Instrumentation Laboratory
A Werfen Company

The Laboratory's Role in the Evaluation of Heparin-Induced Thrombocytopenia

Kathy Shingler MT(ASCP)

Clinical Hemostasis Specialist

Instrumentation Laboratory

Objectives

- Review the pathophysiology of Heparin-Induced Thrombocytopenia (HIT)
- Describe the clinical significance of HIT
- Explore current diagnostic lab tests for HIT
- Discuss benefits of rapid, on-demand HIT testing

Definition of Heparin-Induced Thrombocytopenia (HIT)

- HIT is an adverse affect of heparin therapy causing an immune-mediated disorder that increases risk for venous and arterial thrombosis, and can lead to increased morbidity and mortality
- Caused by the development of platelet-activating antibodies directed against Platelet Factor 4/Heparin complexes

Warkentin TE, Levine MN, Hirsh J, *et al*. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *N Engl J Med.* 1995 May18;332(20):1330-5.

Pathogenesis of HIT

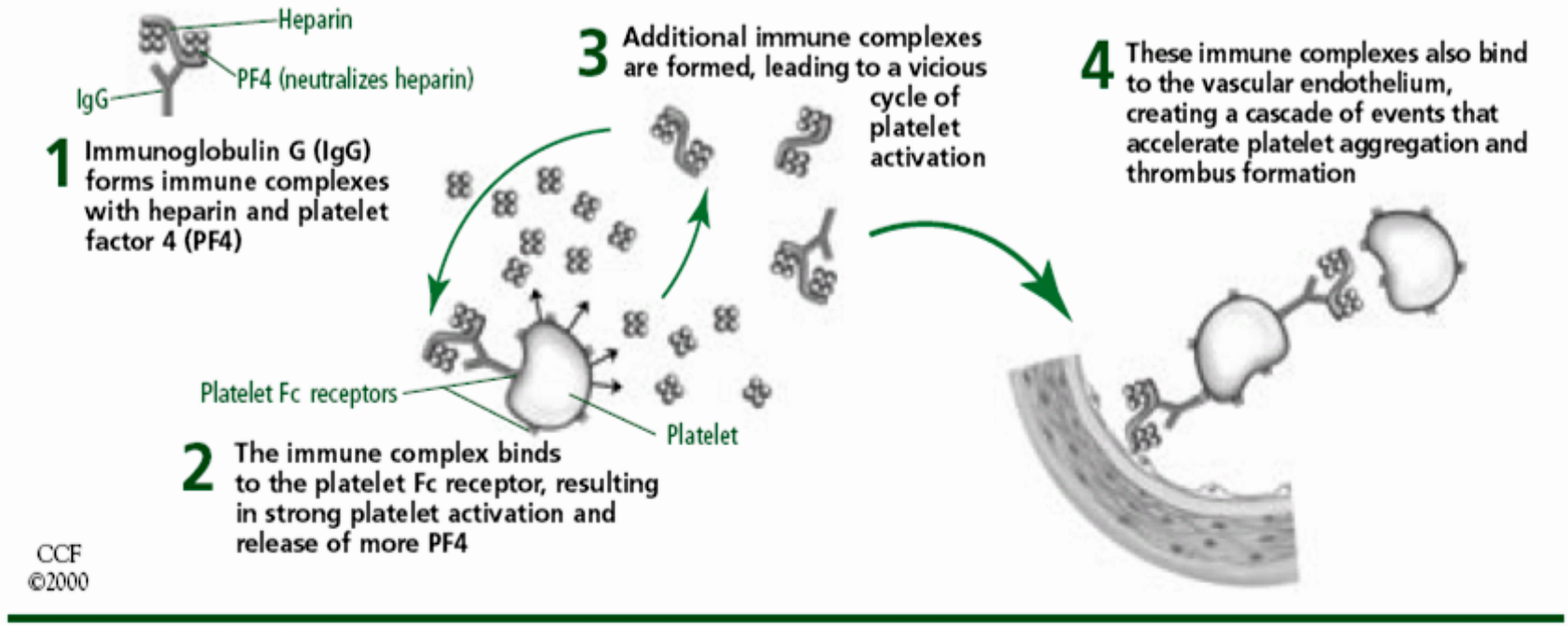
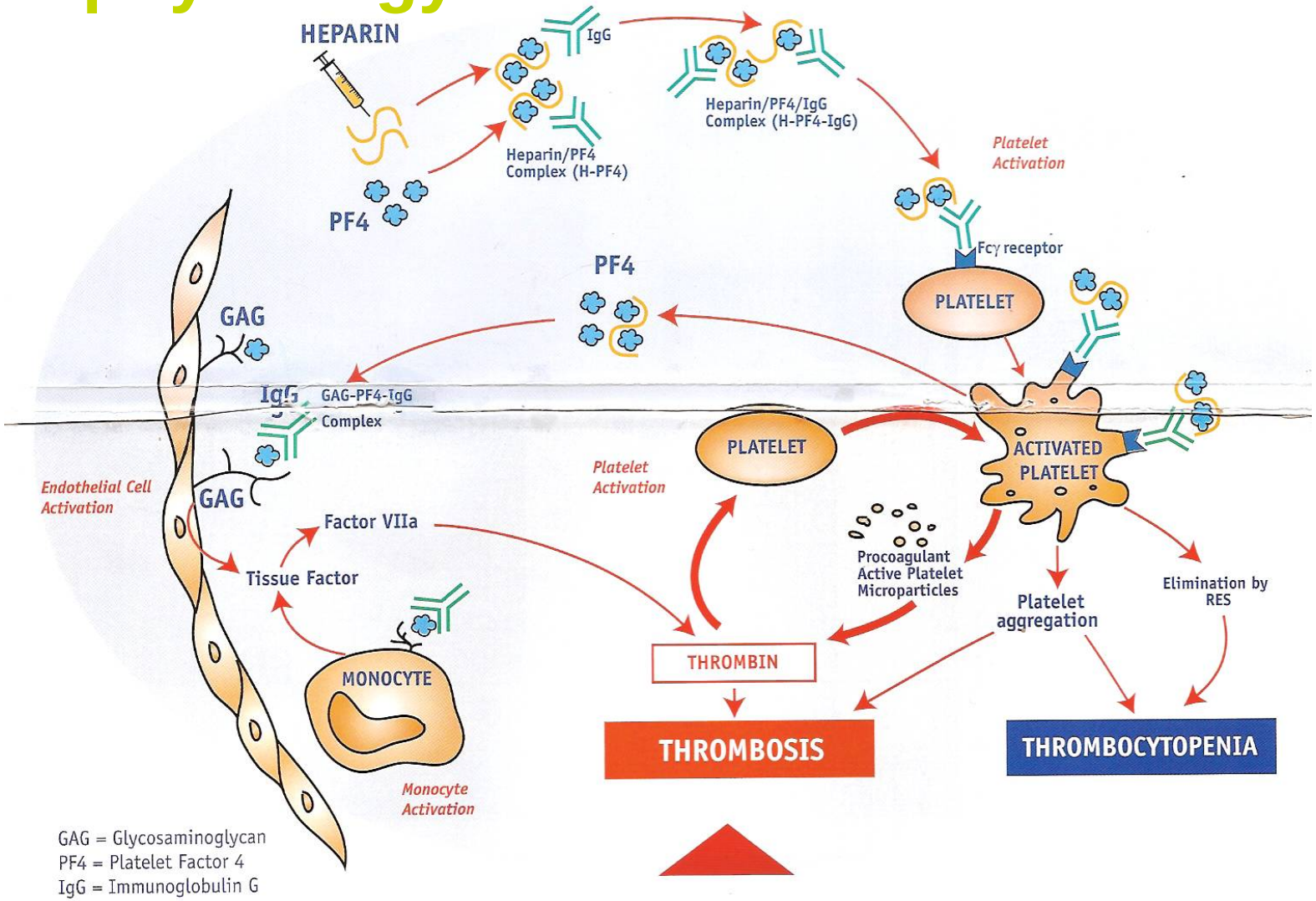


FIGURE 1. Adapted, with permission

Caiola E, Cleve. Heparin-induced thrombocytopenia: how to manage it, how to avoid it. *Clin J Med.* 2000 Sept;67(9):621-4.

Bartholomew JR, *et al.* Heparin-induced thrombocytopenia: principles for early recognition and management. *Clin J Med.* 2005;72(s1):S31-6.

