



HEPARIN INDUCED THROMBOCYTOPENIA



New assays for HIT Diagnosis based on the understanding of Heparin antigens function

Anne Marie VISSAC, AMIRAL Jean

HYPHEN BioMed

AH51
4-2007

Aniara

6560 Gove Court
Mason, OH 45040

Phone: 513-770-1991

Toll Free: 866-783-3797

FAX: 513-573-9241

Web: www.aniara.com

Email: info@aniara.com



INTRODUCTION

- Type II Heparin Induced Thrombocytopenia (HIT), remains the major iatrogenic complication of heparin therapy.
- It is triggered by « Heparin dependent » antibodies targeting heparin-protein (mainly Platelet Factor 4/PF4) complexes.
- It develops more frequently during Unfractionated Heparin (UFH) therapy than during Low Molecular Weight Heparin (LMWH) (ten-fold lower risk).

Aniara

6560 Gove Court
Mason, OH 45040

Phone: 513-770-1991 **Toll Free:** 866-783-3797
Web: www.aniara.com

FAX: 513-573-9241
Email: info@aniara.com



CLINICAL INCIDENCE OF HIT

- 0.5 to 2 % (or more?) with UFH therapy.
- 0.1 to 0.2 % with LMWH therapy.
- Expected at 0 % with pentasaccharide.
- Very high incidence of the clinical context (platelet or endothelial activation, inflammation, malignancy) and the therapy duration.
- Low incidence during ECC, despite presence of antibodies (including IgG); protective effect of heparin in excess???



SEROLOGICAL ASSAYS

Two types of serological assays are available for laboratory detection of HIT antibodies:

- Functional (platelet activation) assays such as the platelet 14C-Serotonin-release assay (SRA) or Platelet aggregation or the Heparin-Induced Platelet Activation (HIPA) test.
- Enzyme-immunoassays employing PF4/heparin or PF4/polyvinylsulfonate complexes. These assays can detect antibodies only when PF4 is the heparin dependent protein. We have now developed a new enzyme-immunoassay using a functionally available heparin coated onto a solid surface.

Aniara

6560 Gove Court
Mason, OH 45040

Phone: 513-770-1991

Toll Free: 866-783-3797

FAX: 513-573-9241

Web: www.aniara.com

Email: info@aniara.com



ASSAY PRINCIPLE

Functionally available heparin is coated onto a solid surface. With this approach:

- If chemokines, exhibiting heparin affinity, are present, they bind to heparin, expose neo epitopes, and capture heparin dependent antibodies.
- In addition, if heparin complexes, are present, they can also directly bind to heparin, through the heparin binding protein.

Aniara

6560 Gove Court
Mason, OH 45040

Phone: 513-770-1991

Toll Free: 866-783-3797

FAX: 513-573-9241

Web: www.aniara.com

Email: info@aniara.com



« Functionally available » Heparin

This can be achieved by different means :

- Coating protamine sulfate in the presence of large excess of heparin.
- Coating streptavidin and biotinylated heparin.
- Other: coating heparin covalently bound to a carrier protein (such as albumin) or a polymer.

