Heparin-induced Thrombocytopenia: Laboratory Diagnosis

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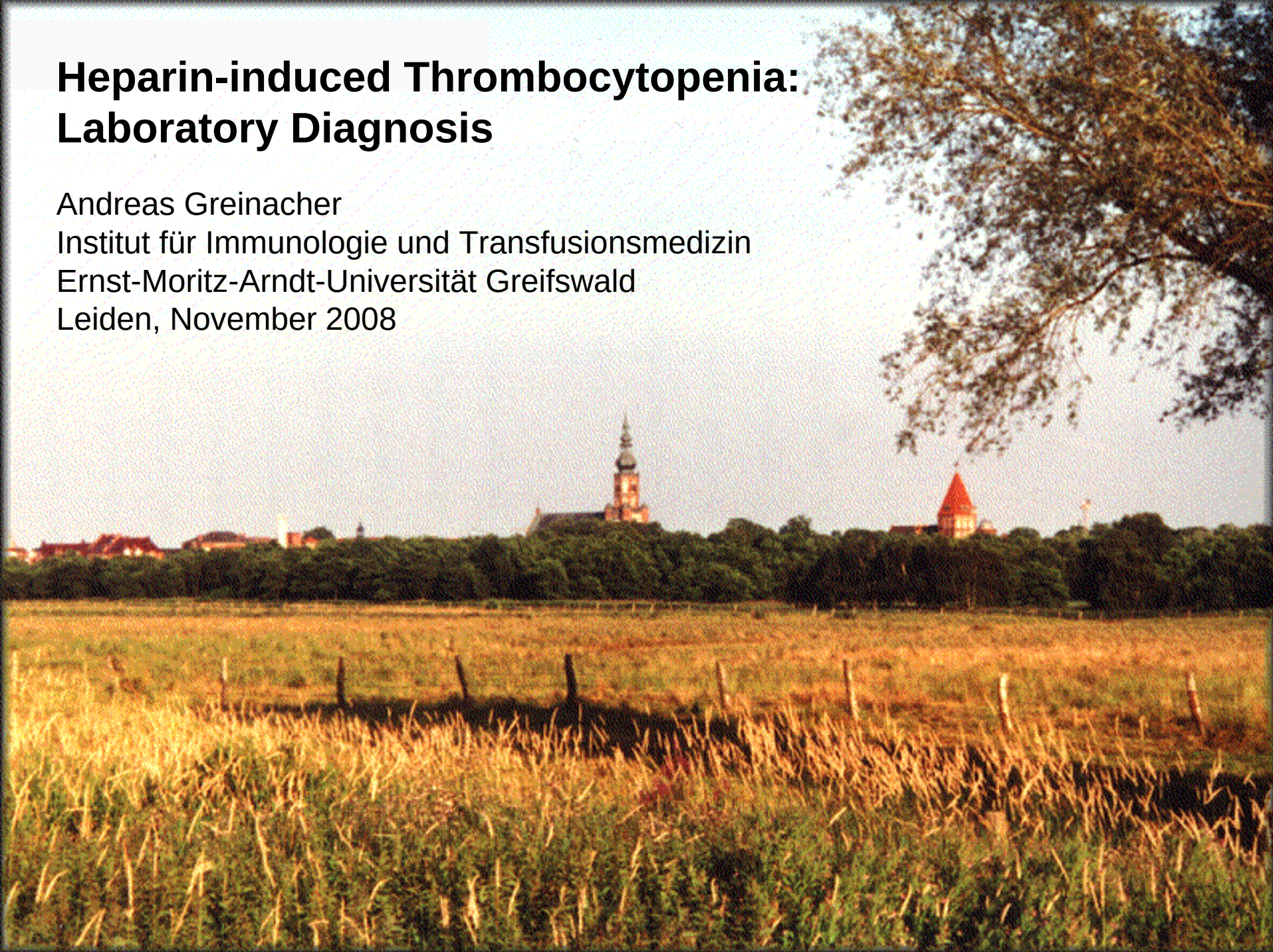


Heparin-Platelet Interactions

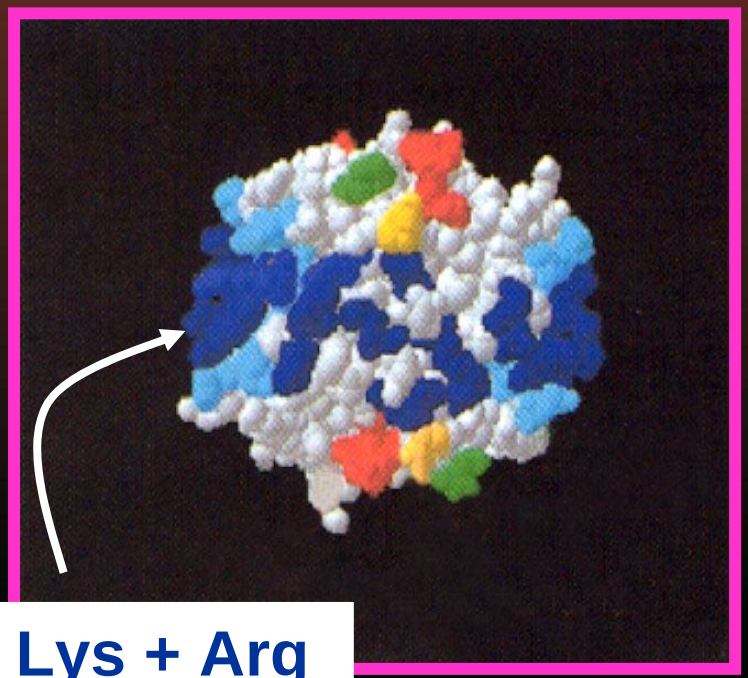
- (Non immune heparin-associated thrombocytopenia (HIT type I))
- **Immune-mediated heparin-induced thrombocytopenia (HIT type II)**

= HIT

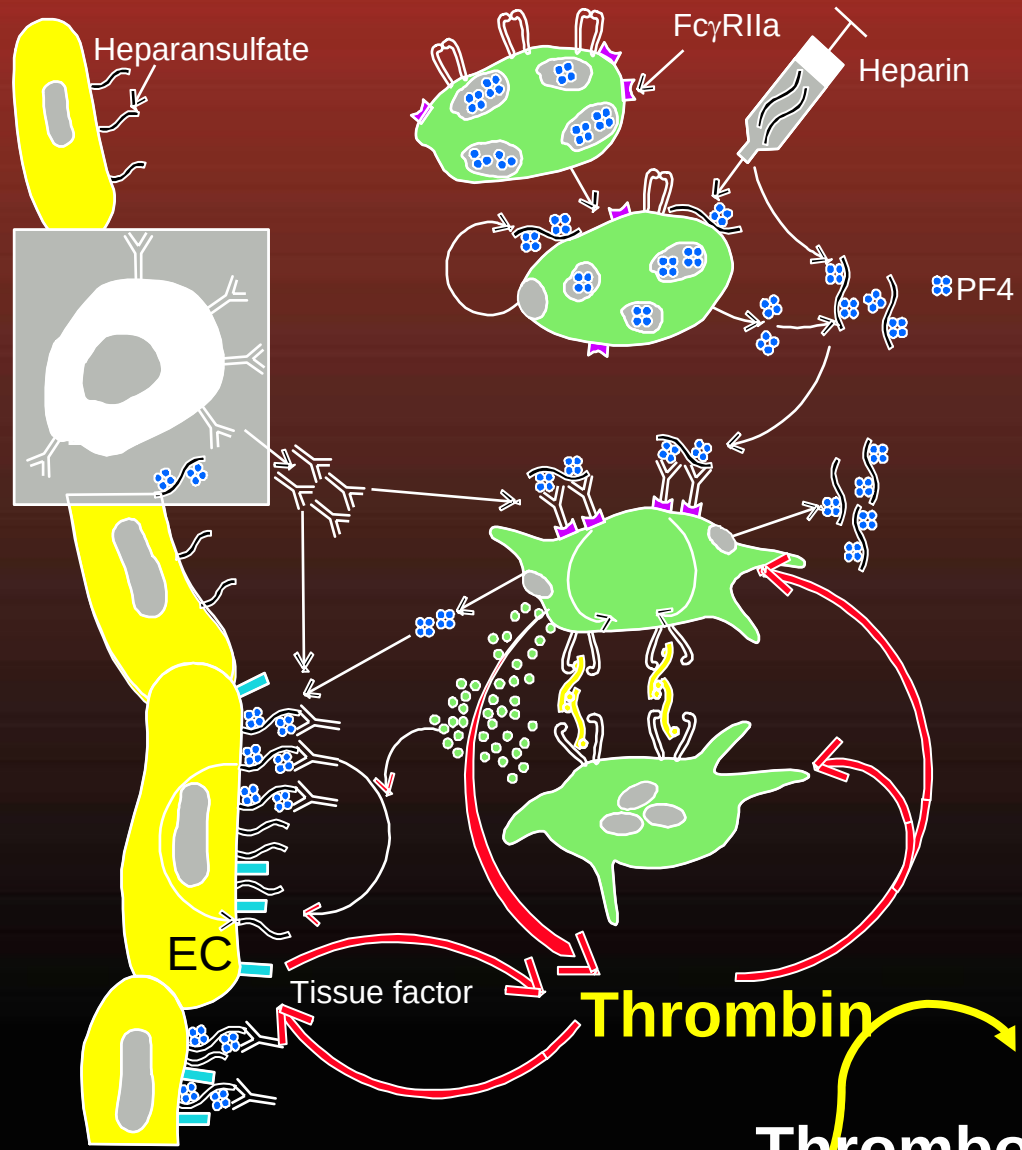
clinico-pathological syndrome

(clinical symptoms + antibodies)