Pathophysiology of HIT



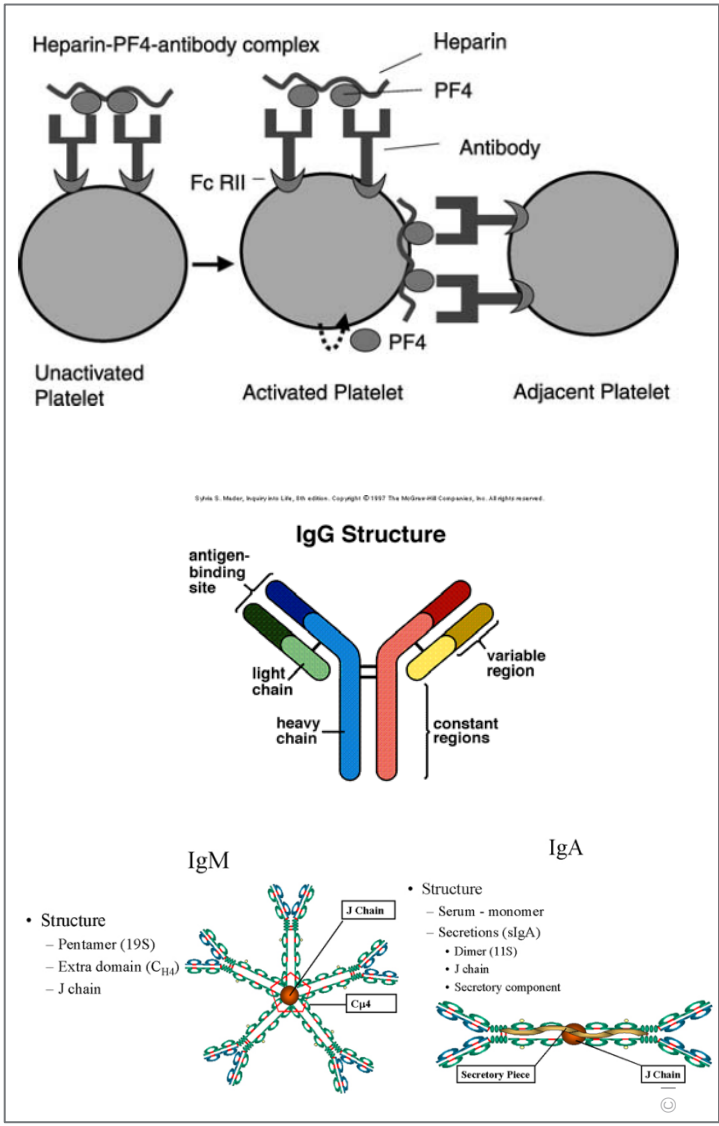
Orgaran - International Guidelines for Diagnosis and Treatment. Poster presented at ISTH, 2005.

Warkentin TE. Heparin-induced thrombocytopenia: pathogenesis and management. *BJH*. 2003;121:535-55.

Warkentin TE, Greinacher A. Heparin-induced thrombocytopenia: recognition, treatment, and prevention. *Chest*. 126:311S-37S.

Antibody Formation in HIT

- PF4 becomes immunogenic when it binds to heparin, eliciting antibody production by circulating B-lymphocytes
- Three classes of antibodies are produced
  - **IgG**, IgM, IgA
- **IgG** appears to be the **pathogenic** platelet-activating antibody
  - Leads to thrombocytopenia via binding to the **FcγRIIa** receptor on platelets
- IgM and IgA are **not** considered to be pathogenic as they should **not** crosslink the FcγRIIa receptor
  - Have been reported to be associated with HIT-related thrombotic complications in a few studies
  - **Heparin can bind <u>nonspecifically to plasma proteins</u> in some patients, leading to antibody binding to heparin chemokine complexes → HIT without typical heparin-PF4 antibodies**



Amiral J, *et al.* Pathogenicity of IgA and/or IgM antibodies to heparin-PF4 complexes in patients with heparin-induced thrombocytopenia. *Br J Haematol.* 1996;92(4):954-9.

Clinical Overview of HIT

- Syndrome associated with the development of immune-mediated thrombocytopenia
  - 8% of patients receiving heparin develop antibody to PF4-heparin
  - 1-5% will develop HIT with thrombocytopenia
- Results in a **prothrombotic diathesis** that may lead to significant morbidity and mortality
  - Up to 50% of patients present with complicating venous or arterial thrombosis
  - 10-20% of patients may require limb amputation
  - 20-30% mortality
- Occurs in
  - Up to 5% of patients receiving UFH
  - Up to 1% of patients receiving LMWH

Heparin-Associated Thrombocytopenia (HAT)

- Benign condition, frequency 10-20% of patients
- **Nonimmune-mediated** mechanism resulting in thrombocytopenia
- No heparin-dependent antibodies present
- Heparin therapy continued

Heparin-Induced Thrombocytopenia
HIT Type II

- Serious, life-threatening condition
- **Immune-mediated** mechanism resulting in thrombocytopenia and thrombosis
- Heparin-dependent antibodies present
- Heparin therapy discontinued

HAT vs HIT Clinical Manifestation

	HAT	HIT
Frequency	10-20%	1-3%
Time of onset	1-4 days	5-10 days
Platelet nadir	**100,000**	**30-55,000 (50% drop in platelet count)**
Antibody-mediated	No—thrombocytopenia due to platelet activation	Yes—antibodies bind to PF4
Thromboembolic sequelae	None	30-80%
Hemorrhagic sequelae	None	Rarely
Management	**Observe**—patient may be maintained on Heparin if indicated	**Immediate cessation** of Heparin—alternate anticoagulation if indicated

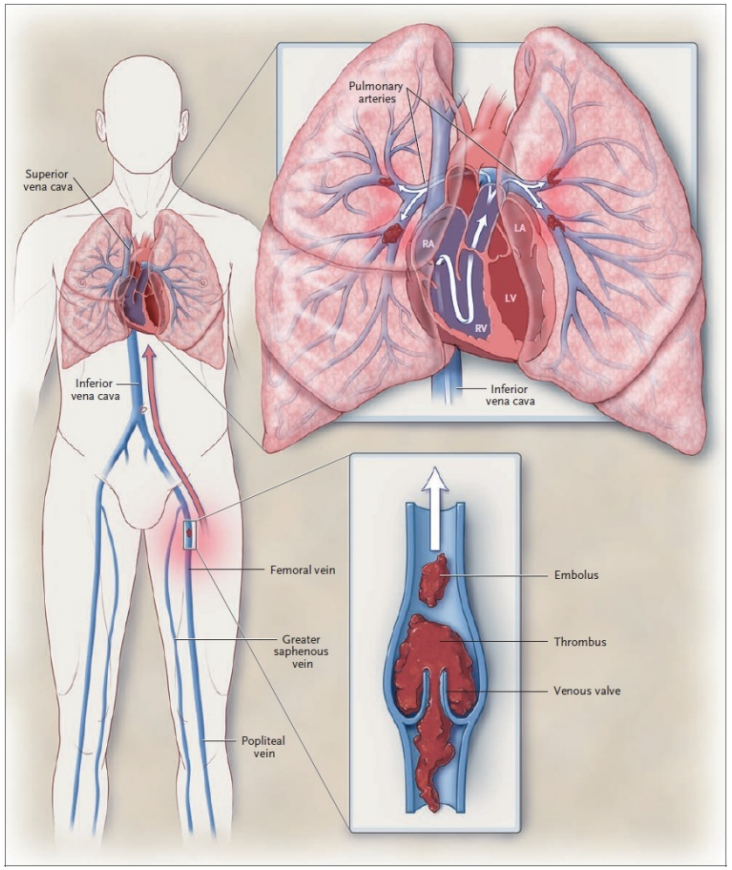
Adapted from Brieger, DB, *et al.* Heparin-Induced Thrombocytopenia. *J Am Coll Cardiol*. 1998;31(7):1449-59.