Aniara

6560 Gove Court
Mason, OH 45040

Phone: 513-770-1991

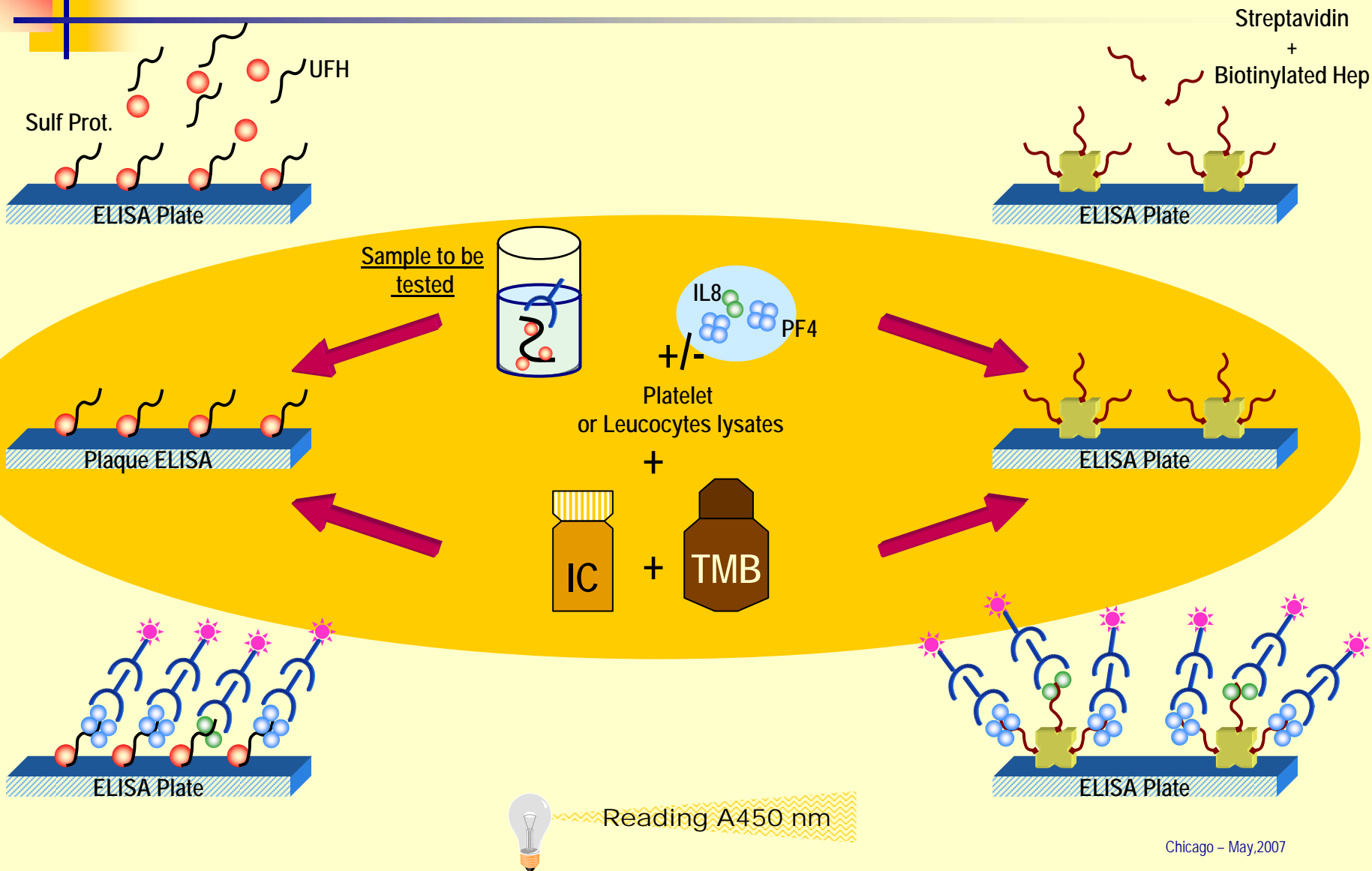
Toll Free: 866-783-3797

FAX: 513-573-9241

Web: www.aniara.com

Email: info@aniara.com

ASSAY PRINCIPLE FOR THE NEW METHOD





Assay Protocol

- The plate is coated with heparin functionally available.
- The patient plasma or serum diluted at 1:100 (or more) is incubated in presence or in absence of platelet lysates.
- The second antibody used for the revelation can be an:
 - Anti IgG,A,M (Screening)
 - Anti-IgG (only for the IgG isotype)
 - Anti-IgM (only for the IgM isotype)
 - Anti-IgA (only for the IgA isotype)
- Substrate: TMB/H₂O₂ , the coloration (5 min.) is stopped with sulfuric Acid.
- The absorbance is measured at 450 nm.



Specificity

- Normal Plasma (N= 90)