Heparin-induced thrombocytopenia a link between immune system and hemostasis



Lys + Arg



Warkentin TE & Greinacher A. Heparin induced thrombocytopenia, 3rd ed, Marcel Dekker, New York 2004

Clinical features of HIT

Platelet count decrease > 50%
and/or
new thrombotic complications

between day 5-14 of heparin

Diagnosis - pretest probability: the *4 T's*

		2	1	0
A	Thrombocytopenia	> 50 % platelet count fall to nadir ≥ 20	30-50 % platelet count fall to nadir 10-19	<30 % platelet count fall to nadir ≤ 10
B	Timing of fall in platelet count or other sequelae	Onset d 5-10 or < 1 d (if heparin exposure within 30 d)	> d 10, or timing unclear, or < d 1 with recent heparin 31-100 d	Platelet count fall < d 4 (without recent heparin exposure)
C	Thrombosis or other sequelae	New thrombosis; skin necrosis; post-heparin bolus acute systemic reaction	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis – not confirmed	None
D	OTher cause for thrombocytopenia	No other cause for platelet count fall is evident	Possible other cause is evident	Definite other cause is present

Lo et al. J Thromb Haemost **2006**

Diagnosis - pretest probability
Interpretation of *4 T's* score

Score 0 - 3: very unlikely to be HIT (<5%)

Lo et al. J Thromb Haemost **2006**

http://www.medizin.uni-greifswald.de/transfus/transfus.htm/

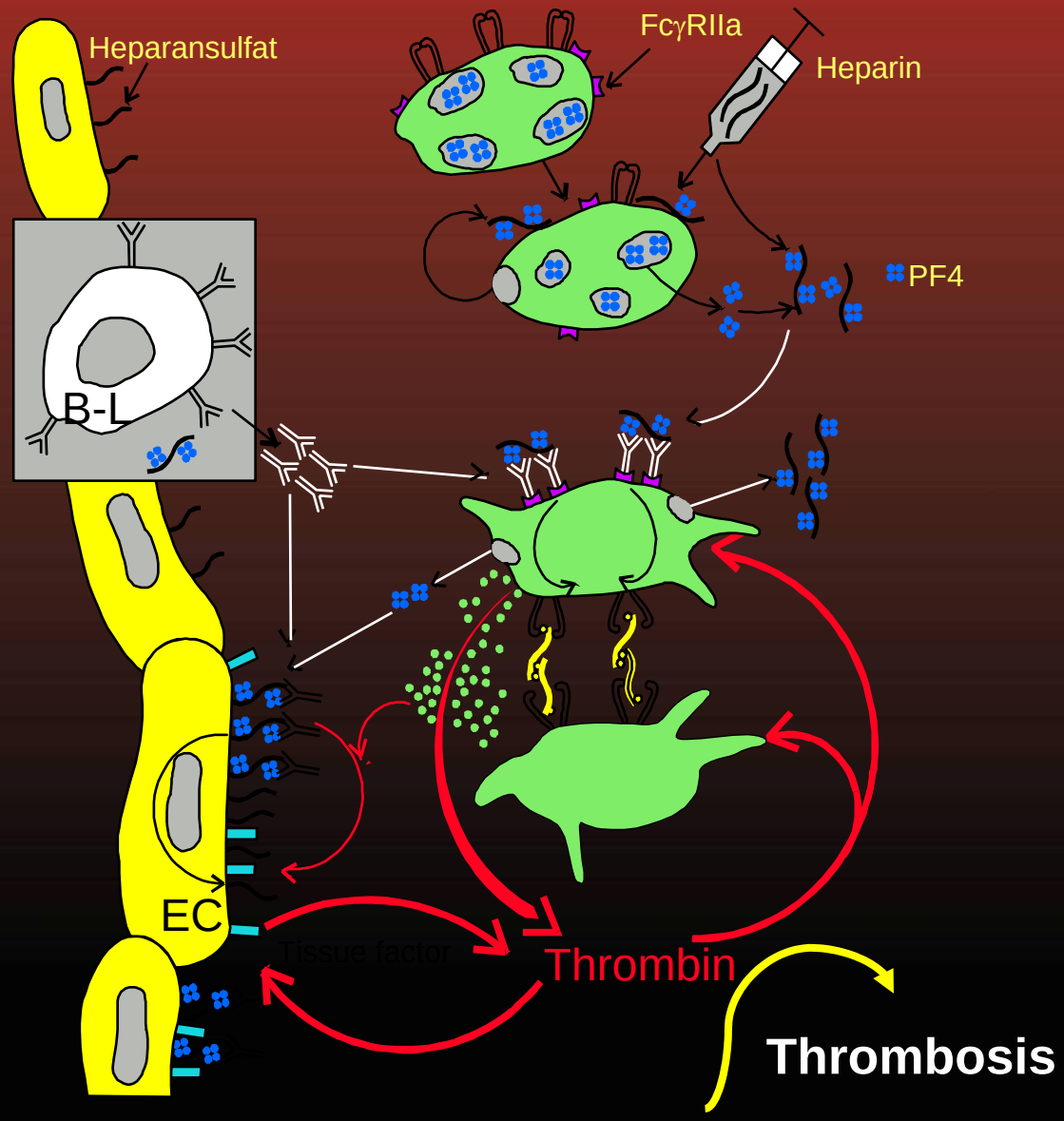
Laboratory Tests for HIT antibodies

- Antigen tests
  - rPF4/heparin IgG/M/A (Stago)
  - PF4/polyvinylsulfonate IgG/M/A, IgG (GTI)
  - Heparin/platelet lysate (HIA; Hyphen)
  - PF4/heparin IgG, IgA, IgM (Greifswald)
  - (Fluid phase ELISA)
  - Microcolumn IgG/A/M (Diamed)
  - PIFA rapid test
- Functional assays
  - Platelet aggregation using PRP
  - Platelet activation using washed platelets
    - Optical assessment heparin induced platelet activation (HIPA) test
    - Serotonin release: ^{14}C serotonin release (SRA), HPLC, ELISA
    - Flow cytometry: microparticles, annexin V
  - miscellaneous

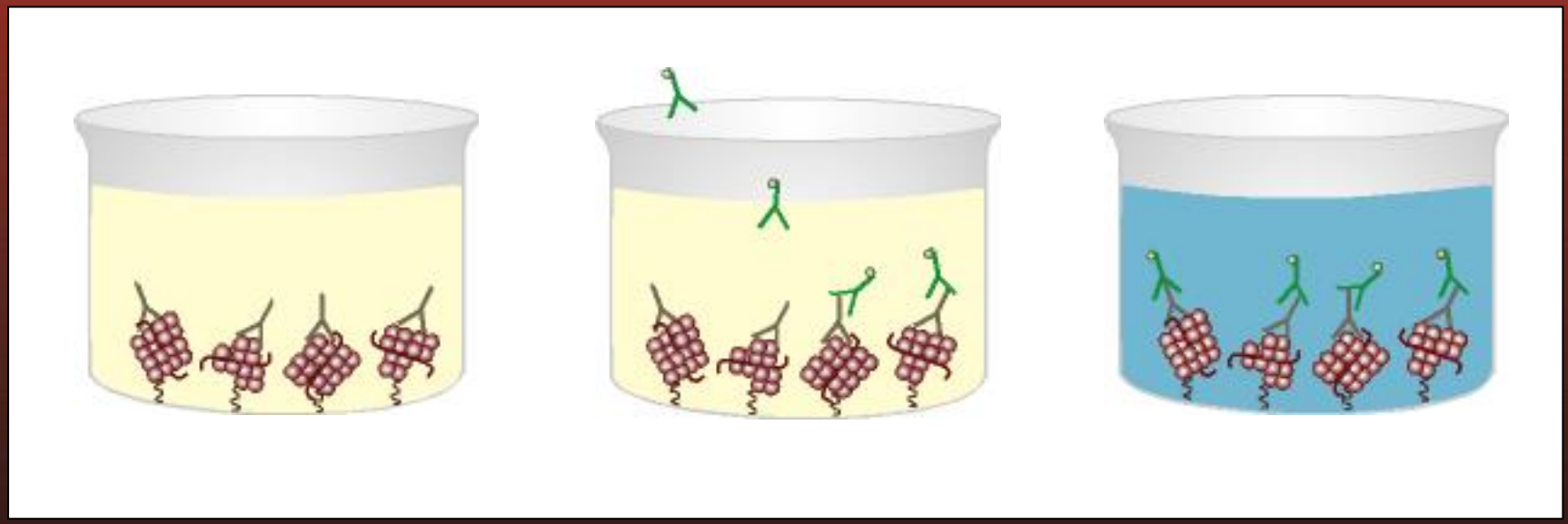
Antigen test
PF4/heparin ELISA
microcolumn

