Heparin Induced Thrombocytopenia (HIT) Diagnostic Testing

Types of Assays

- Serologic Assays: detect circulating antibodies
  - ***Enzyme-linked immunosorbent assay (ELISA)***
  - Immunofiltration assays (IFA)

- Functional Assays: measure platelet activation
  - ***Serotonin release assay (SRA):***
  - Heparin-induced platelet activation assay (HIPA)
  - Platelet aggregation test (PAT)

Enzyme-linked Immunosorbent Assay (ELISA or HIT Ab)

- Detects IgG, IgM, and IgA antibodies against the platelet factor 4 (PF4)/heparin complex
- Reported as optical density (OD) which is an indication of antibody strength
  - Antibody present in the patient sample binds to the heparin-PF4 coated wells leading to a color-producing reaction. A higher antibody concentration leads to more color production and a higher OD reading. The positive cut-off is 0.4 OD **(our lab uses 0.3 OD as lower cutoff)**, and high titer antibodies may yield values up to 4 OD.
  - Magnitude of OD Result (41 patient study**)**
    - OD <0.30 -0.40 — SRA positive in 0.0 to 0.1 percent
    - OD 0.40 to <1.00 — SRA positive in 1 to 5 percent
    - OD 1.00 to <1.40 — SRA positive in 18 to 30 percent
    - OD 1.40 to <2.00 — SRA positive in 19 to 46 percent
    - OD >2.00 — SRA positive in 89 to 100 percent
- Sensitivity: > 95%
- Specificity: 50-89%

Seratonin Release Assay (SRA) – Gold Standard

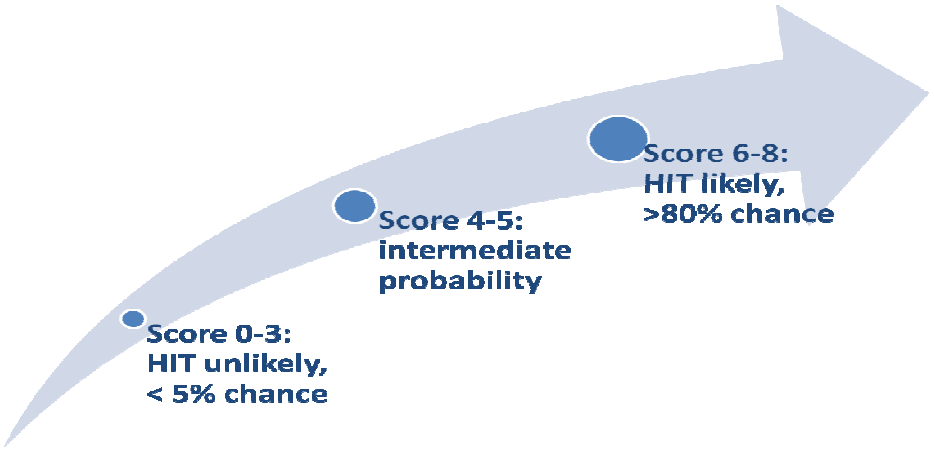
- Measures heparin-dependent platelet activation
- Normal donor platelets are radiolabeled with ^{14}C serotonin and incubated with patient's serum and either low or high concentration of heparin
  - If HIT Antibody present in the patient serum, it will bind and activate donor platelets, releasing radiolabeled serotonin from the platelet granules
  - Low dose heparin (0.1 U/ml): A positive SRA is expected to show >20% release of the ^{14}C serotonin when mixed with patient serum and low dose heparin
  - High dose heparin (100 U/ml): When mixed with high dose heparin, it must reduce the % release obtained with low dose heparin by at least 50% in order to demonstrate that the platelet activation is heparin dependent.
- Sensitivity: 88-100% (Note: combined sensitivity of ELISA + SRA = 99%)
- Specificity: 89-100%