- DO : $< 0,10$

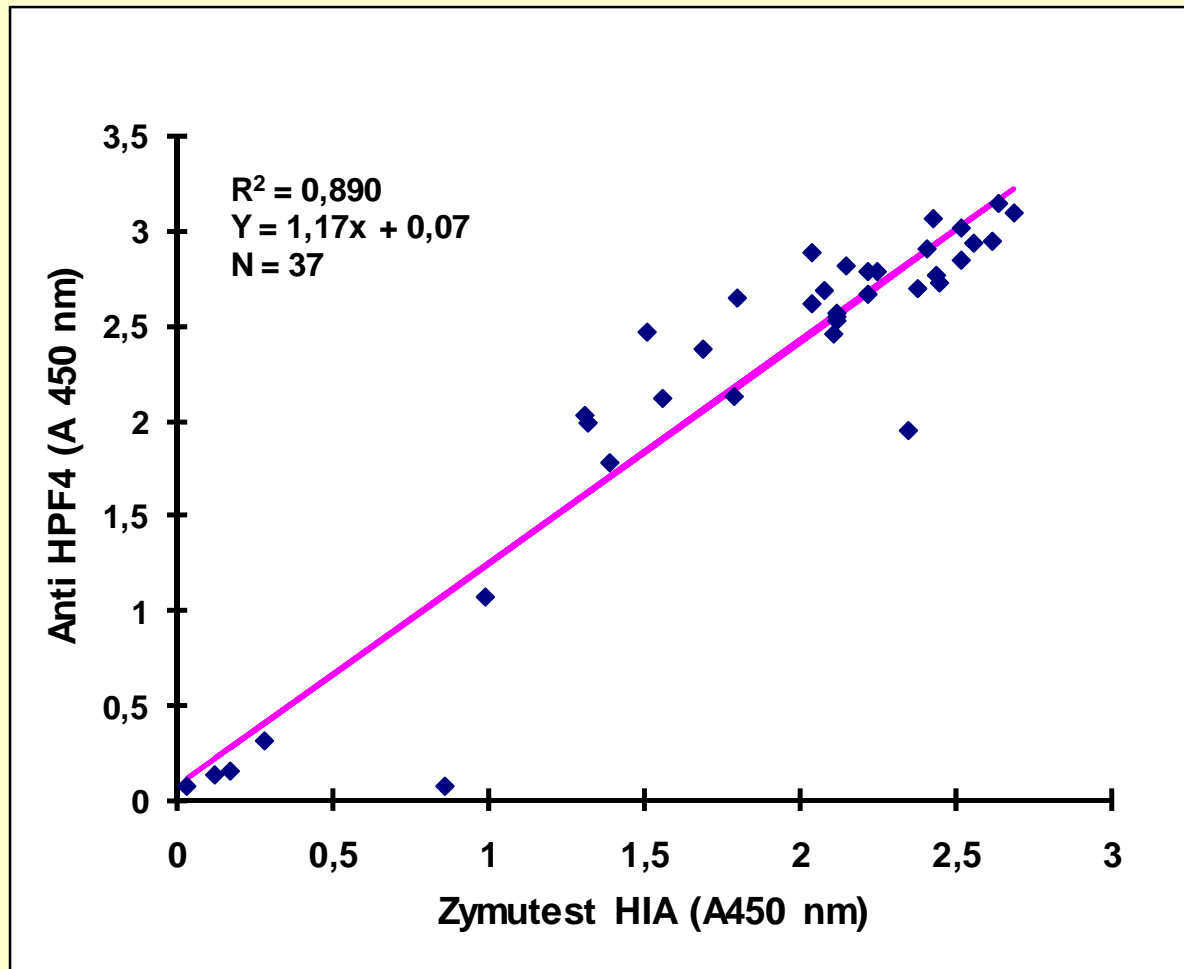
- SD : 0.04

- Pathological plasma (N= 37): HIT or suspicion.

