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## ZYMUTEST HIA

### A NEW TEST FOR THE DIAGNOSIS OF HEPARIN DEPENDENT ANTIBODIES ASSOCIATED WITH TYPE II HEPARIN INDUCED THROMBOCYTOPENIA (HIT)

Measurement of antibodies to heparin-antigen complexes (mainly heparin-PF4 in most of the cases) allows diagnosing the risk of developing Heparin Induced Thrombocytopenia, or to confirm this iatrogenic complication of heparin therapy, when thrombocytopenia develops, during the course of treatment.

A new assay was developed in order to measure antibodies to heparin-antigen complexes by reproducing the mechanism matching the closest the conditions occurring in pathology. Different options are available for measuring the whole of heparin dependent antibodies, or only the IgG isotype, or to proceed to a full isotyping of IgG, IgM and IgA isotypes.

### ASSAY CHARACTERISTICS

- Easy to perform and cost effective.
- Highly sensitive and specific.
- Good correlation with platelet agregation tests and measurement of anti-H-PF4 antibodies.
- Potentially sensitive to various antigenic targets of antibodies (studies in progress).
- Possible measurement of circulating complexes "heparin-protein-antibody".

Form AH30  
4-2007

**Aniara**

6560 Gove Court  
Mason, OH 45040

**Phone:** 513-770-1991

**Web:** [www.aniara.com](http://www.aniara.com)

**Toll Free:** 866-783-3797

**FAX:** 513-573-9241

**Email:** [info@aniara.com](mailto:info@aniara.com)

Nov 2006/REF 027

# Diagnosis of heparin dependent antibodies associated with type II Heparin Induced thrombocytopenia (HIT)

## (ZYMUTEST HIA)

### ASSAY PRINCIPLE

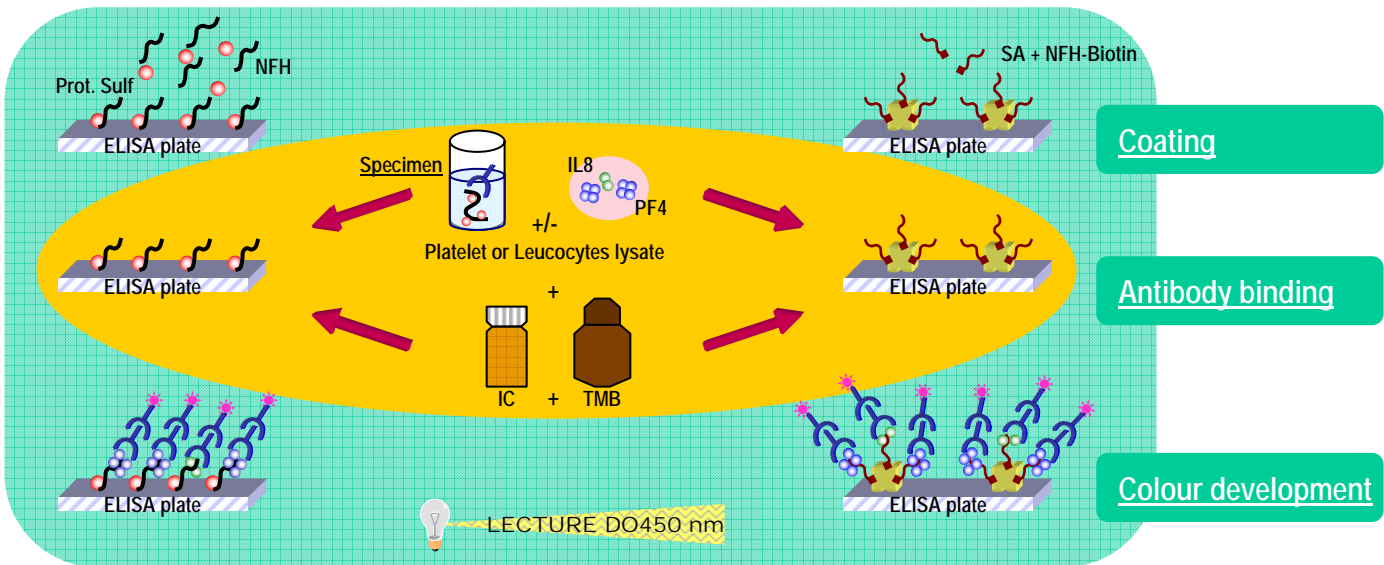
- Heparin is immobilised onto a solid reactive surface (plate or other) by either:
  - Protamin sulfate complexed with an excess of heparin or streptavidin complexed with biotinylated heparin.
  - Alternatively, heparin can be chemically coupled to a high molecular weight molecule (natural or synthetic) or polymer, then coated.
- Immobilised heparin remains "functionally available" for reacting with proteins.
- "Functionally available" heparin:
  - Captures chemokines present into the plasma/serum of the patient (or supplied exogenously) and then forms the reactive auto-antigen, which binds the heparin dependent antibodies.
  - Can fix "heparin-protein-antibody" immuno-complexes generated in blood circulation.
- When required, enhancement of assay sensitivity and specificity by addition of platelet (or a platelet leucocyte pellet) lysate.

### ASSAY PROTOCOL

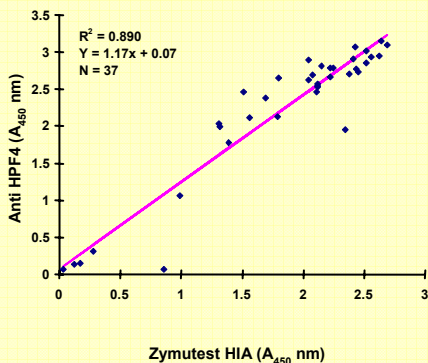
- Plate coated with "functionally available" heparin.
- 1:100 (or more) diluted plasma or serum ± lysate.
- Second antibody (peroxidase labelled):
  - Anti-IgGAM (screening)
  - Anti-IgG (IgG isotype only)
  - Anti-IgG, Anti-IgA and Anti-IgM (total isotyping)
- TMB/H<sub>2</sub>O<sub>2</sub> substrate and reaction stopped with sulfuric acid.
- OD measurement at 450 nm.

### SPECIFICITY

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

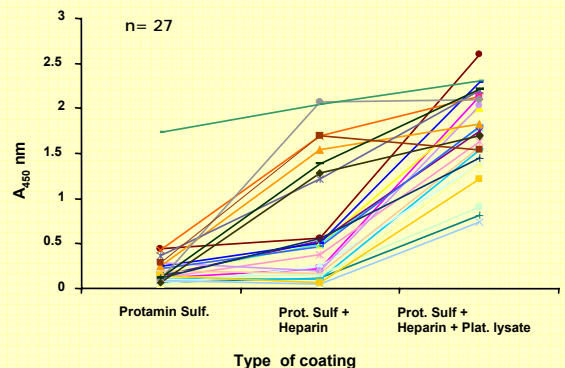


### Correlation with anti-HPF4 antibodies (anti- IgG)



Excellent correlation with antibodies to HPF4 in most of the cases

### Effect of platelet lysate addition



Two groups of patients are identified: those enhanced by platelet lysate and those not.