Functional test
HIPA-test
^{14}C-SRA

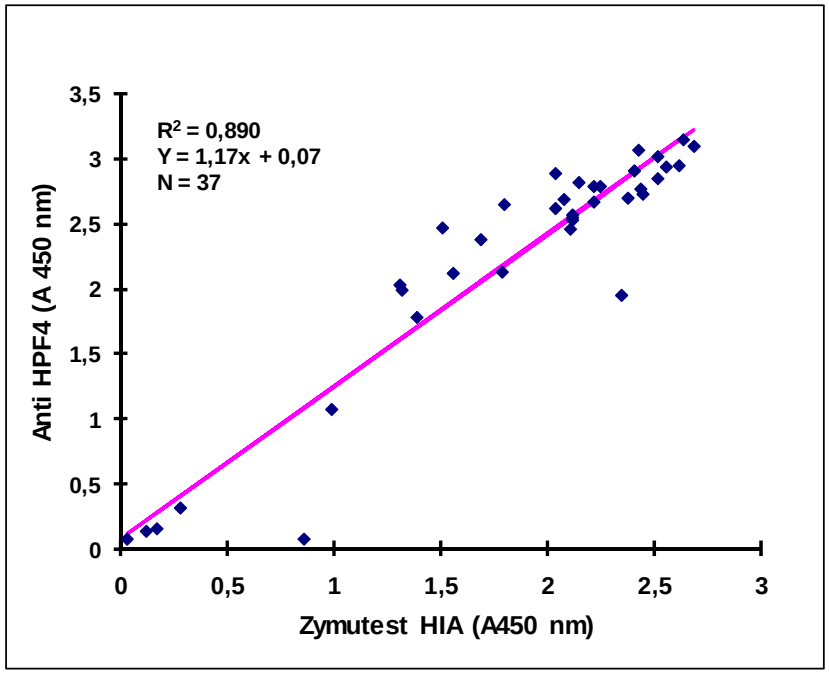
Sonography



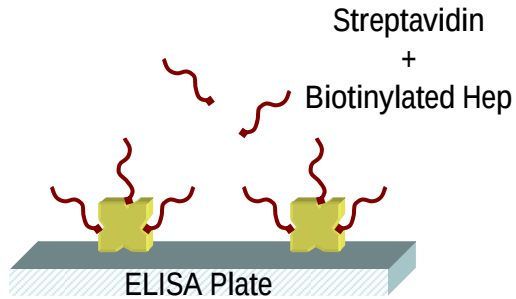
PF4/heparin Antigen Tests



+ Detect IgG, IgA, and IgM antibodies
+ Somewhat standardized
- Detect only PF4 dependent antigens
- Control of sensitivity/specificity of different lots??

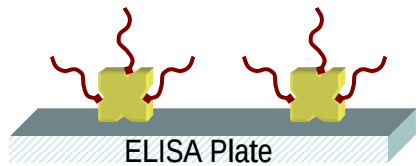


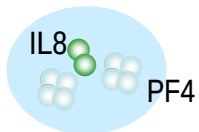
Zymotest HIA



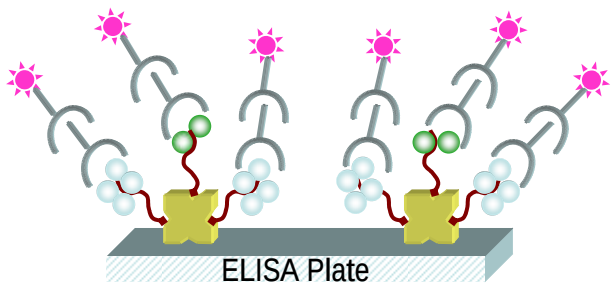
+/-

Platelet
or Leucocytes lysates

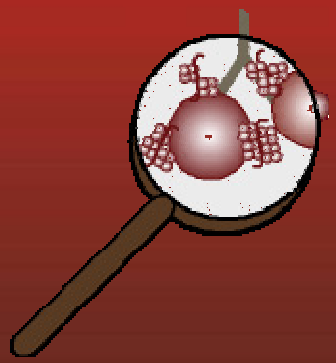




+ additionally detects anti IL8 antibodies



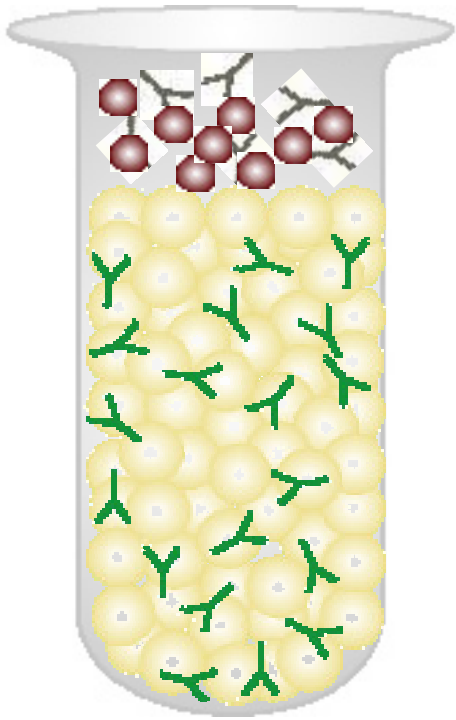
Immuno Beads Assay PaGIA (Diamed)