Factors Associated with HIT

Risk Factor	Highest Risk	Moderate Risk
Administration	IV (high dose)	SC (low dose)
Type	UFH	LMWH
Source	Bovine	Porcine
Patient	Surgical, Coronary Artery Bypass Grafting, Orthopedic	Medical

Clinical Presentation of HIT

- Platelet count fall > 50% from baseline
- Venous and/or arterial thromboses
- Skin necrosis
- Anaphylactic reactions



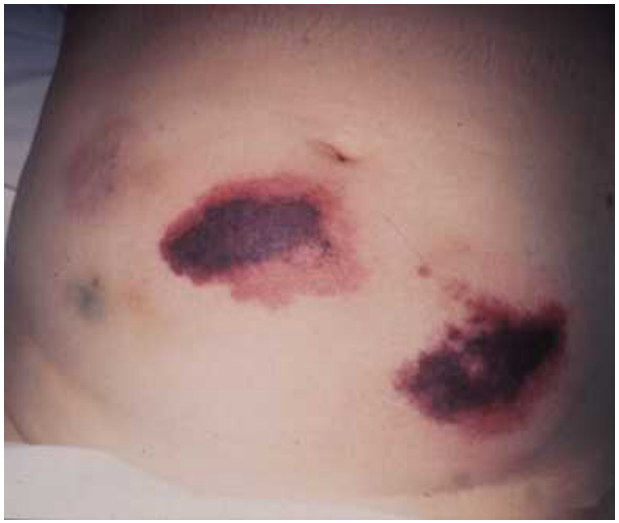
Medical illustration of Venous Thrombosis and Pulmonary Embolism

Clinical Presentation

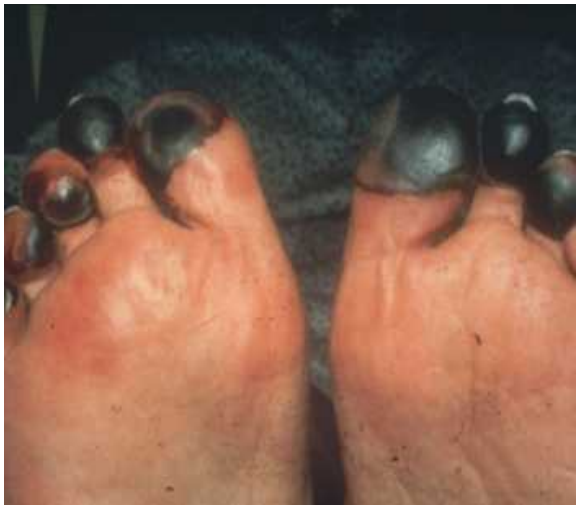
- Thrombocytopenia is the most common clinical manifestation of HIT
- Important features of the thrombocytopenia include
  - Timing of the onset of the thrombocytopenia
  - Severity of the thrombocytopenia
  - Course of the platelet count
    - Following withdrawal of heparin
    - While still on heparin

Manifestations of HIT

Skin Necrosis



Venous Limb Gangrene



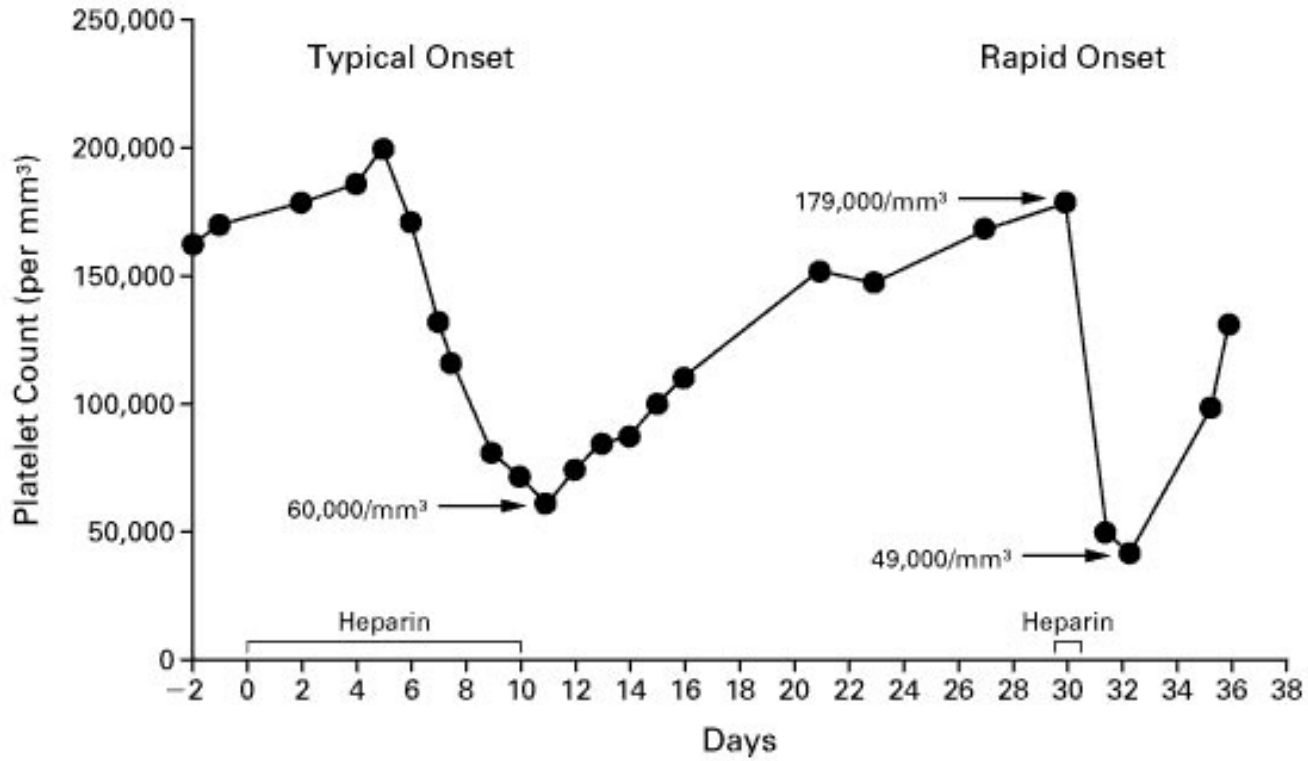
Warkentin TE. Heparin-induced thrombocytopenia: pathogenesis and management. *Br J Haematol.* 2003;121(4):535-55.
Warkentin TE, *et al.* The pathogenesis of venous limb gangrene associated with heparin-induced thrombocytopenia. *Ann Intern Med*. 1997;127(9):804-12.

Typical Onset HIT

- Thrombocytopenia
  - Generally occurs **5-10 days** after the initiation of heparin therapy
  - Platelet count rarely drops to severe levels (<50), unlike other types of immune-mediated thrombocytopenia (*i.e.,* ITP, TTP)

- Thrombosis
  - Major clinical presentation

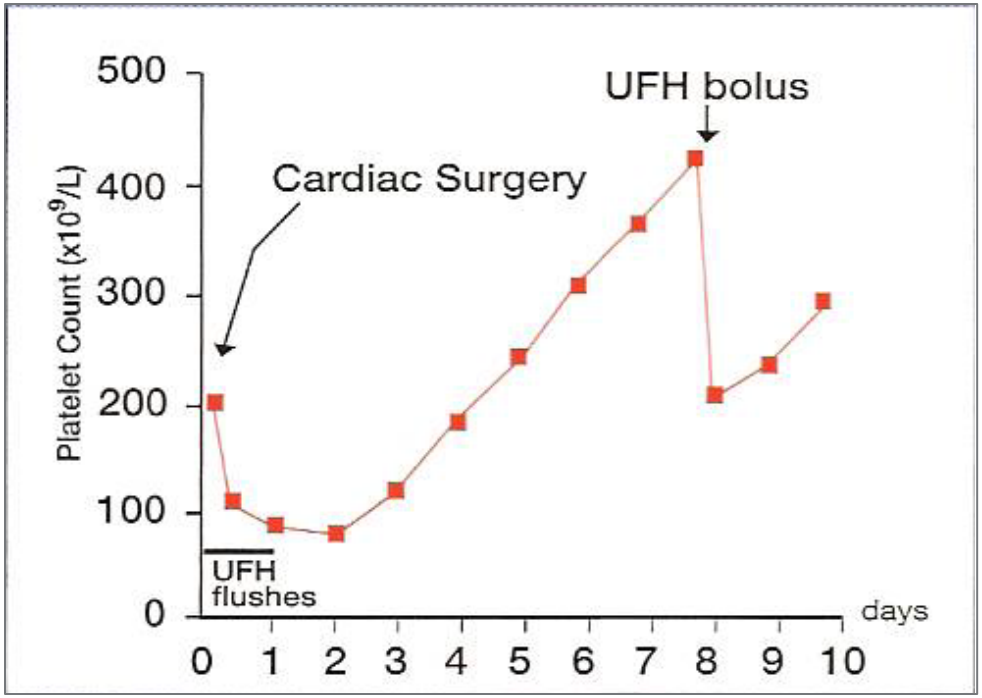
Typical Platelet Count Drop with HIT



Warkentin T. New Approaches to the Diagnosis of Heparin-Induced Thrombocytopenia. *Chest.* 2005;127:35S-45S.

