Platelet Aggregometry

References

• Rao AK. Chapter 54
• Kottke-Marchant K, Corcoran G. The Laboratory Diagnosis of Platelet Disorders. Arch Pathol Lab Med 2002;126:133-46.
  • Coller BS. *Forward*; Refaai MA. Chapter 19; Steinhubl SR. Chapter 21
• Accumetrics www.accumetrics.com
• Bio/Data Corporation www.biodatacorp.com
• Chrono-Log www.chronolog.com
• Helena Laboratories www.helena.com
Aggregation

First Descriptions
Born, GVR. Nature 1962:194:927-9

Principle
Platelet function is determined in either whole blood or platelet rich plasma (PRP) and is proportional to changes in either turbidity (PRP) or impedance (whole blood). The lesser the turbidity or the greater the impedance, the better are platelets in responding to various stimulating agents.

If an initial evaluation of platelet function reveals a potential defect, further investigation using aggregometry will help unmask the abnormality. Platelet aggregation testing assess platelet adherence (Glycoprotein Ibα [GPIbα]), secretion (secondary wave or increase in luminescence), and aggregation (α_{IIb}/β_3). Generally six agonists are used to activate platelets via various receptors and associated signaling pathways: arachidonic acid (thromboxane pathway via cyclooxygenase), collagen (integrin α_2/β_1 & GPVI receptors), ADP (receptors P2Y_1 & P2Y_12), epinephrine (α_2-adrenergic receptor), ristocetin (GPIbα), and thrombin receptor activating peptide (Protease Activated Receptor [PAR] 1 and 4).
Whole Blood

**Turbidimetric**
- Chrono-Log Aggregometers
  - Electrical resistance is measured across two metal wires (probe)
  - Whole blood sample initially exposed to small electric current that coats wires with monolayer of platelets (stable impedance)
  - Upon addition of agonist, platelets form aggregates on the monolayer adding electrical resistance (ohms) to the circuit
  - Change in impedance is measured as a function of time

**Impedance**
- Accumetrics Ultegra® RPFA
  - Patient sample platelets are activated, in vitro, with iso-TRAP (modified thrombin receptor activating peptide) causing agglutination of polystyrene beads coated with fibrinogen in direct proportion to number of available GPIIb/IIIa receptors
  - Used for monitoring therapy with aspirin or GPIIb/IIIa antagonists

**Luminescence**
- Helena Plateletworks™
  - Measures change in platelet count due to aggregation of functional platelets
  - Determine baseline platelet count
  - Add whole blood to separate agonists tubes containing ADP and collagen
  - Perform platelet counts to obtain “unaggregated” counts (non-functional platelets fail to aggregate)
  - Calculate % aggregation between baseline and each agonist tube (the greater the percentage, the lower is platelet count in the agonist tube)

- Chrono-Log Lumi-Aggregometers
  - ATP secreted from dense granules binds with firefly luciferin-luciferase producing light that can be measured
  - Luminescence due to released ATP is compared to luminescence induced by 2 nMoles of ATP standard
  - Luminescence is proportional to platelet ATP secretion thereby permitting agonist (except ristocetin) induced aggregation and dense granule secretion to be monitored simultaneously

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Platelet Rich Plasma

Optical

• Turbidimetric aggregometry measures light transmission through platelet rich plasma (PRP) that initially is turbid but allows for increasing transmittance of light as larger and larger aggregates form subsequent to stimulation with an agonist.
• Primary waves of aggregation are noted with arachidonic acid, collagen, and ristocetin.
• Biphasic aggregation tracings are seen with ADP and epinephrine resulting from platelet granule secretion and further recruitment of additional platelets.
• Instrumentation is available from Bio/Data Corporation, Helena Laboratories, and Chrono-Log Corporation (instruments can also measure luminescence using PRP).

Chrono-Log Lumi-Aggregometers
• ATP secreted from dense granules binds with firefly luciferin-luciferase producing light that can be measured.
• Luminescence due to released ATP is compared to luminescence induced by 2 nMoles of ATP standard.
• Luminescence is proportional to platelet ATP secretion thereby permitting agonist (except ristocetin) induced aggregation and dense granule secretion to be monitored simultaneously.

Luminescence