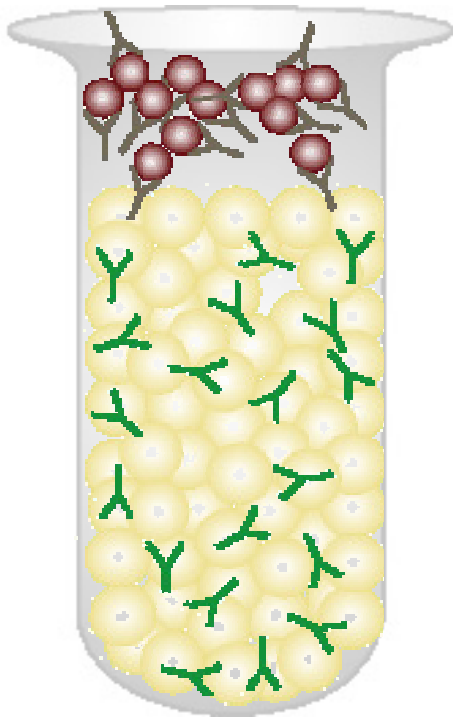
= PF4/heparin coated beads

= human antibody

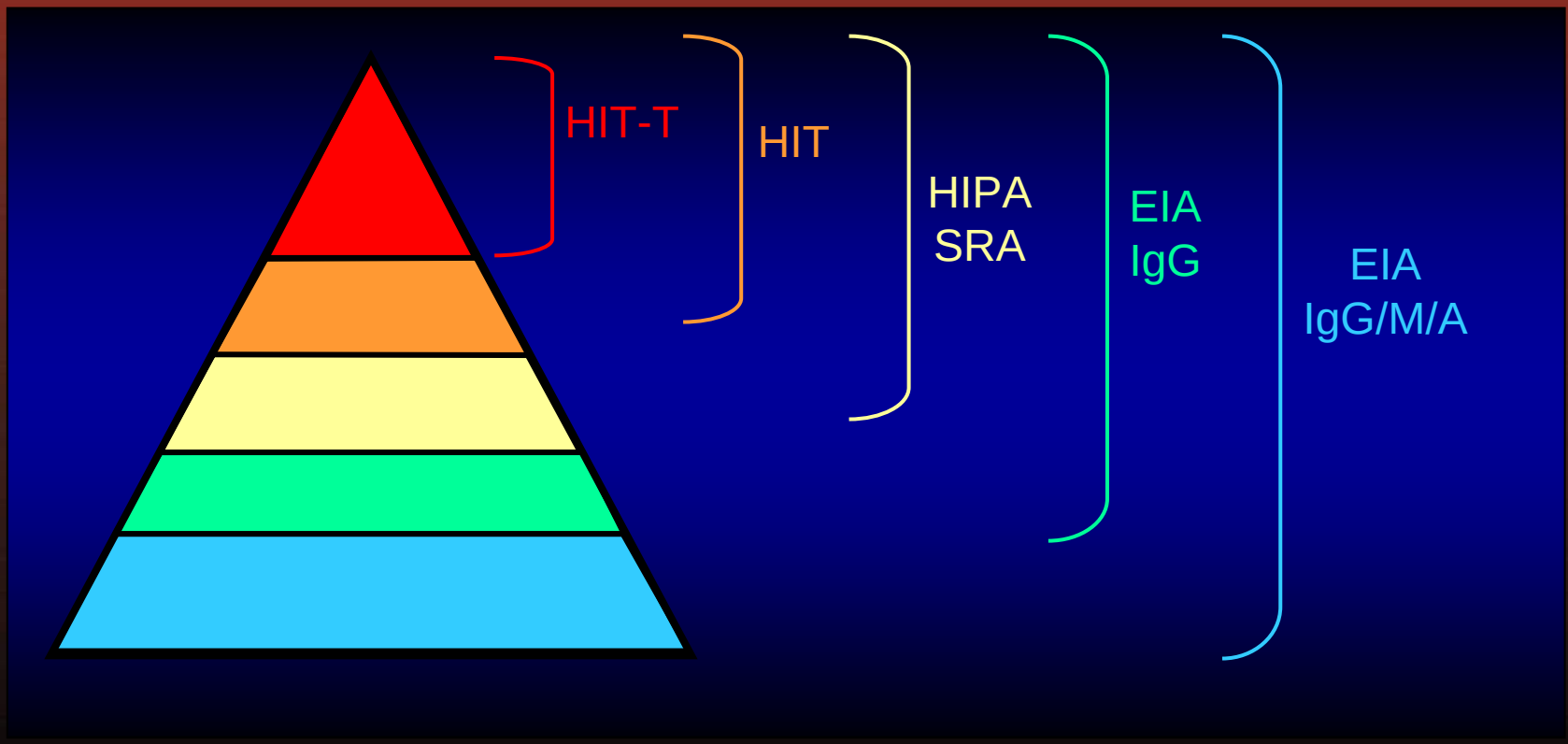
= anti-human Ig



negative



positive



Modified from Warkentin TE, in HIT, Warkentin and Greinacher 2007

How to improve specificity of antigen tests?

Test for anti-PF4/heparin IgG.

IgM and IgA antibodies are of minor relevance

Greinacher et al JTH 2007;5:1666-73

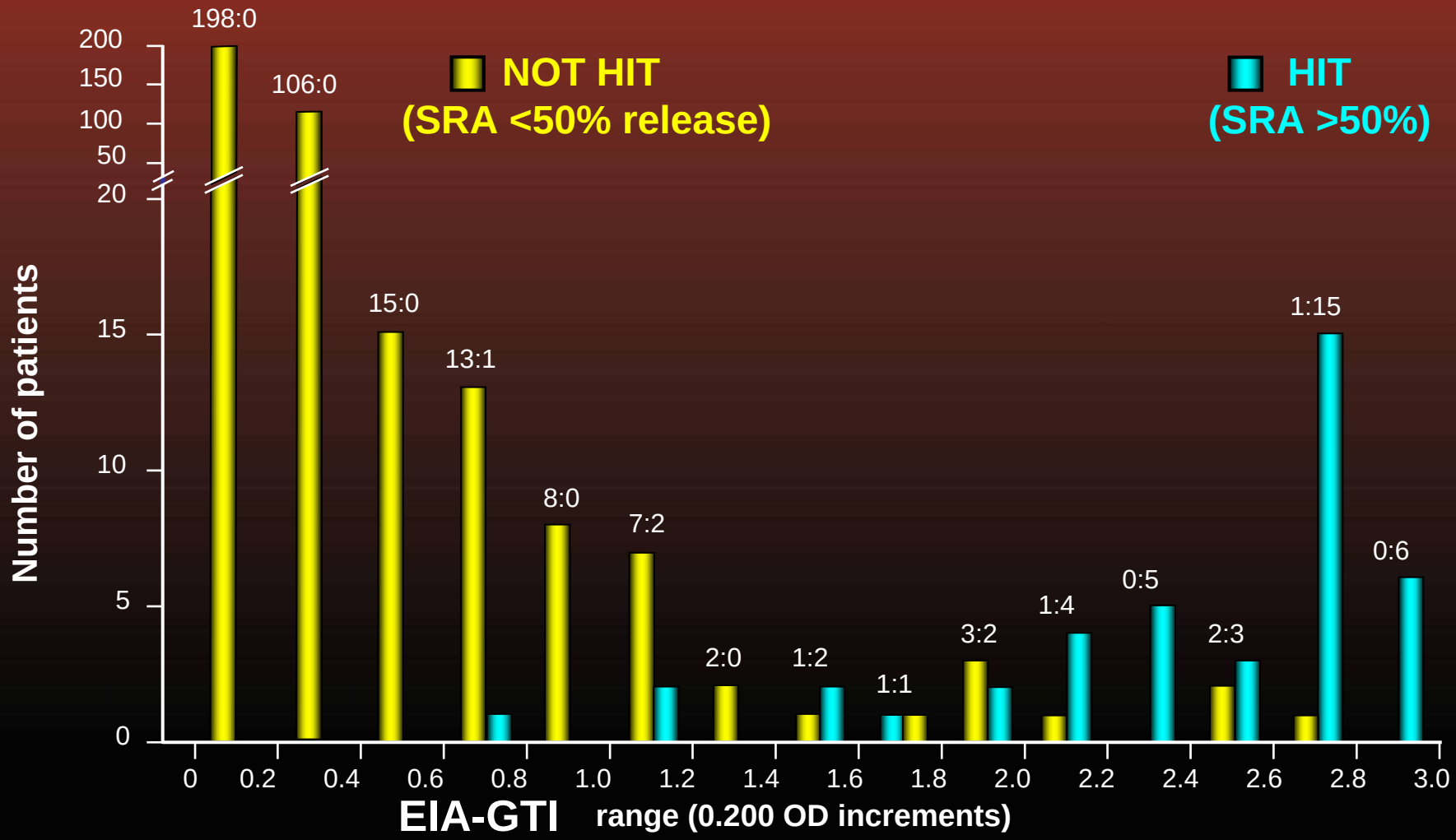
How to improve specificity of antigen tests?

Use a higher cut off. OD>1.0 is more likely to be of clinical relevance than an OD<1.0

Zwicker et al JTH 2004;2:2133-7

– few functionally very active antibodies show an OD < 1.0

EIA-SRA Relationship: GTI Assay (n=405)



Warkentin et al. *J Thromb Haemost* 2008; 6: 1304-1312.

PaGIA Microcolumn Test

Serial dilution of positive sera (2-fold steps)

- Clinically unlikely HIT:
  - titers ≥ 4: 2/85 (2%)
- Clinically probable or highly probable HIT:
  - titers ≥ 4: 39/54 (72%)
- Thrombosis:
  - negative or titer < 4: 8%
  - titer 4–16: 55%
  - titer ≥ 32: 74%

Alberio L et al. Am J Med 114:528-536, 2003

How to improve specificity of antigen tests?