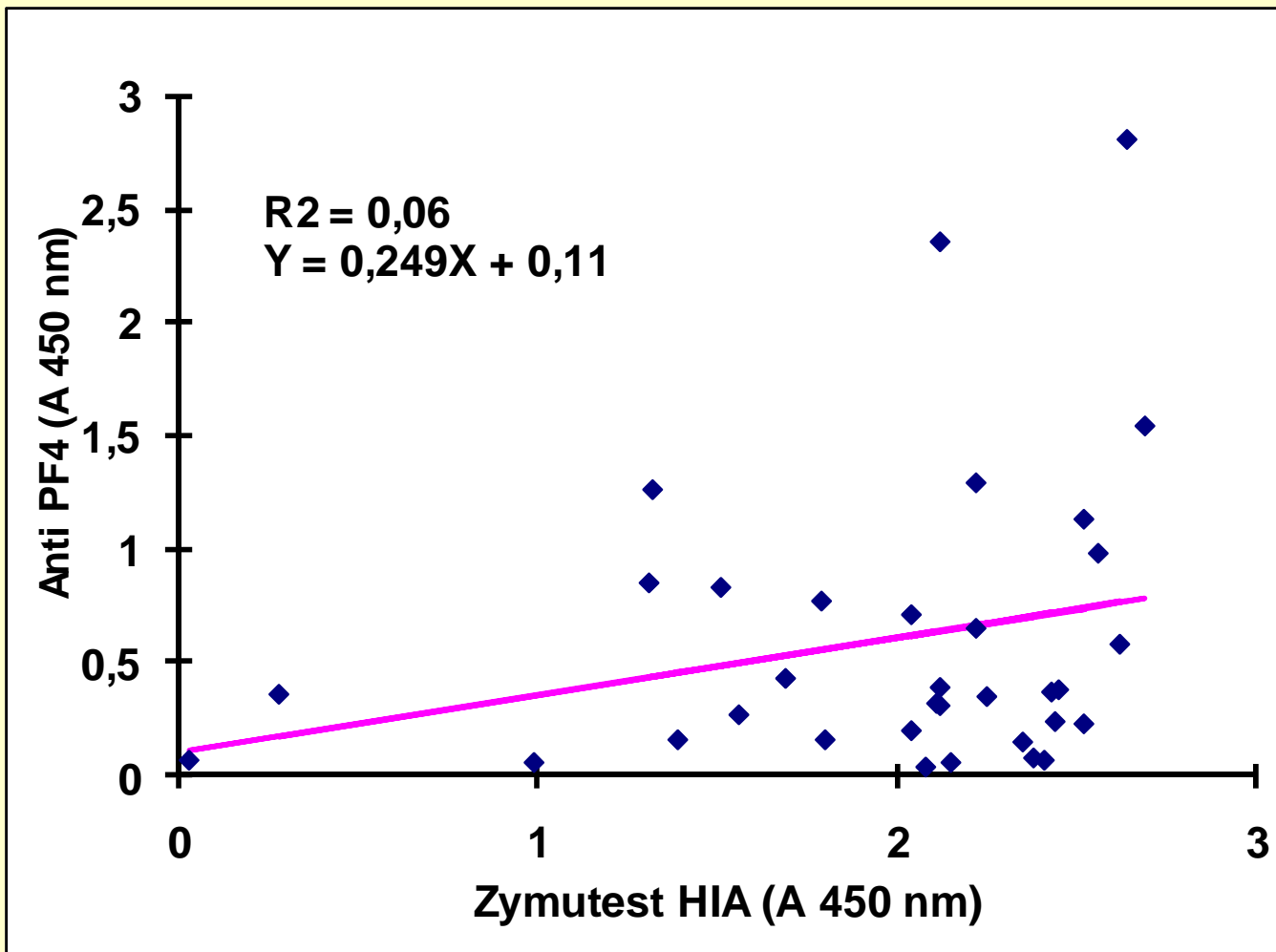
- A450 ≥ 1

- Extremes: 1.02 to > 3.00

Correlation with antibodies anti-HPF4 (anti-IgG)