Rapid-Onset HIT

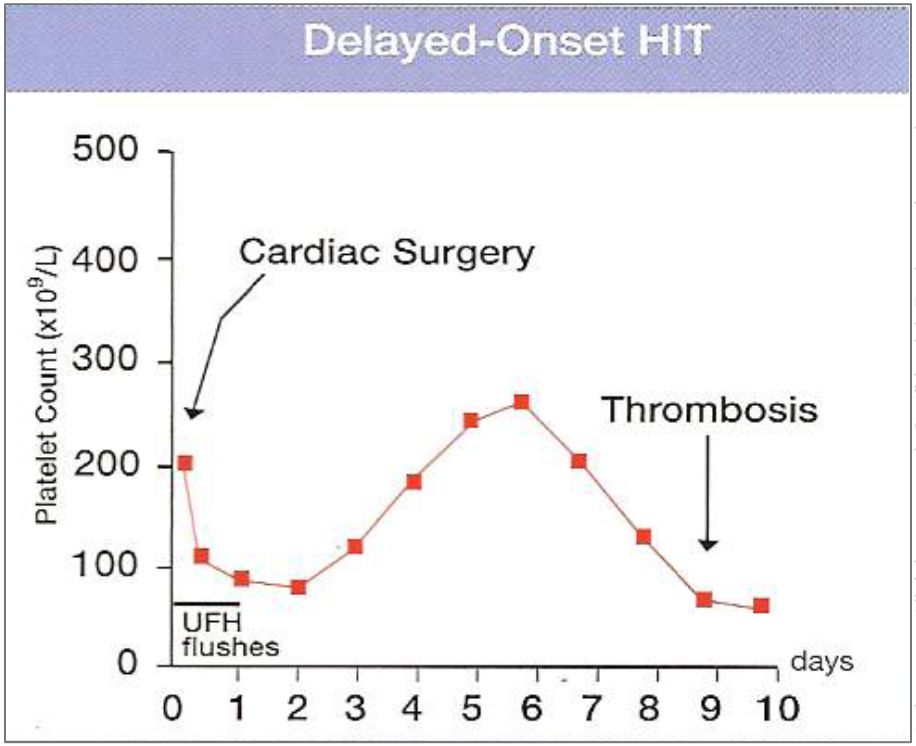
- Recent prior exposure to heparin
- The following can occur in patients sensitized to heparin within 5–30 minutes:
  - Fever, chills
  - Tachycardia, hypertension
  - Flushing, headache
  - Chest pain, dyspnea
  - Nausea, vomiting, large-volume diarrhea
  - Transient global amnesia
  - Sudden “anaphylactoid” death



Warkentin T. New Approaches to the Diagnosis of Heparin-Induced Thrombocytopenia. *Chest.* 2005;127:35S-45S.

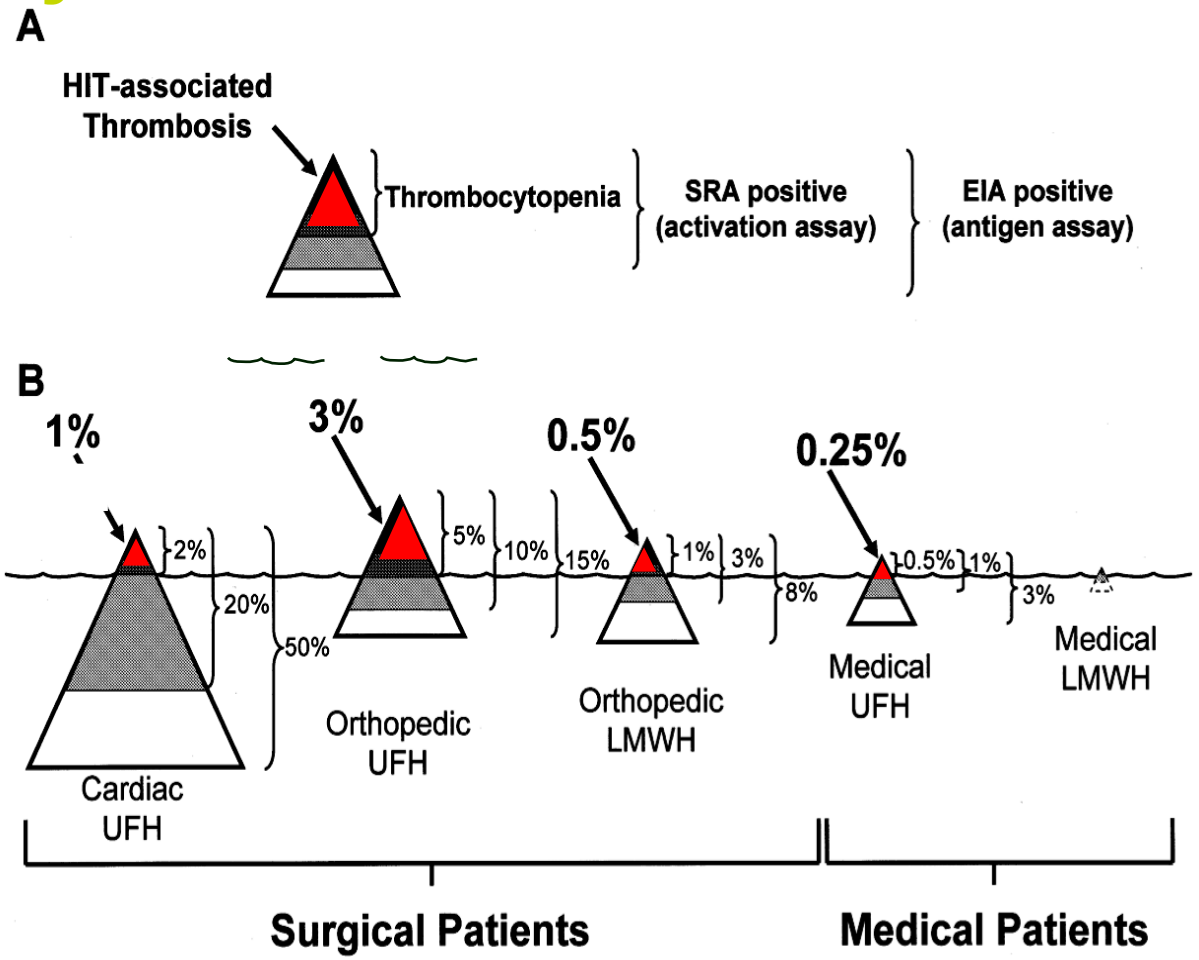
Delayed-Onset HIT

- Occurs a **week or more** after heparin cessation
- Patients typically demonstrate a further decrease in platelet count
- Should be suspected in a patient with recent heparin exposure who presents with:
  - thrombosis or
  - thrombocytopenia
- Immune-mediated



Warkentin T. New Approaches to the Diagnosis of Heparin-Induced Thrombocytopenia. *Chest. 2005;*127:35S-45S.

Frequency of HIT



- Activation assays are more specific than antigen assays for clinical HIT
- Incidence of HIT varies with clinical setting

Warkentin TE. Heparin-induced thrombocytopenia: pathogenesis and management. *Br J Haematol.* 2003;121(4):535-55.

Evaluation of HIT

Recommended Procedure for HIT Diagnosis

1. Clinically assess probability for HIT (*e.g.,* 4T score).
   a. Patients with Low probability (≤ 3) do not need further testing and Heparin can be maintained.
2. If clinically indicated, order HIT screening test
   a. If HIT antibodies not present, HIT is unlikely and heparin can be maintained if clinical pre-test probability is not high
   b. If HIT antibodies present, confirmatory testing is needed

Greinacher A. Heparin-Induced Thrombocytopenia. *J Thromb Haemost.* 2009 July13;7:9-12.