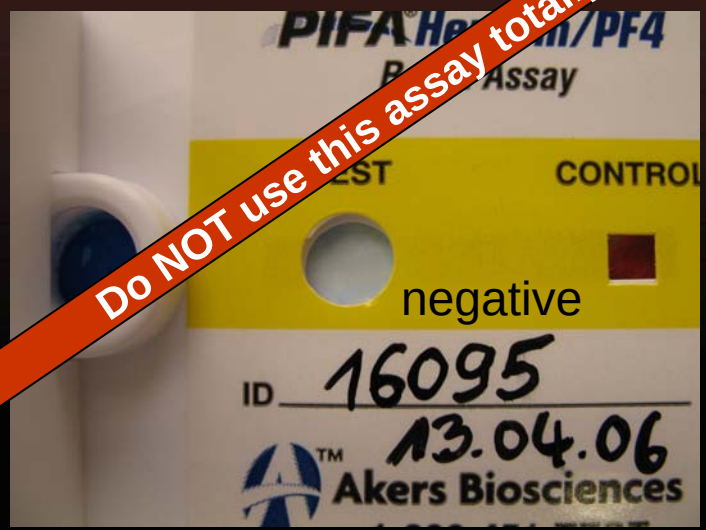
Add a confirmatory step with high heparin to show specificity for PF4/heparin complexes

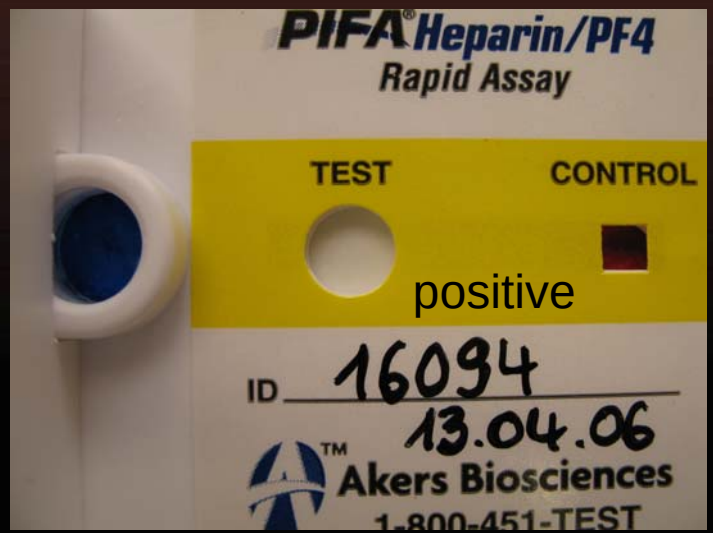
Whitlatch et al. Thromb Haemost 2008;100;678-84

– especially high affinity binding HIT antibodies may not be inhibitable, valid for ODs < 1.0

PIFA Heparin/PF4 Rapid Assay

Do NOT use this assay totally random results = non-informative assay JTH 2007





Functional assays for HIT

- Require fresh platelets
- Washing is critical
- Internal controls are important
- Require experienced staff
- Time consuming
- Not available as test kit

Platelet aggregation test

120 µl PRP

75 µl heat inactivated serum

10 µl heparin/buffer

+Relatively easy to perform

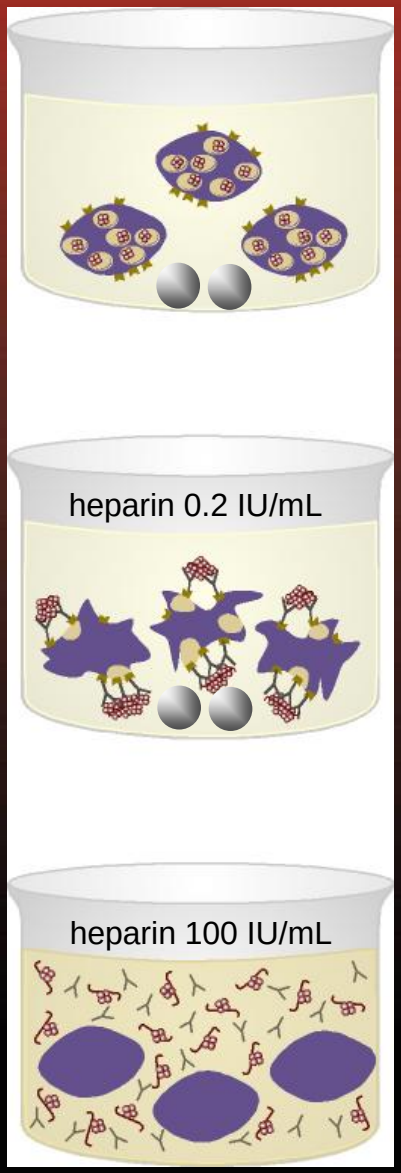
+Antigen independent

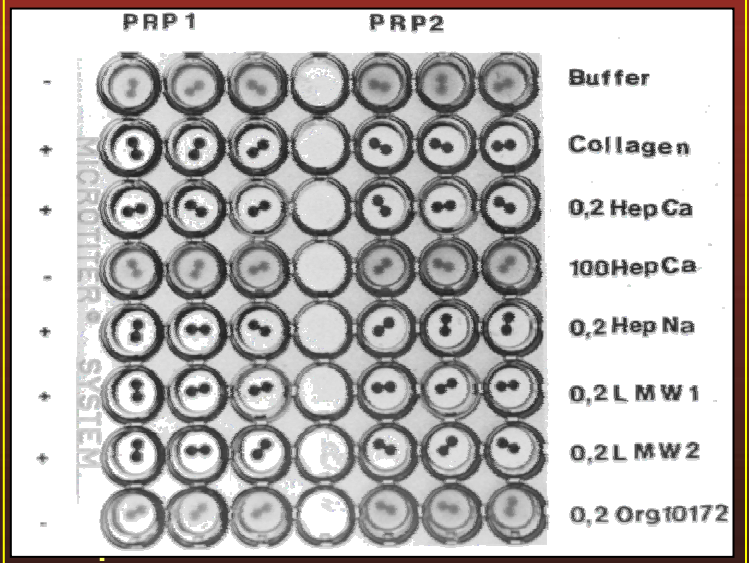
- Low sensitivity

- False positive reactions

Greinacher et al. Transfusion 34:381-385, 1994

HIPA test





+ 10 µl heparin

0.2 U f.c or 100 U f.c.

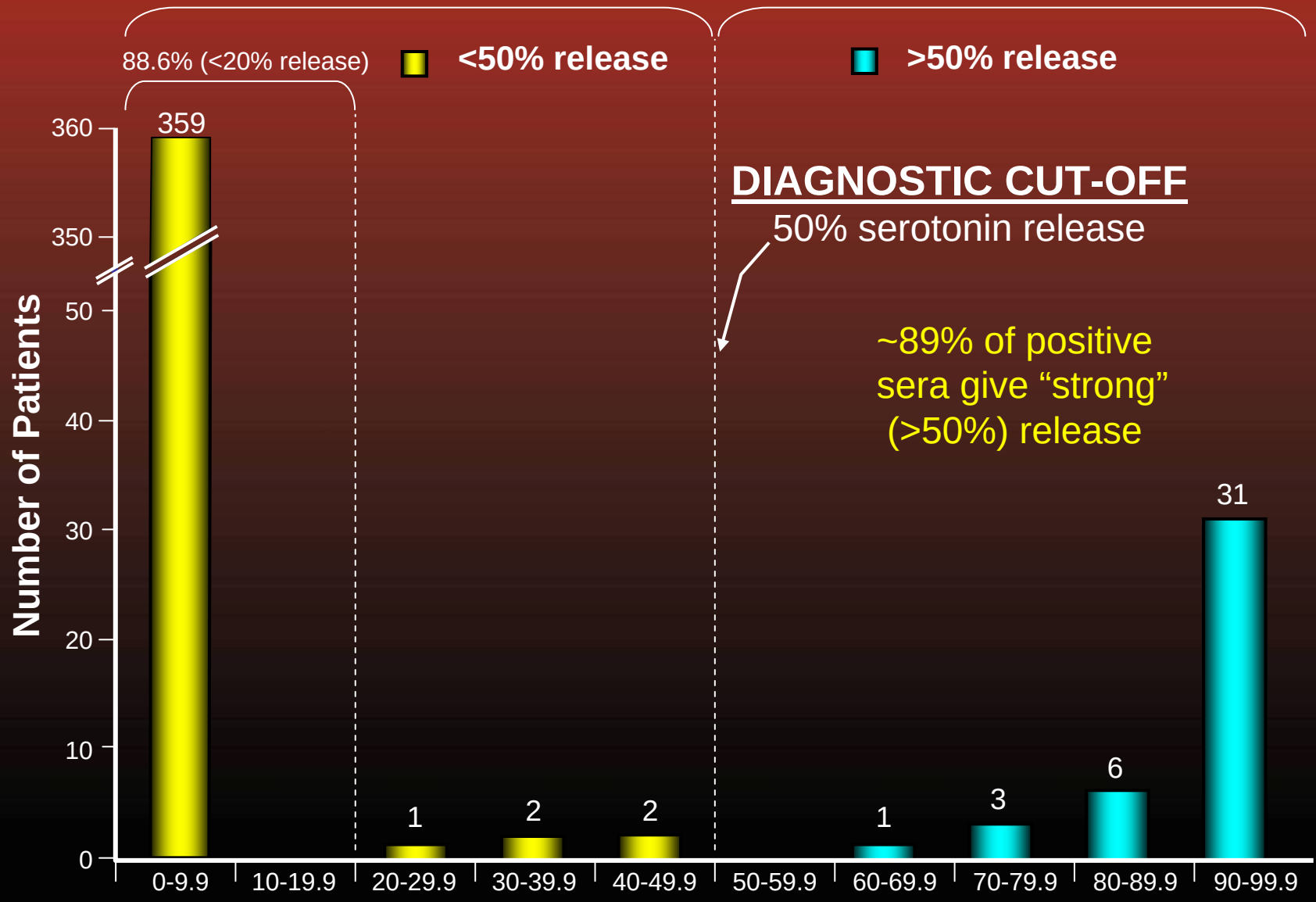
- **high heparin:** heparin dependency;
- **hirudin:** eliminates thrombin effects;
- **moab IV.3:** Fc-RIIa dependency