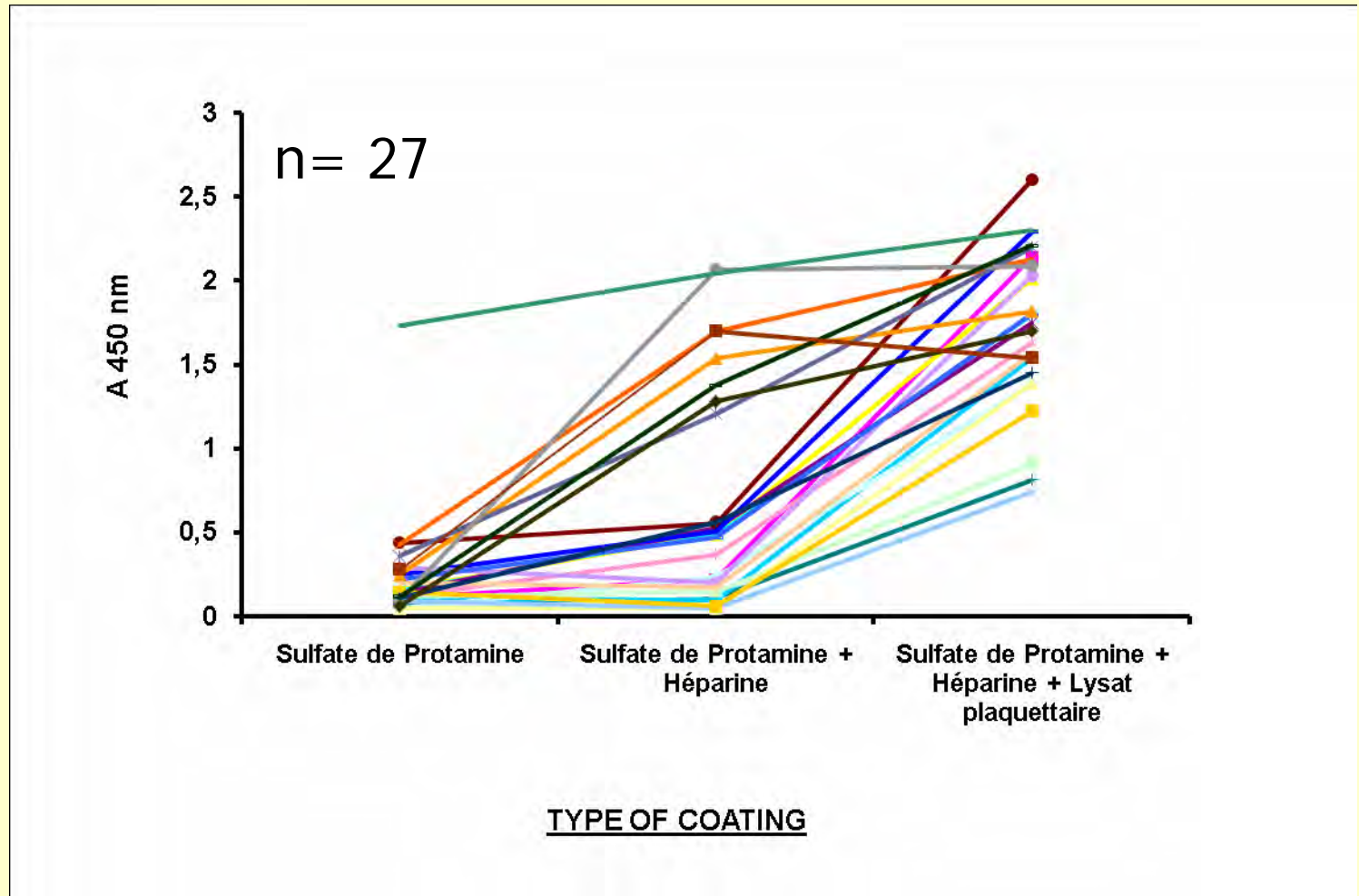


Correlation with antibodies anti-PF4 (IgG)



