IMPORTANT OF LABORATORY DOCUMENTATION OF HIT ANTIBODIES

Immune-medicated Heparin-Induced Thrombocytopenia (HIT) is a clinicopathologic syndrome; diagnosis should be based on two criteria: 21

- Occurrence of clinical symptoms, characteristically thrombocytopenia, with or without thrombosis, in the setting of current or recent heparin therapy
- Detection of antibodies to complexes of Heparin/Platelet Factor 4 (PF4)

HIT may occur within minutes or hours of heparin re-challenge, if patient had a prior heparin exposure within the previous 100 days and Heparin/PF4 antibodies are circulating 22. Correlation of clinical diagnosis with laboratory tests may be helpful in the management of HIT 22.

References

4. PF4 Enhanced Package Insert. GTI, Inc.
5. Asserachrom® HPIA Package Insert. Diagnostica Stago, Inc.
6. HealthTest™/Heparin PF4 Antibody Assay. Health-Chem Diagnostic, LLC

LABORATORY METHODS FOR

HEPARIN INDUCED THROMBOCYTOPENIA

For Training Purposes Only
LABORATORY METHODS FOR HEPARIN INDUCED THROMBOCYTOPENIA

CLASSIFICATION OF DIAGNOSTIC ASSAYS

ACTIVATION/FUNCTIONAL ASSAYS

**Principle:**
Measurement of the platelet-activating potential of Heparin/Platelet Factor 4-antibody complexes

**Testing Methods:**
- Serotonin-Release Assay (SRA)
- Platelet Aggregation Test (PAT)
- Heparin-Induced Platelet Activation Assay (HIPA)
- Flow Cytometry*

*Flow Cytometry, a whole blood assay, is often restricted to use in the research setting. As a result, assay information will not be presented in detail.

ANTIGEN ASSAYS/IMMUNOASSAYS

**Principle:**
Detection of antibodies of the three major immunoglobulin classes (IgG, IgM, IgA) against PF4 bound to heparin or a heparin substitute

**FDA-cleared tests:**
- Enzyme Linked Immunoassay (ELISA)
  - PF4 Enhanced® (GTI, Inc., Brookfield, WI)
  - Asserachrom® HPIA
    (Diagnostica Stago, Inc., Parsippany, NJ)

**Particle ImmunoFiltration Assay (PIFA®)**

- HealthTest™/Heparin PF4 Antibody Assay
  (Health-Chem Diagnostics, LLC, Pompano Beach, Fla.)

**NOTE:**
If there is a strong suspicion of HIT, stopping heparin and initiating treatment with an alternative anticoagulant should not be delayed while awaiting the results of laboratory tests.

The combined use of antigen and functional assays may give the best chance of identifying a positive HIT patient. Caution is advised for all assays; none is optimal or 100% accurate.

ACTIVATION/FUNCTIONAL ASSAYS

**Advantages:**
- Higher probability of identifying cases clinically diagnosed as HIP positive
- Assays that incorporate washed donor platelets result in enhanced sensitivity and specificity
- Well-documented clinical history
- If testing performed when HIT clinically suspected, washed platelet activation assays have a high sensitivity for diagnosing HIT

LABORATORY METHODS FOR HEPARIN INDUCED THROMBOCYTOPENIA

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**Disadvantages:**

- Often technically demanding and time consuming\(^2\)
- Lower sensitivity for the detection of Heparin/PF4 complex antibodies\(^9,10\)
- Variability of donor platelets major factor influencing test sensitivity\(^2\)
- Large interassay variation\(^2\)

**SEROTONIN-RELEASE ASSAY**

**Advantages**

- Specificity and sensitivity reported as high as 90% and >80% respectively\(^12,13\)
- Use of washed platelets improves specificity for HIT antibodies\(^1\)
- Documented as reference procedure among activation assays\(^14\)

**Disadvantages**

- Radioactive material needed; requires special lab license\(^1\)
- Labor-intensive; requires well-trained, experienced technicians for high-quality, reproducible results\(^8\)
- Frequently performed in reference laboratories; increases turnaround time and cost per assay

**HEPARIN INDUCED PLATELET ACTIVATION ASSAY (HIPA)**

**Advantages**

- Good correlation with SRA test if both methods incorporate washed platelet \(^15\)
- No radioactive materials needed
- Published clinical history

**Disadvantages**

- Requires careful validation with known positive and negative controls to control for differences in donor platelet responsiveness\(^16\)

**Platelet Aggregation Test (PAT)**

**Advantages**

- Easier handling than other activation assays\(^2\)
- Specificity for HIT >90%\(^8\)

**Disadvantages**

- Sensitivity ranges from 40-60% but can be optimized to 80% in the hands of an experienced technician\(^13\)
- Only a limited number of test conditions and patient/control samples can be tested at one time\(^3\)

**Antigen Assays/Immunooassays**

**Advantages**

- Commercially available assay kits with standardized reagents
- Less technically challenging than functional assays and more suitable for hospital laboratories\(^17\)
- Screening tests with high sensitivity for the detection of 3 classes Heparin/PF4 complex antibodies (1gG, 1gA, 1gM)\(^1\)
- If testing performed when HIT clinically suspected, antigen assays have a high sensitivity for diagnosing HIT\(^11\)

**Disadvantages**

- Diagnostic specificity for clinical HIT dependent on patient population\(^18\)
- More likely to detect clinically insignificant HIT antibodies\(^3\)
- Does not measure ability of antibodies to cause platelet activation, a functional response associated with HIT\(^8\)
- Different antigen complex employed by each testing method; may contribute to differences in assay sensitivity and specificity\(^8\)
ELISA METHODS
(PF4 Enhanced® & Asserachrom® HPIA)

Advantages
• Sensitivity and specificity generally reported in the 85-95% range\textsuperscript{19,20}
• Contain positive and negative controls
• Published clinical history

Disadvantages
• Capital equipment purchase required (ELISA reader)\textsuperscript{2}
• Minimum 2-to-3 hour turnaround time when processed in-house\textsuperscript{45}; stat results challenging
• Microtiter plate configuration varies cost efficiency and makes single patient determinations expensive

PIFA® METHOD – (PIFA® HEPARIN/PF4 RAPID)

Advantages
• Specificity and sensitivity reported as 98.1\% and 91.3\% respectively versus ELISA\textsuperscript{6}
• Unit-use device facilitates single-patient determinations; relatively low, fixed assay cost
• Approximate 10 minutes turnaround time facilitates stat results\textsuperscript{6}
• Internal device control provided with each test run to help ensure proper functioning\textsuperscript{6}
• Manual assay that is accessible to all hospital laboratories

Disadvantages
• New market entry with limited clinical history
• Positive and negative controls not provided with assay