Greinacher A, et al. Thromb Haemost 1991

^{14}C SRA

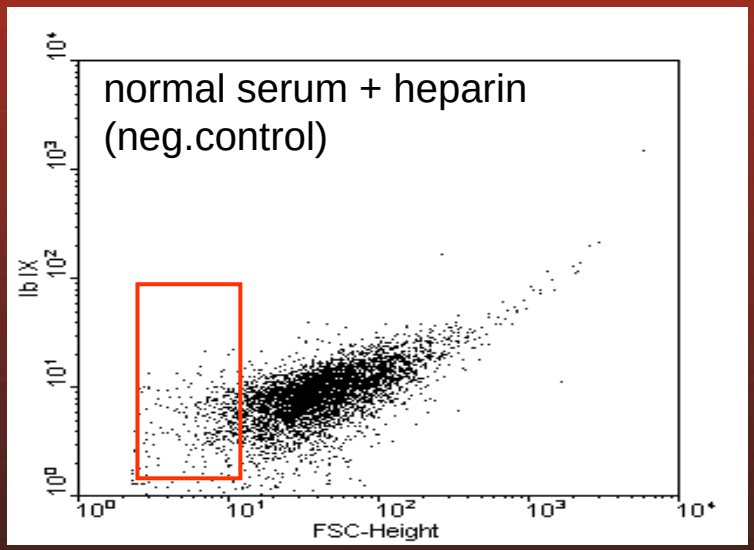
Sheridan D, et al. *Blood* 1986

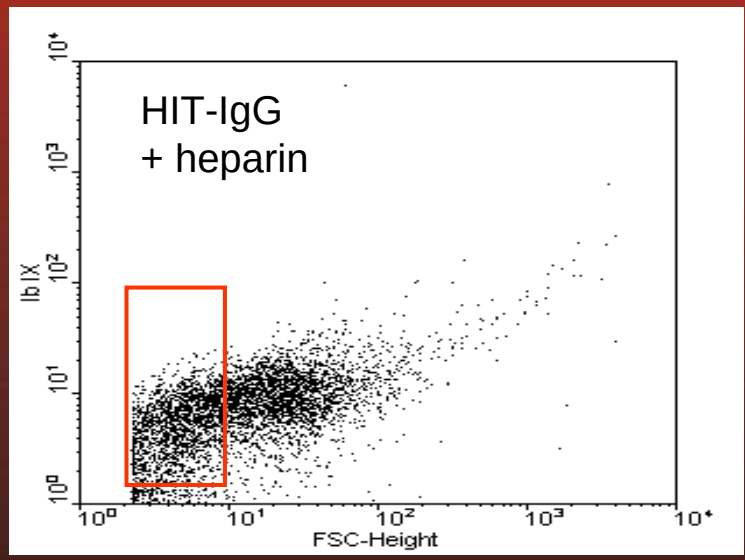
SRA is a “Dichotomizing” Assay



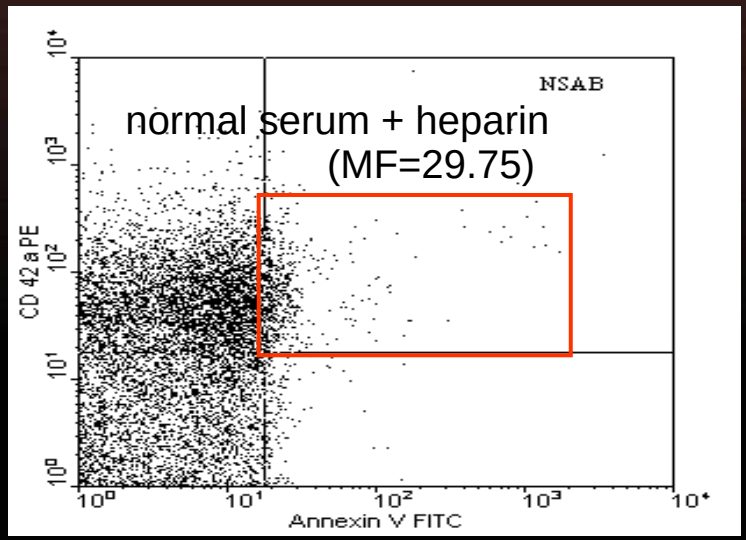
Warkentin et al. JTH 2008

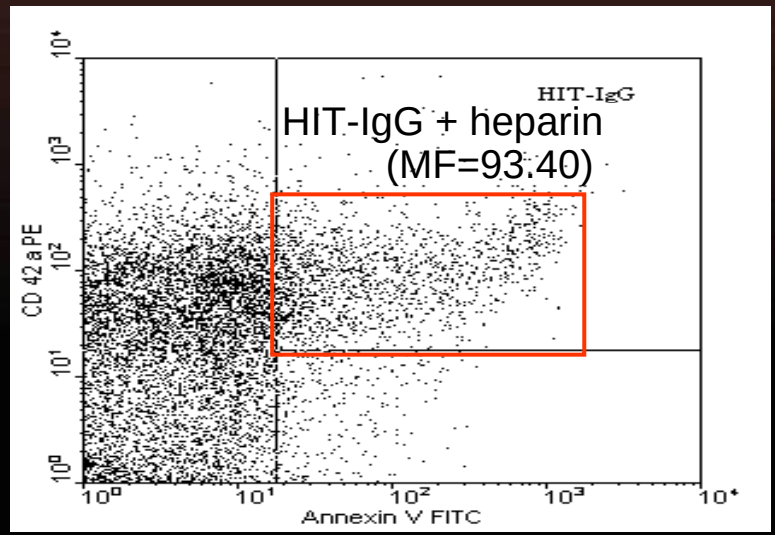
Generation of microparticles





Binding of Annexin V





Frequency of HIT in Patients Suspected to Have HIT

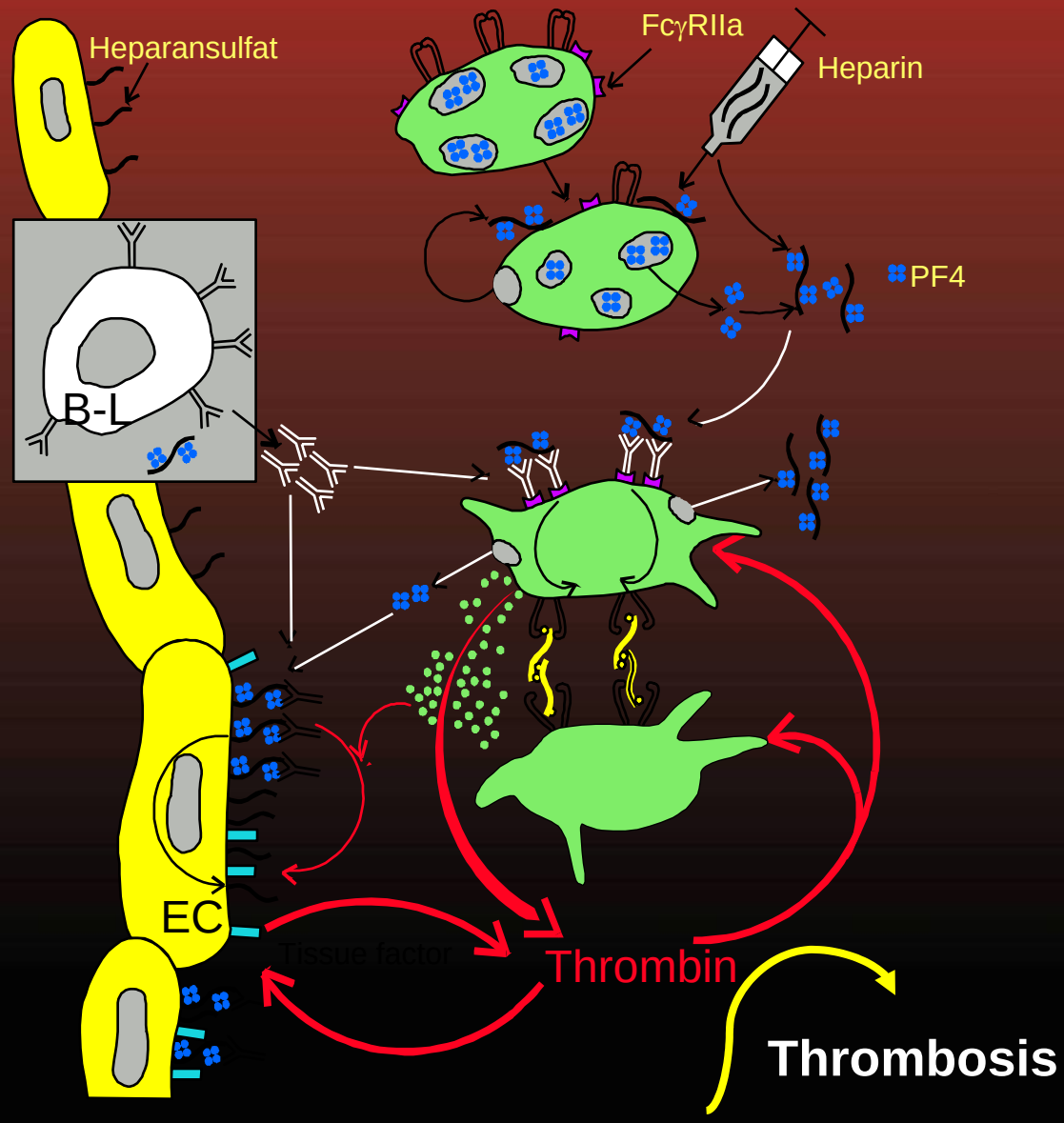
- Reference laboratory experience
- Consecutive patients referred with clinical suspicion of HIT: 2405
- Positive antigen test: 309 (12.8%)