4T Score

- Estimates the *probability* of HIT based on clinical findings

Category	2 points	1 point	0 points
Thrombocytopenia	> 50% fall in PLTS or nadir of 20–100 x 109	30–50% fall or nadir of 10–19 x 109 cells/L	< 30% fall or nadir of < 10 x 109 cells/L
Timing of platelet count fall	Days 5–10 or ≤ 1 day if heparin exposure within past 30 days	Beyond day 10 or unclear or ≤1 day if heparin exposure 30-100 days ago	≤ 4 days without heparin use in the past 100 days
Thrombosis or other seqeulae	Proven thrombosis, skin necrosis, or acute systemic rxn after heparin bolus	Progressive, recurrent, or suspected thrombosis; erythematous skin lesions	None
Other causes of Thrombocytopenia	None apparent	Possible	Definite

- Total Score Interpretation



References

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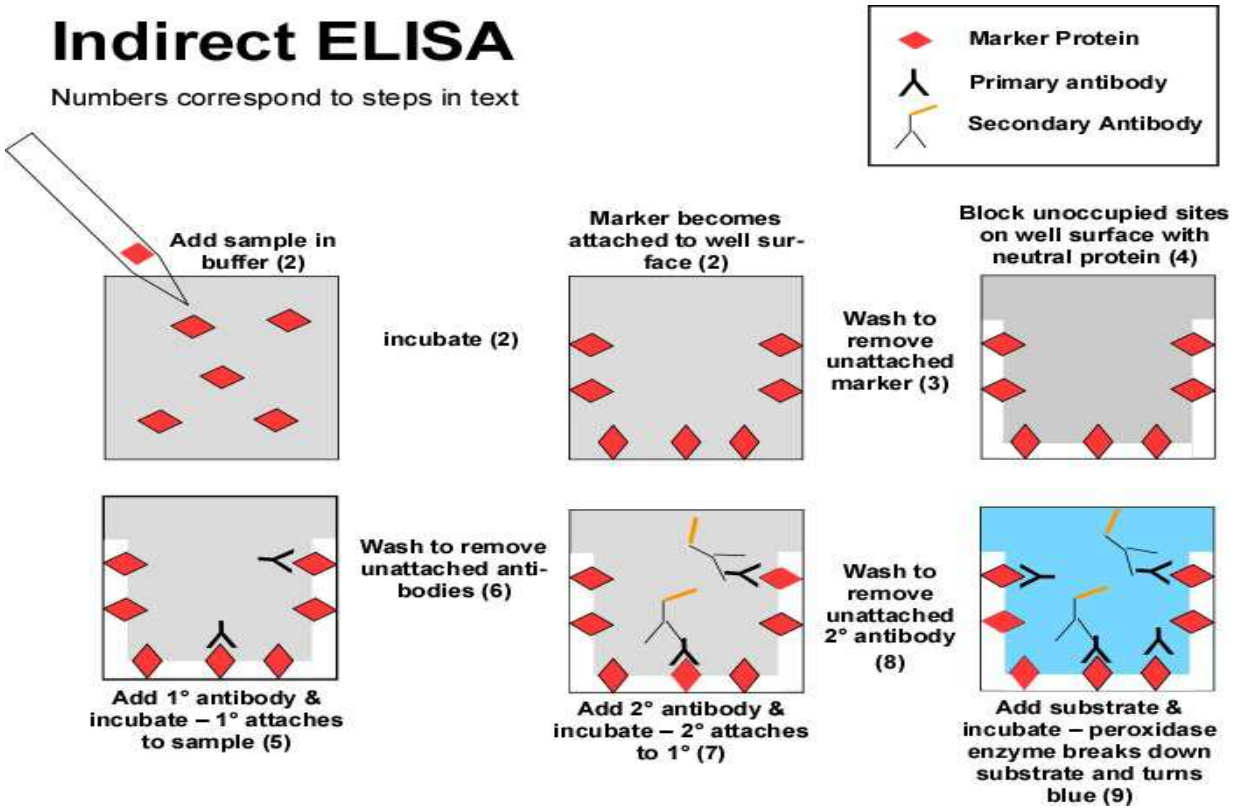

*Marker = heparin/PF4
*Primary antibody = patient's serum sample (if has heparin/PF4 Ab, will attach to marker)
*Secondary antibody = enzyme linked