of 1ml. Be sure to keep these cuvettes separate to avoid dilution errors.
7. Place cuvettes in warming slots for 10 min, no blank needed, set aggregometer to Impedance, press select until Gain appears, enter patient info in software.
8. Fill a cuvette with saline and place in a warming slot for each channel. Place the probes in these cuvettes. Cooling probes affects baseline.

Things to remember:

- Use 100µl Chrono-Lume
- Use WB test information
- You **DO** need to close the channel covers
- You **WILL** need to increase Gain
- You do not need to use the probes for ATP standard and Thrombin
- Make sure probes are all the way down, and plugged in.
- Probes are numbered for which well they are calibrated to
- Use a saline filled cuvette in a warming slot to keep probes warm between runs
- When setting baseline, aggregation trace only goes down to 50
- When cleaning probes between tests: swish in water first. The plt clump will usually fall off. Gently wipe dry with a Kimwipe. Be sure to not bend the wires. Verify the plt clump is removed. Rinse in saline.

Agonists WB

	Agonist	Amount µl	Notes	Storage location
No probe	ATP standard	5	Adjust Gain so peak is 80-40	SF251 -75
	Thrombin	100	If no release, use high dose, see 6-pThrombin in SOP	SF029 Coag freezer
Probe	Arachidonic Acid	10	Set baseline before measuring aggregation for next 5 aggonists	SF251 -75
	ADP	5	Slow to release Need to adjust start time due to small amount of ATP in reagent	
	Collagen	2	**If you need to rerun any aggs, do it now before Ristocetin**	SR267 SH fridge
	Ristocetin High	8	No Chrono-Lume for Ristocetin Aggregation is normal	SF029 Coag freezer
	Ristocetin Low	2	No aggregation is normal See RIPA .jad if Aggregation occurs	

- Report Print
- Before storing probes, clean with bleach
  Into 1 cuvette add: stir bar, 1500µl dH_2O, 100µl 8.25% bleach.
  With probe unplugged, place in cuvette then place cuvette in well (to stir the bleach) for 30 seconds. Rinse probe good with dH_2O, wipe with Kimwipe. Clean probe 2 with same bleach/ cuvette. Allow to air dry.
- Well inserts are numbered for when you return them
- Return Aggregometer setting to OPTICAL, and GAIN to 0.005