Clinical Probability: The 4Ts

Points for each category; maximum score = 8			
	2	**1**	**0**
Thrombocytopenia	>50% fall or platelet nadir 20-100 x 10^9/L	30-50% fall or platelet nadir 10-19 x 10^9/L	Fall <30% or platelet nadir <10 x 10^9/L
Timing of platelet count fall or other sequelae	Clear onset between days 5-10; or <1 day (if heparin exposure within past 100 days)	Consistent with immunization but not clear (*ie.,* missing platelet counts) or onset of thrombocytopenia after Day 10	Platelet count fall too early (without recent heparin exposure)
Thrombosis or other sequelae (ie. skin lesions)	New thrombosis; skin necrosis; post-heparin bolus acute systemic reaction	Progressive or recurrent thrombosis; erythematous skin lesions; thrombosis not yet proven	None
o**T**her causes for thrombocytopenia are not evident	No other cause for platelet count fall is evident	Possible other cause is evident	Definite other cause is present

Probability of HIT score:
6-8 = High; 4-5 = Intermediate; 0-3 = Low

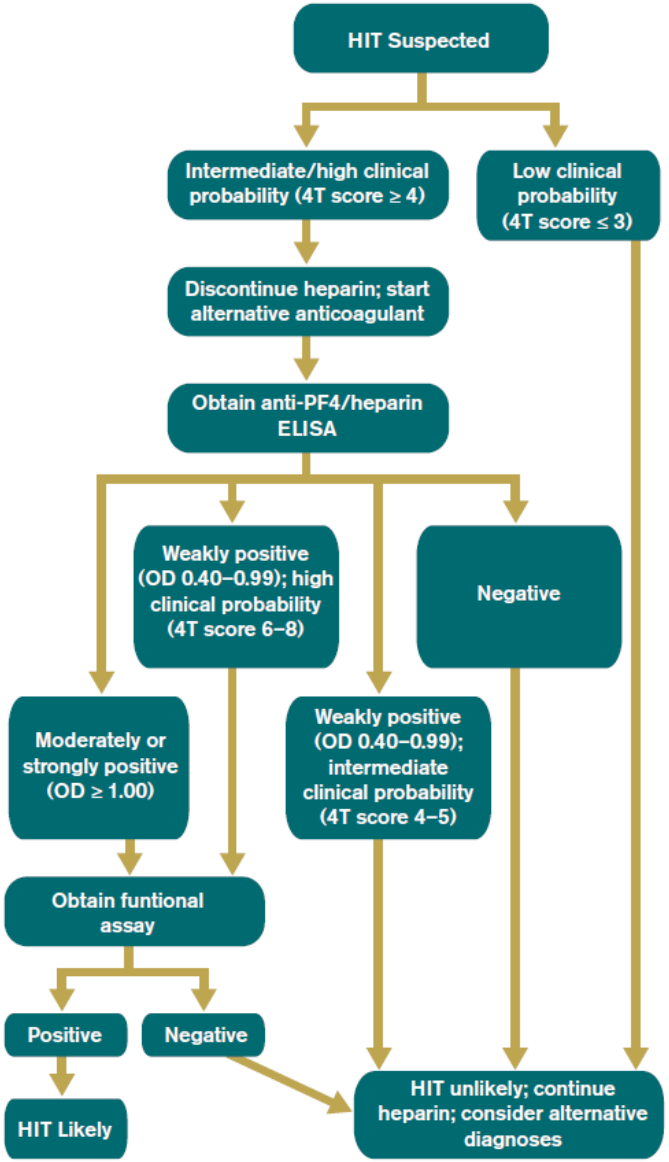
Pouplard C, *et al.* Prospective evaluation of the '4Ts' score and particle gel immunoassay specific to heparin/PF4 for the diagnosis of heparin-induced thrombocytopenia. *J Thromb Haemost*. 2007;5:1373-9.

Laboratory Testing

- Detection of antibodies against PF4/Heparin complexes
  - ELISA
    - Commercial
    - Home-brew
  - Particle-based Immunoassays
  - HemosIL® assay
- Functional assays
  - Serotonin Release Assay (SRA)
  - Heparin-Induced Platelet Aggregation (HIPA)

American Society of Hematology (ASH) Guidelines (2013)

- Recommends:
  - 4Ts scoring
  - HIT antibody testing
  - Functional assay testing



Cuker A, Crowther M. 2013 Clinical practice guideline on the evaluation and management of adults with suspected Heparin-Induced Thrombocytopenia (HIT). *Washington, DC: ASH (American Society of Hematology).* 2013;4.

Recognition and Treatment of HIT

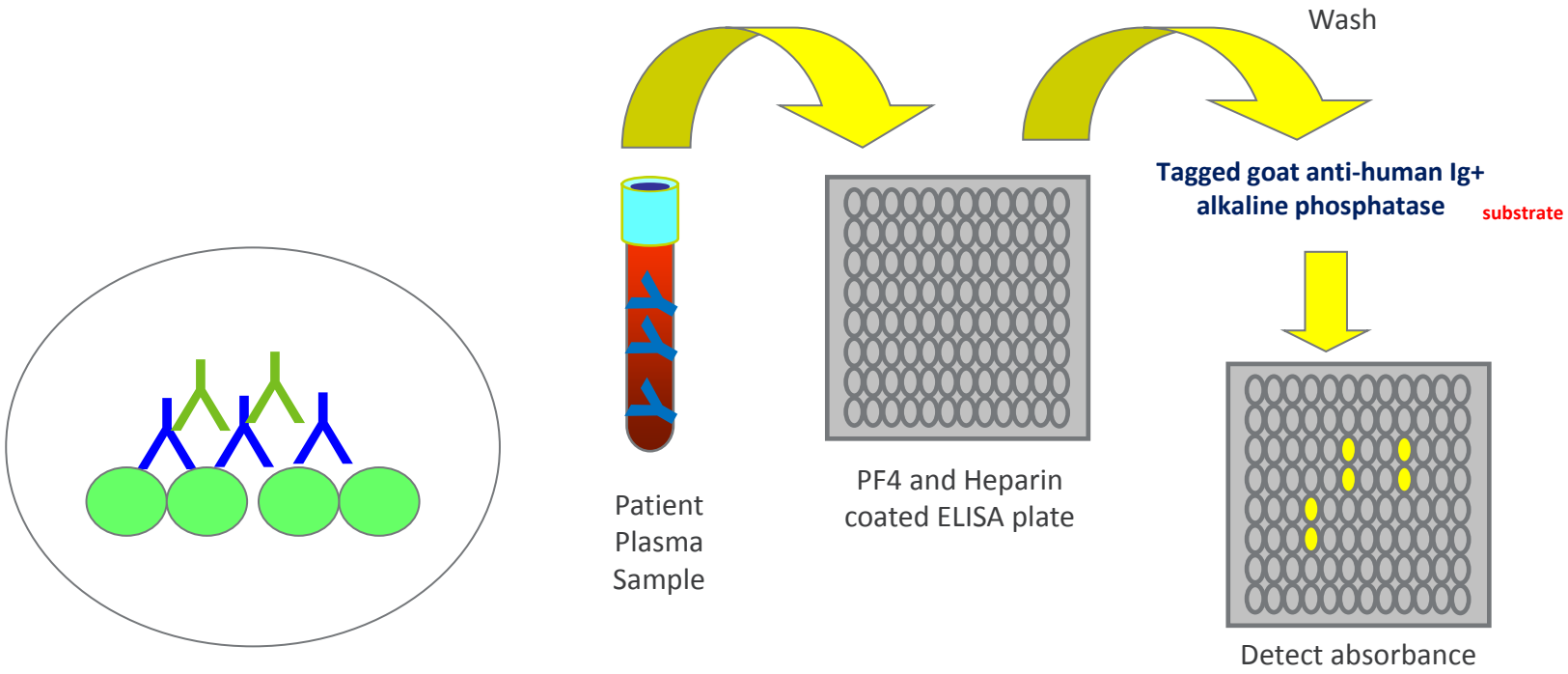
- Prompt recognition is key because heparin therapy must be immediately suspended
  - Patients should be treated with alternative, non-heparin anticoagulants (*i.e.,* argatroban, bivalirudin, fondaparinux)
- Challenges:
  - Alternative drugs are more expensive than heparin
  - Alternative drugs have an increased bleeding risk
  - Patient management with alternative drugs can be very challenging:
    - No antidote
    - Transition to warfarin (Direct thrombin inhibitors prolong PT)

Laboratory Evaluation of HIT

Immune-Based Assays

- ELISA-based assay
- Particle Immunofiltration or particle immumoagglutination
- Immunoturbidimetry

ELISA-Based Assay



PIFA-4

- **Particle Immunofiltration Assay**
- Dyed microparticles coated with purified PF4
- Sample is moved through a filter medium to react with PF4
- Reactive/non-reactive

TEST Window	CONTROL Window	RESULT
NO Blue	ANY Red Area*	Positive/Reactive
ANY Blue Area*	ANY Red Area*	Negative/Non-reactive

http://akersbiosciences.com/pdf/pifa_instruction%20sheet_ce_v3_4_26_05.pdf

HemosIL HIT-Ab(PF4-H)

The first, on-demand, qualitative, fully automated assay for the detection of anti-platelet factor 4/heparin (PF4/H) antibodies in human 3.2% or 3.8% citrated plasma on the ACL TOP Family of instruments in a laboratory setting.*

