NEW APPROACH FOR DETECTION OF HEPARIN DEPENDENT ANTIBODIES AND RISK ASSESSMENT FOR HEPARIN INDUCED THROMBOCYTOPENIA

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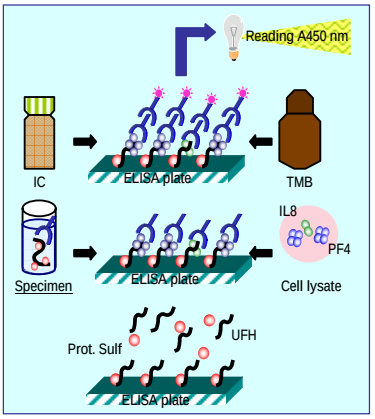
Introduction

- This assay uses the potential of immobilized and biologically active heparin to focus and catch antibody-protein (mainly PF4)-heparin complexes. It then mimics the conditions occurring in vivo when heparin dependent antibodies are generated and can induce Heparin Induced Thrombocytopenia (HIT).
- A new assay for measuring heparin dependent antibodies, involved in the development of HIT was developed.
- Various presentations are proposed for the measurement of total antibodies (IgGAM), or for specifically measuring IgG isotypes, or for the total isotyping of IgG, IgA and IgM isotypes.

Assay principle

- Heparin, immobilized onto a solid reactive surface (plate or other), but «functionally available»:
  - Capts chemokines present into the patient plasma/serum (or supplied exogenously as a platelet lysate), and then forms the reactive auto-antigen, which binds heparin dependent antibodies.
  - Can also bind «heparin-protein-antibody» complexes present in blood circulation.
- «Functionally available» heparin uses one of the following coating procedures:
  - Protamine sulfate complexed with a large excess of heparin.
  - Streptavidin complexed with biotinylated heparin.
  - Heparin chemically coupled to a high molecular weight molecule (natural or synthetic) or polymer.



Assay Protocol

- Plate coated with «functionally available» heparin.
- 1:100 (or more) diluted plasma or serum ± lysate.
- Second antibody (peroxidase labeled):
  - Anti-IgGAM (Screening) ➔ HIT risk assessment
  - Anti-IgG (IgG isotype only) ➔ Confirmation of HIT
  - Anti-IgG, Anti-IgA and anti-IgM (total isotyping) ➔Research studies
- TMB/H2O2 substrate and reaction stopped with Sulfuric Acid.
- Measurement of absorbance at 450 nm (A450).

Conclusions

- New highly sensitive and specific assay for the diagnosis of heparin dependent antibodies involved in HIT, easy to perform and cost effective, offering automation possibilities.
- Good correlation with platelet aggregation tests and measurement of anti-H-PF4 antibodies.
- Potentially sensitive to the various antigenic targets for heparin dependent antibodies (studies in progress).
- Possible measurement of circulating complexes «heparin-protein-antibody» and assay mimicking the heparin dependent antibody binding mechanisms occurring in vivo.
- Very “flexible” assay principle for all laboratory immunological studies on heparin dependent antibodies, which can cause HIT.

Results

Patients: Citrated plasmas from:

– 60 normal individuals

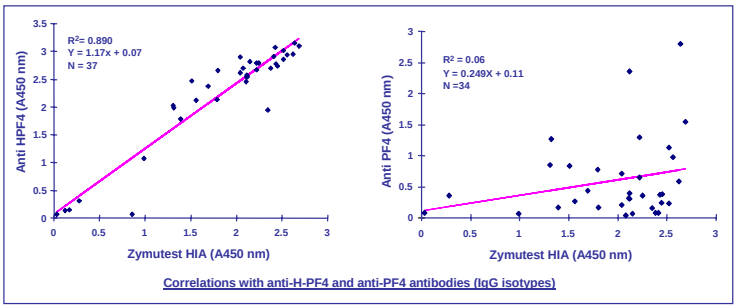
– 37 patients with a clinically diagnosed HIT (platelet course kinetics, positive platelet aggregation tests at low but not at high heparin concentration, recovery of platelet count following heparin withdrawal).

Table 1 :

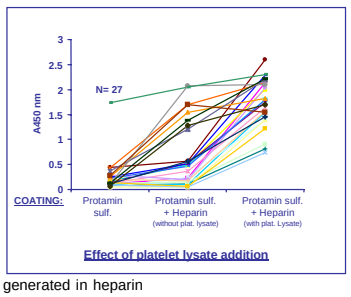
A450 in normals and patients with HIT

Specificity	A450	SD
NI Plasmas (N=60)	<0.10	0.03
HIT Plasmas (N=37)	≥ 1.00	Range: 1.02 to >3.00

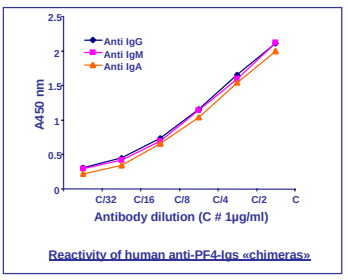
Assay performances



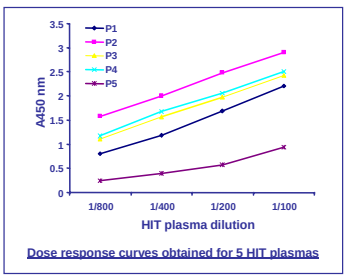
Correlations with anti-H-PF4 and anti-PF4 antibodies (IgG isotypes)



Effect of platelet lysate addition



Reactivity of human anti-PF4-Igs «chimeras»



Dose response curves obtained for 5 HIT plasmas

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Form AH66 7-2007
Abstract n° 1999
ISTH 2007 Geneva