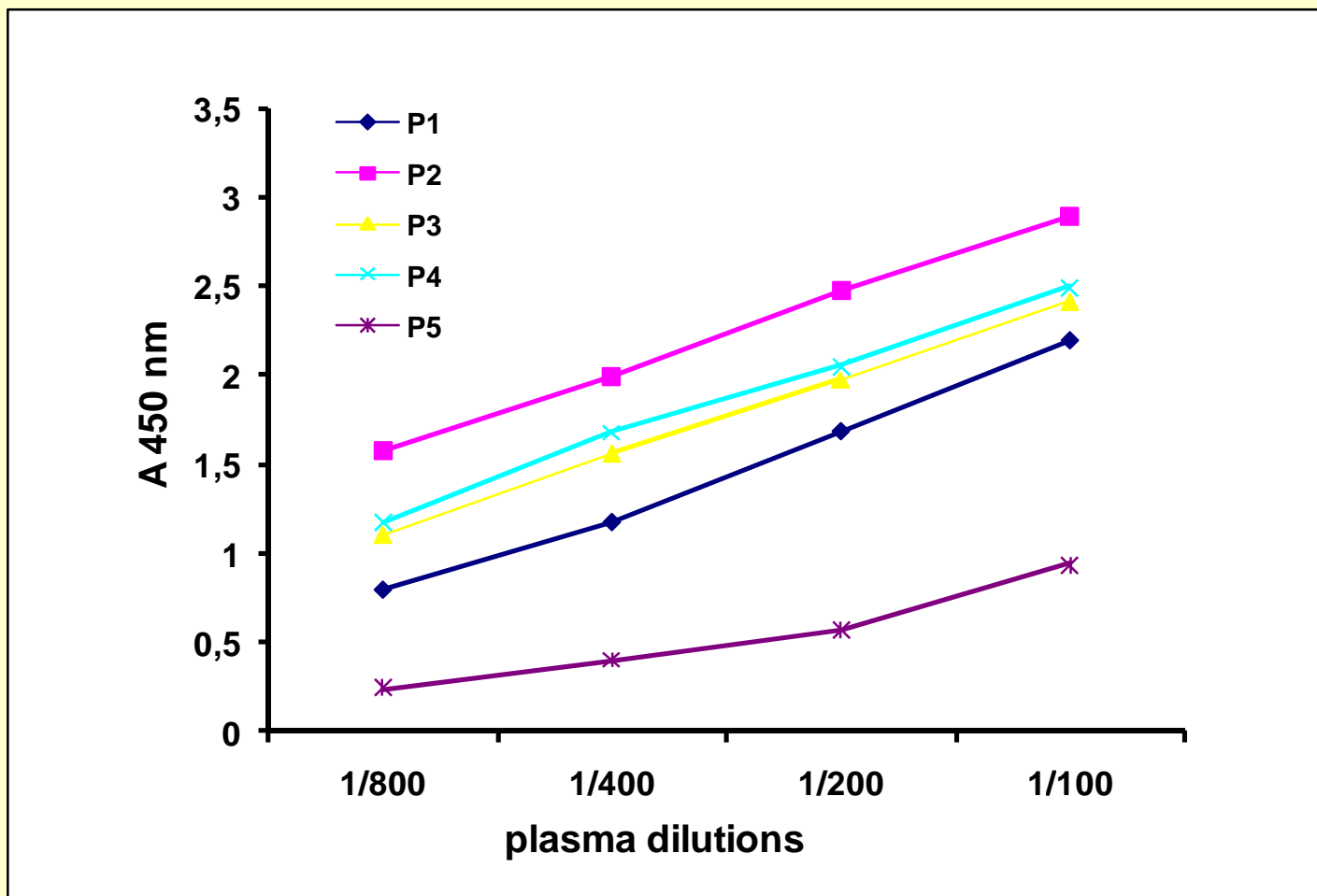
Chicago - May, 2007

Effect of addition of platelet lysates



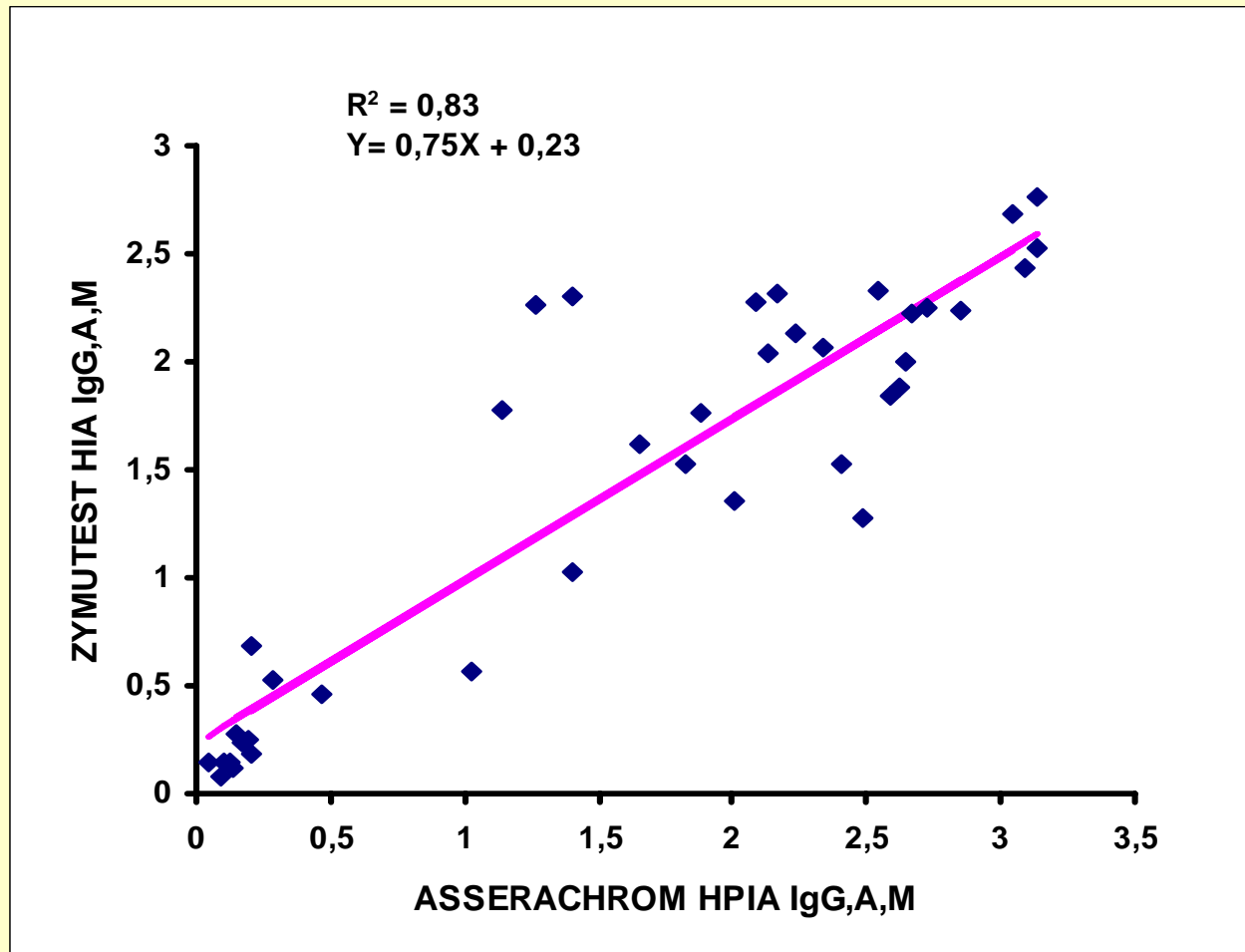
Chicago - May, 2007

Curve dose response obtained for 5 pathological Plasmas



Chicago - May, 2007

Correlation with Asserachrom HPIA



Chicago - May, 2007

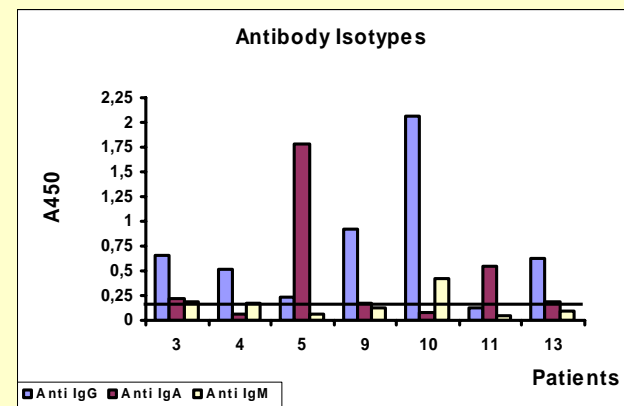
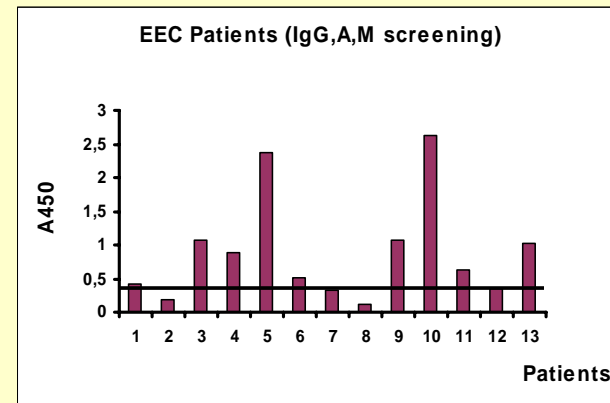


HEPARINE DEPENDENT ANTIBODY ISOTYPES IN PATIENTS WITH HIT

- 50 patients with HIT (Thrombocytopenia induced by heparin, positive PAT, +/- thrombosis).
 - 31 only IgG
 - 12 IgG and IgA
 - 2 IgG and IgM
 - 4 IgG, IgA and IgM
 - 1 IgM only

CLINICAL INCIDENCE OF HIT DURING ECC

- 13 patients under ECC have been tested. 54% were positive in IgG,A,M screening.
- 5 / 7 have IgG isotypes. Despite the presence of IgG, there is a low clinical incidence of HIT. (Possible protective role of large excess of heparin in plasma) .





Conclusions

- The new assay for heparin dependent antibodies, responsible for HIT, is easy to carry out as well as economical and soon, it will be automated.
- Good correlation with the functional assay (platelet aggregation) and the current test measuring anti H-PF4 antibodies.
- Potentially sensitive to different antigen targets of the antibodies (studies in progress).
- Possibility of measuring the circulating complexes "Heparin-protein-antibodies".