The on-demand solution for fast PF4-H antibody detection

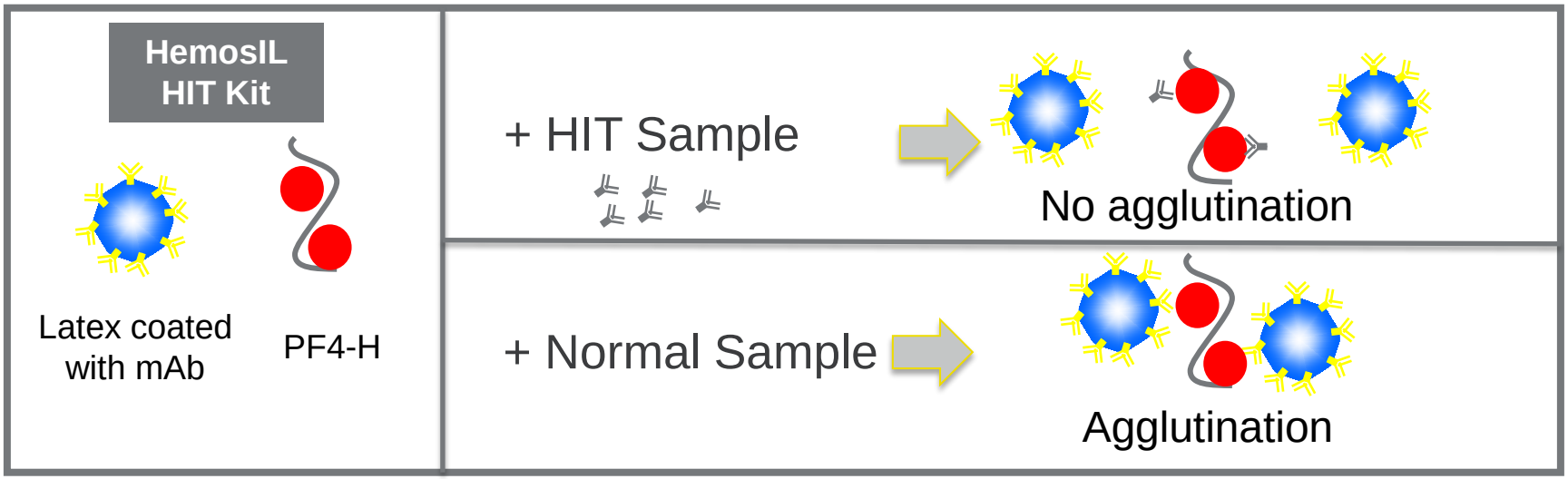
- Simple, Fast
  - Fully automated, liquid, ready-to-use
  - Results available 24 hours/day, 7 days/week
  - Results in minutes

*For adult population. To be used in conjunction with other laboratory and clinical findings.

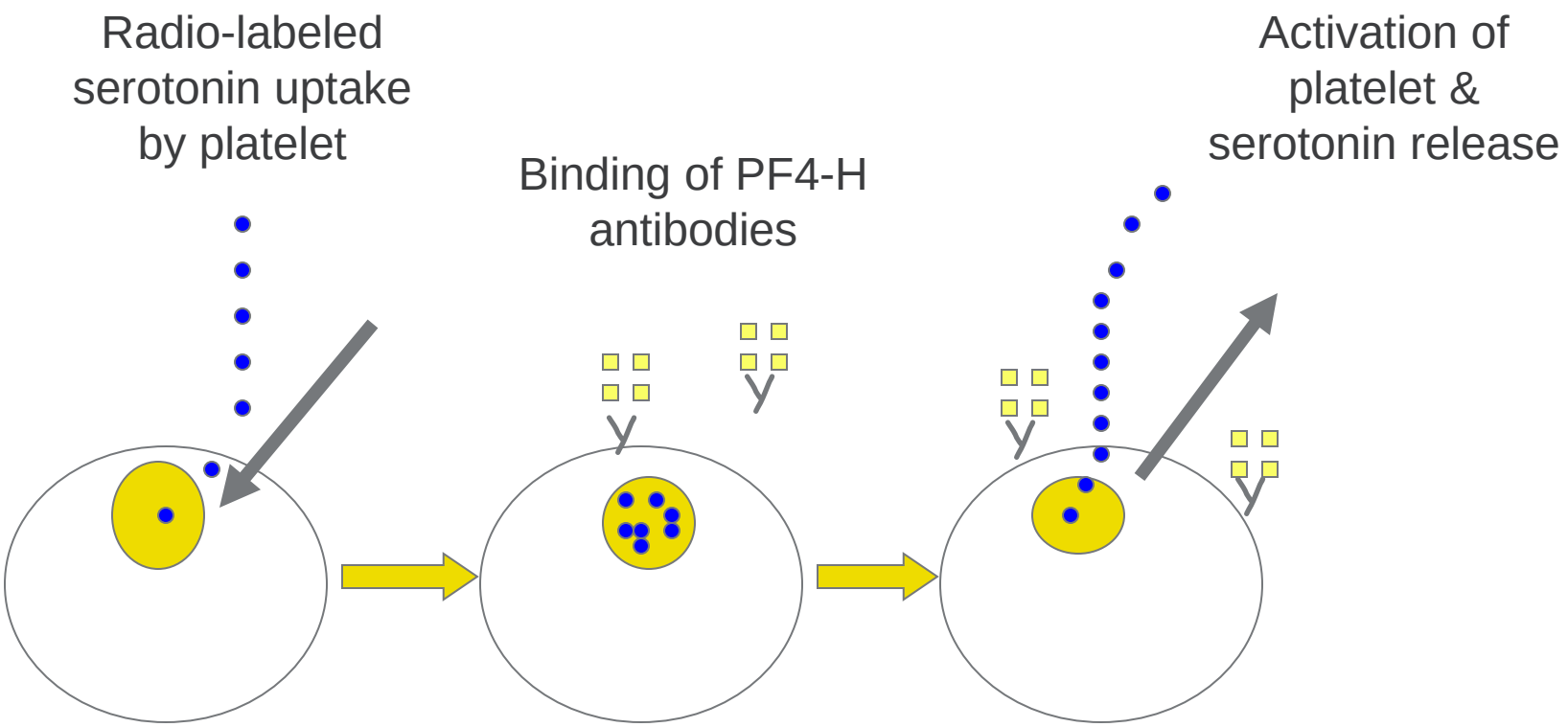
HemosIL HIT-Ab(PF4-H)

- Analytical excellence
  - Detects total immunoglobulin against PF4-H complexes
  - Dedicated controls for a complete quality management program
  - Excellent agreement with commercially available ELISAs
- Cost-effective
  - Fully automated—no manual processes required
  - Faster response impacts cost and quality of care

