

Platelet Aggregometry to Quantify Platelet Function in LVAD Plasma

1. Platelet Aggregometer (Chrono-log light transmittance aggregometer)
 - a. Turn on 30 minutes prior → warm up
 - b. Turn on computer
 - c. Turn on box to computer
2. Centrifuge
 - a. Switch on back
 - b. Use plastic (NO GLASS) rounded bottom tubes
 - i. Transfer pipettes
 - ii. 2-3 ml whole blood/tube
 - c. **Spin 210g x 12 minutes** (g not rpm)
3. Platelet Aggregometer
 - a. Silicone coated glass tubes
 - b. Add stir bar
 - c. Place in machine to warm
 - d. Switch: can use 1 control for all, or separate control for each
4. Make PRP
 - a. Centrifuge
 - b. 15ml polypropylene tubes x 2
 - c. Pull off PRP
 - i. Should be 50/50 rbc/plasma, minimal hemolysis
 - d. Get ~ 5ml PRP
 - e. If too concentrated dilute with spin at 400g x 5 minutes
5. Count Platelets
 - a. 200ul PRP + 2ml PBS
 - i. Dilution factor of 10
 - b. Use hemocytometer
 - i. Cover slip
 - ii. Let sit for 5 minutes to let platelets settle
 - c. Use microscope
 - d. Count 5 small squares out of 25 at 20x
 - i. Average 5 squares x dilution factor of 10 x 25 squares x 10,000
 - e. Target concentration of $2-4 \times 10^9$ platelets/ml
6. Make PPP
 - a. Centrifuge
 - b. Use left over rbc and plasma
 - c. **Spin at 1000g for 15 minutes**
 - d. Place 250ul of PPP in aggregometer as control (without stir bar)
7. Aggregation Studies
 - a. Aggrolink (Computer Program)
 - b. Test procedure under Aggregometer
 - c. “Default settings”
 - i. 700rpm in program and on machine dial
 - ii. “4” channels – “optical” density

- d. Run "New Patient"
 - i. Name, date
 - e. PRP
 - i. 250ul
 - ii. Stir bar
 - iii. **Add Ca++**
 - 1. **Use 5ul=2mM**
 - 2. Stock 1M (1.47g/10ml Distilled Water)
 - f. Calibrate
 - i. Push/hold button on aggrenometer
 - ii. Goes to zero
 - iii. Push "Run Next"
 - g. **ADP** (10min)
 - i. **Use 12.5ul = 10uM**
 - ii. Stock 200um (1ml ADP + 1ml distilled water)
 - h. **Collagen** (20min)
 - i. **Use 25ul=10ug**
 - ii. 5ul=2ug
 - iii. 15ul=6ug
 - iv. Stock 100ug/ml
 - i. **Ristocetin** (10min)
 - i. **Use 25ul=4mg/ml**
 - ii. Stock 40mg/ml (10mg ristocetin/250ul distilled H₂O)
 - j. **Epinephrine** (10min)
 - i. 4.1ul=50uM
 - ii. 8.2ul=100uM
 - iii. 12.3ul=150uM
8. Washed Platelets
- a. **Spin 2ml PRP at 400g for 5 min => Pellet**
 - b. Pull off plasma
 - c. Resuspend in 2ml PBS (pH 7.4)
 - d. **Spin 2ml PRP at 400g for 5 min => Pellet**
 - e. Pull off PBS
 - f. Resuspend in 2ml PBS
9. Fix Platelets
- a. **Spin left over PRP at 400g for 5 min => Pellet**
 - b. Pull off plasma
 - c. Add 10% buffered formalin
 - d. Resuspend platelets
10. Analysis
- a. Collagen – 20 min
 - b. ADP – 10 min
 - c. Epinephrine – 6 min
 - d. Ristocetin – 10 min