- Positive functional assay: 151 (6.3%)

> 90% of patients did not have HIT

Greinacher et al. JTH 2007

Antigen test
PF4/heparin ELISA
Microcolumn
IgG/A/M

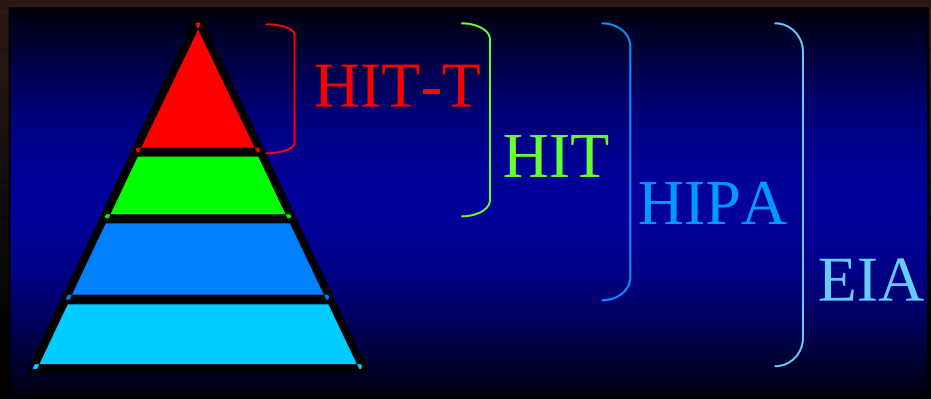
Functional test
HIPA-test
^{14}C-SRA
IgG/ other antigens



Frequency of “HIT”: Platelet Fall >30% and/or Thrombosis (Pos HIPA test)

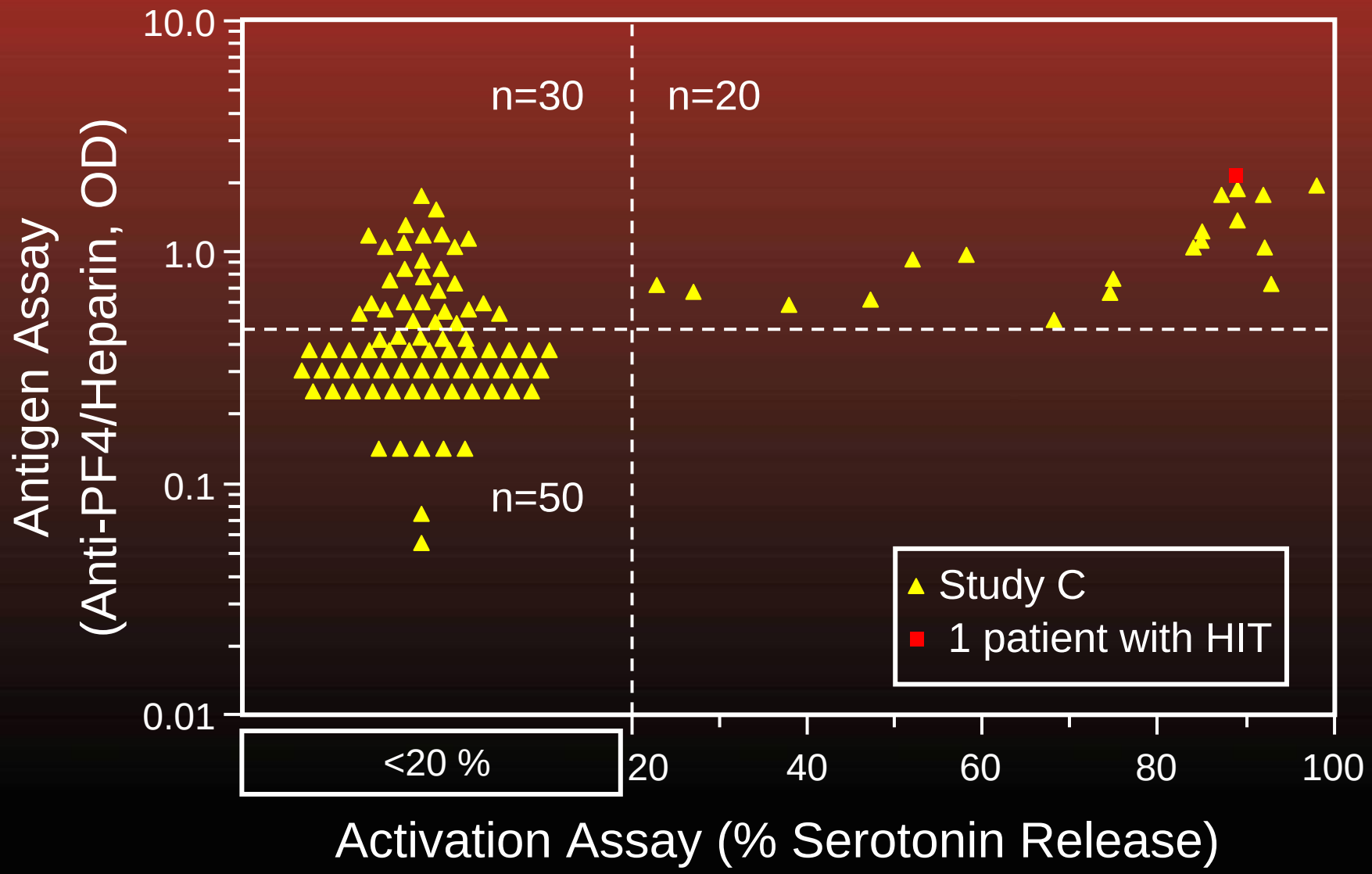
	UFH	LMWH*	
HIT-T	6/231 (**2.6%**)	0/271 (**0.0%**)	*P*<0.0092
HIT	12/231 (**5.2%**)	0/271 (**0.0%**)	*P*=0.00008
HIPA	25/202 (**12.4%**)	13/238 (**5.5%**)	*P*=0.0101
EIA	46/196 (**23.5%**)	19/228 (**8.3%**)	*P*=0.00002