HemosIL HIT-Ab$_{(PF4-H)}$ Assay Principle

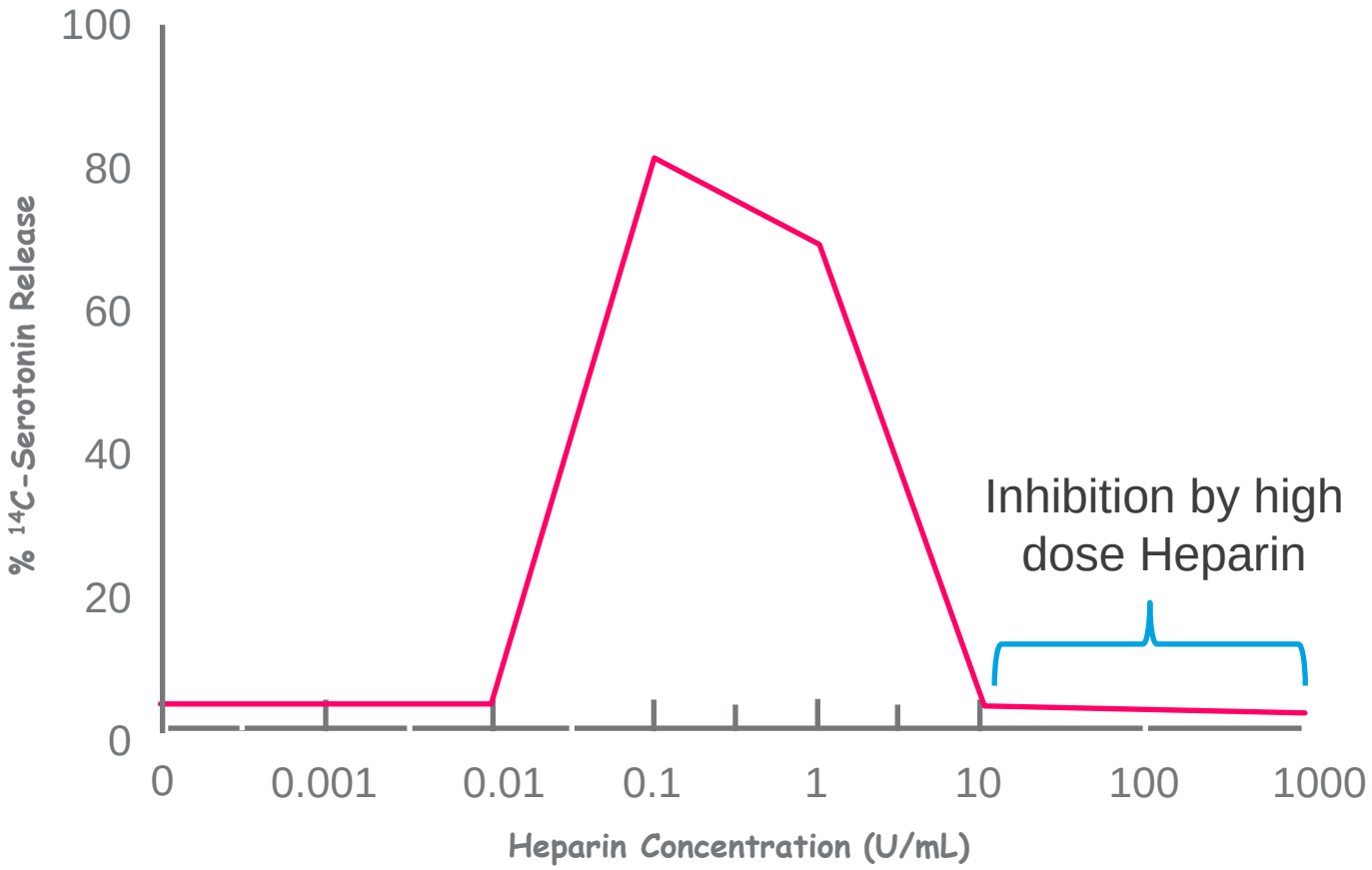


Serotonin Release Assay (SRA)



Warkentin T. New Approaches to the Diagnosis of Heparin-Induced Thrombocytopenia. *Chest. 2005;127:35S-45S.*

SRA and Heparin Dose



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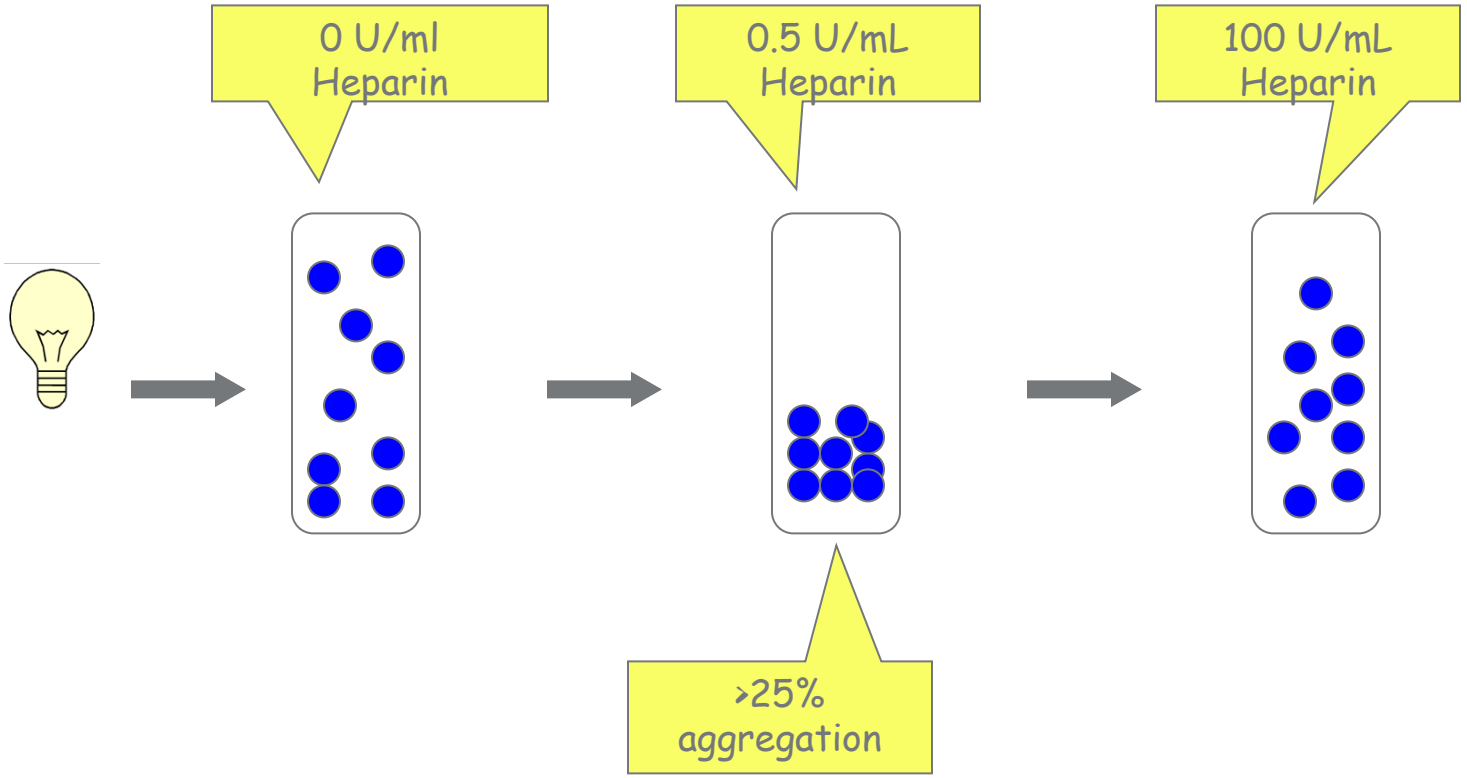
Heparin-Induced Platelet Aggregation (HIPA)

- HIPA examines platelet aggregation at 3 different heparin concentrations



Laboratory Testing for HIT

Test	Advantages	Disadvantages
ELISA	HIGH Sensitivity (75-90%) Technically easy 3 hr TAT	LOW Specificity False positives (for some populations)
PIFA	HIGH Sensitivity HIGH Specificity Technically easy Rapid TAT	Limited Clinical History Pos/Neg controls not provided
Immunoturbidimetric	On demand 24/7 Technically easy Liquid, ready to use Rapid TAT Excellent agreement with ELISA	Dedicated instrumentation
SRA	HIGH Sensitivity, HIGH Specificity (~99%) False positives rare	Technically demanding Radioisotopes Not routinely available
HIPA	HIGH Specificity	**LOW** Sensitivity (29-82%) Technique dependent

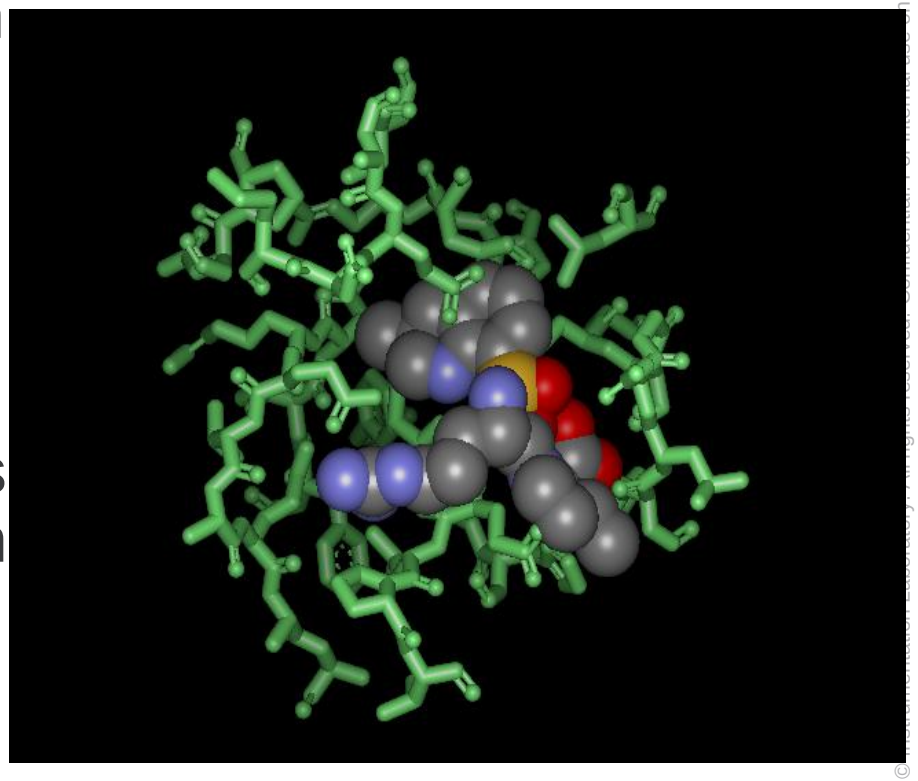
Treatment of HIT

Treatment of HIT

- Discontinue all heparin immediately, including
  - Heparin flushes
  - Heparin-coated pulmonary catheters
  - Heparinized dialysate and any other medications or devices containing heparin
- Initiate alternative anticoagulation therapy
- *Confirm diagnosis of HIT with appropriate laboratory tests*
- Monitor patient carefully for thrombosis
- Monitor platelet counts until recovery
- Avoid prophylactic platelet transfusions

Direct Thrombin Inhibitors used to treat HIT

- Argatroban
  - Synthetic and reversible, direct thrombin inhibitor from L-arginine
  - Half-life—40 minutes
  - Monitored by aPTT
  - Elevates PT/INR
  - Excreted by liver
  - Approved for the prophylaxis or treatment of thrombosis in patients with HIT
  - Bivalirubin and Fondiparinux also used. Less common



Greinacher A. Heparin-Induced Thrombocytopenia. *New England Journal of Medicine*. 2015;373:252-261.

HIT Diagnosis in Current Practice

1. In current clinical practice two scenarios may occur:
   - Patients with > 50% drop in platelet count have heparin immediately suspended and alternative anticoagulants are administered with no HIT testing
   - **Result: Overuse of alternative anticoagulant treatments**
2. Patients with >50% drop in platelet count have heparin immediately suspended and alternative anticoagulants are administered with HIT testing
   - HIT antibodies testing is immediately performed in hospital with ELISA methods
     Low efficiency in reagent use, very high testing costs
   - HIT antibodies testing is performed in batch or sent out to reference labs
     Delay in diagnostic response, overuse of alternative drugs, revert back to Heparin if patient is negative

Warkentin T. Demand on-demand testing for the diagnosis of heparin induced thrombocytopenia. *ThrombRes.* 2016 Apr;140:163-4.

Value of HIT Antibody Assays

- Evaluating patients suspected of HIT
  - Sensitivity:
    ~99%
  - Specificity:
    50-75% (IgG/M/A)
    55-90% (IgG)

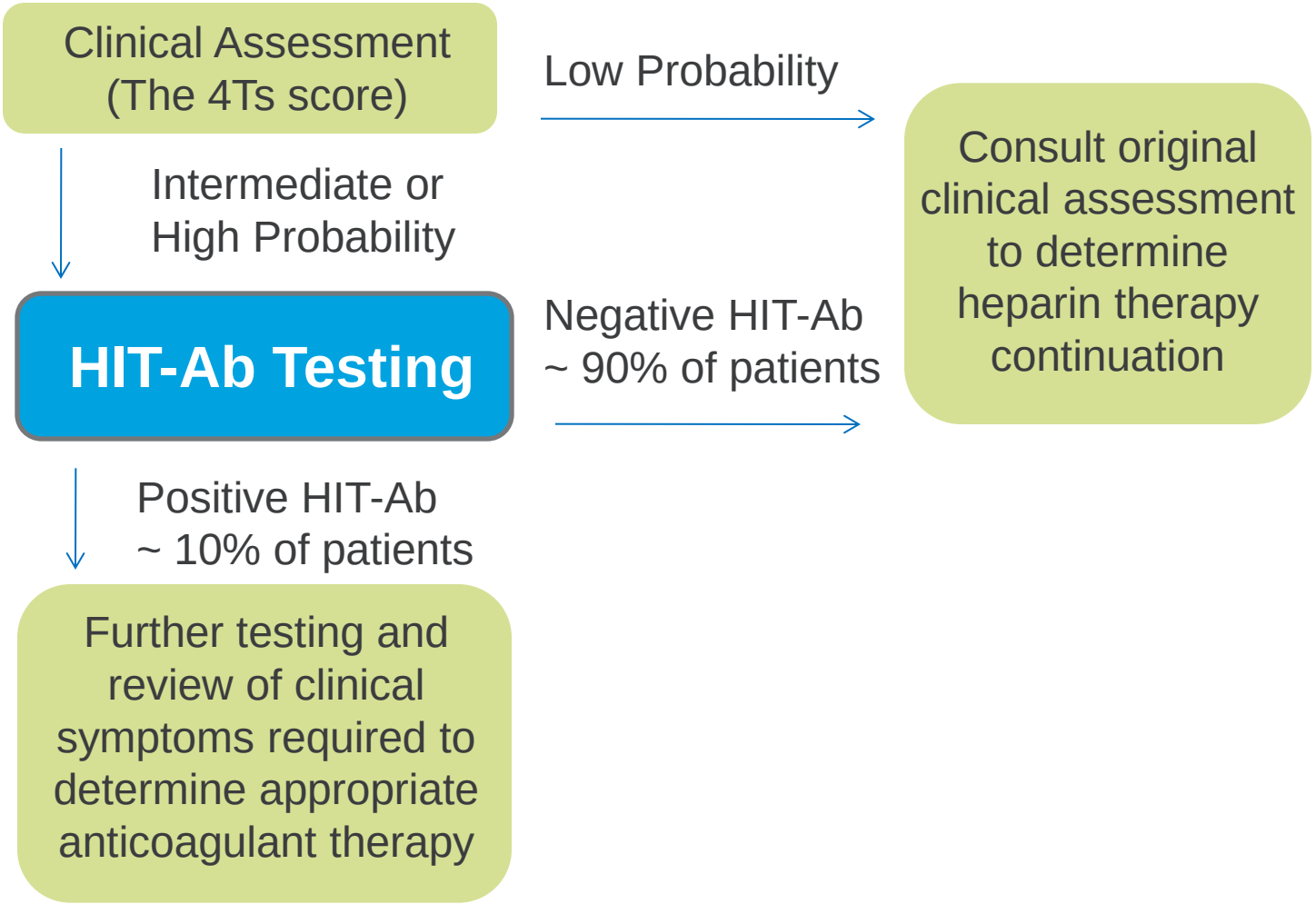
Greinacher A. Heparin-Induced Thrombocytopenia. *J Thromb Haemost.* 2009 July13;7:9-12.

Determining Risk of HIT

- A rapid, 24/7, fully automated, reliable test can promptly identify patients without HIT antibodies in approximately 90% of suspected patients and provide physicians with highly relevant information before determining anticoagulant alternatives
- ~10% antibody positive samples will require further functional tests for confirmation
- Approximately 50% of patients testing positive with HIT IgG tests are negative with functional assays

Greinacher A. Heparin-Induced Thrombocytopenia. *J Thromb Haemost.* 2009 July13;7:9-12.

Testing Algorithm



Importance of HIT Antibody Testing

- If HIT antibodies are present, the clinicians must be informed immediately
- With this information, they can then decide to continue heparin or suspend it and replace with alternative anticoagulants

Why Can't They Just Switch to New Anticoagulants?

- Expensive
- More difficult to manage
  (*e.g.,* bleeding, no antidote, transition to warfarin)
- A HIT diagnosis increases length of stay; thus, an erroneous diagnosis leads to unnecessary hospital costs
- **HIT Antibody testing improves patient care and can save costs**

When to Suspect HIT and When to Test

Has the patient:

- Been treated with heparin (UFH or LMWH)
- Experienced a platelet-count drop >50% within 5-10 days of heparin exposure
- Presented with thrombosis, skin necrosis, anaphylactic response

Which Test?

- HIT Antibody testing is key in determining the presence or absence of PF4-H antibodies, which are those ultimately responsible for the thrombocytopenia
- IgG HIT antibodies are considered more specific since this isotype can activate platelets.
- However, cases of HIT with IgM and IgA isotypes have also been reported

Which Test?

The value of HIT antibody testing relies mainly on negative predictive value rather than positive predictive value. That is, identifying the patient population that does not have HIT:

- IgG positive samples are not necessarily HIT (≈ 50%)
- 90% of patients suspected of HIT are negative, which makes HIT antibody testing extremely valuable independent of the isotype

Conclusions

- HIT is a serious condition that requires fast results
- A rapid, on-demand fully automated test can:
  - Improve patient safety and the quality of patient care
  - Result in significant hospital cost savings
  - Improve the lab's service to clinicians

Our Passion.

Your Results.

Instrumentation Laboratory