* Enoxaparin



Greinacher et al. *Blood* 2005;106:2921-2

Cardiac Surgery Unfractionated Heparin

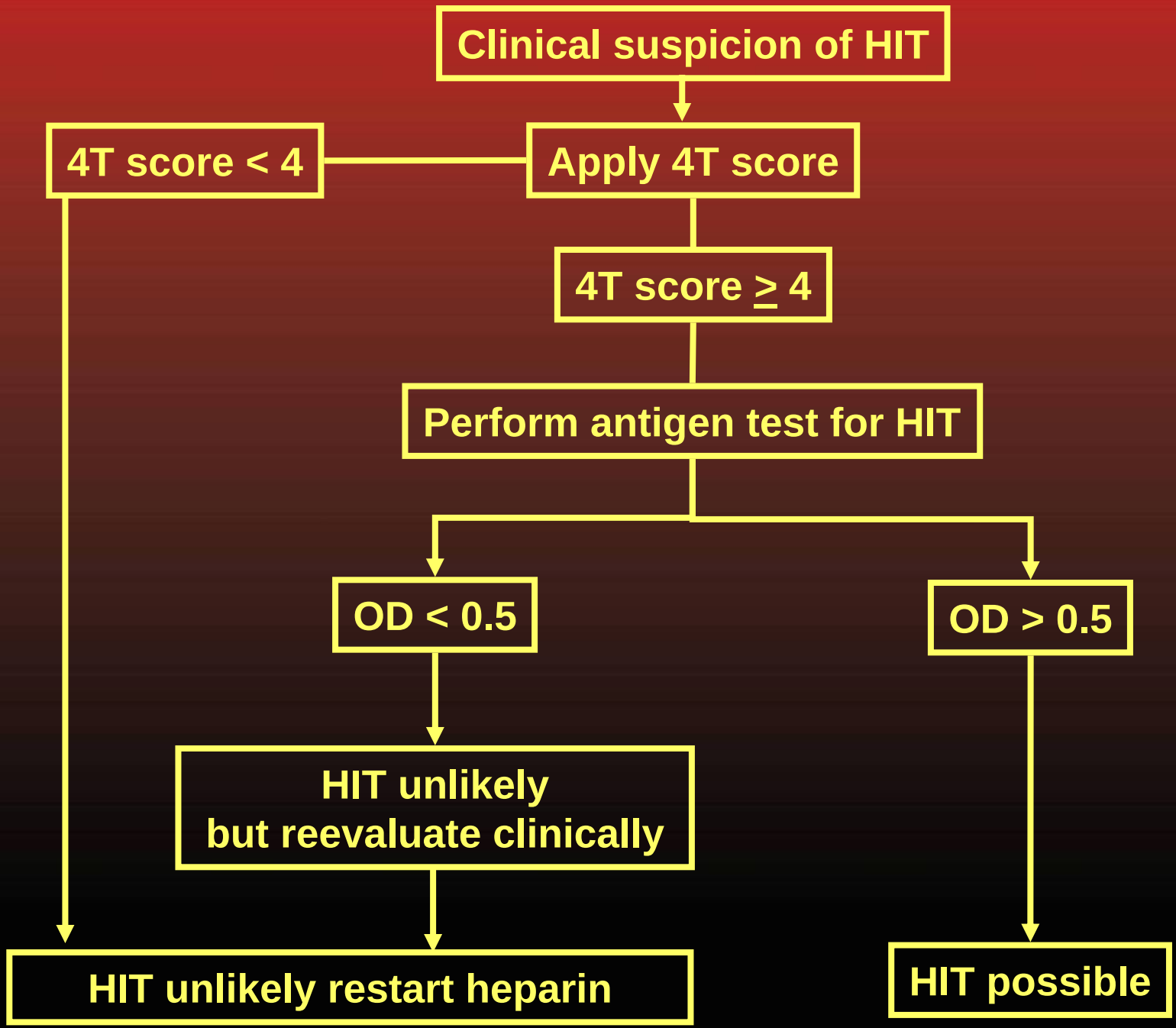


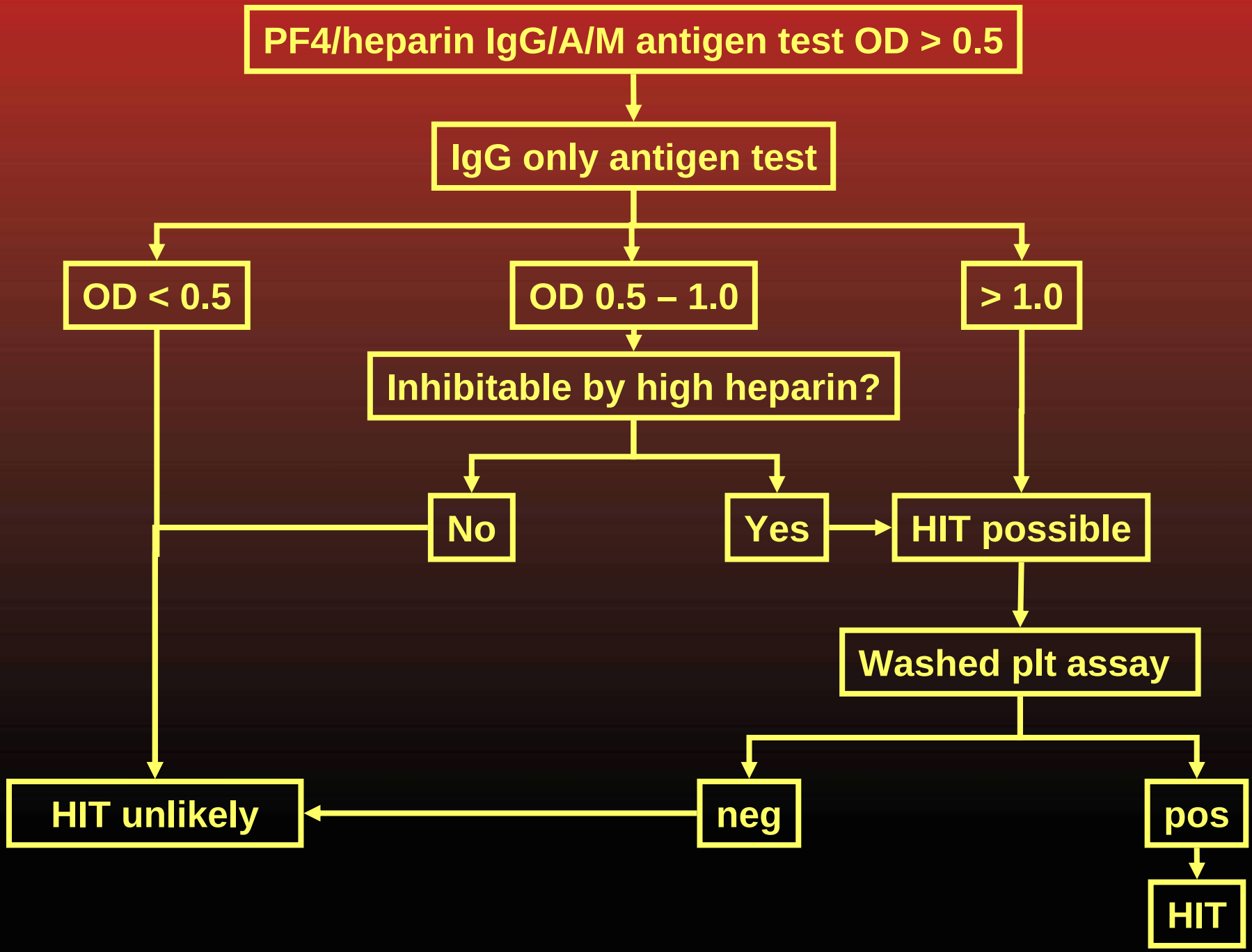
Warkentin et al. *Blood* 2000;96:1703 and Warkentin & Heddle *Curr Hematol Rep* 2003; 2: 148

A Practical Approach

- Do not screen for PF4/heparin antibodies
- Include the 4T score in the clinical assessment

- HIT antibody test:
  - for exclusion of HIT use antigen test with high negative predictive value
  - for confirmation of HIT use functional test with high positive predictive value


Clinical suspicion of HIT
Apply 4T score
4T score < 4
4T score ≥ 4
Perform antigen test for HIT
OD < 0.5
OD > 0.5
HIT unlikely
but reevaluate clinically
HIT unlikely restart heparin
HIT possible


PF4/heparin IgG/A/M antigen test OD > 0.5
IgG only antigen test
OD < 0.5
OD 0.5 – 1.0
> 1.0
Inhibitable by high heparin?
No
Yes
HIT possible
Washed plt assay
HIT unlikely
neg
pos
HIT

The Greifswald Approach

- PF4/heparin EIA IgG + high heparin control

  (IgA and IgM only to explain a positve result in an external laboratory)
- HIPA test with platelets of 4 donors
- Same day results (Mo-Fr)

- Charging: 100 €

P. Eichler
N. Lubenow
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C. Blumentritt
U. Strobel
B. Fürll
T. Lietz
S. Schenk

T.E. Warkentin