

## MEASUREMENT OF HIRUDIN AND SYNTHETIC THROMBIN INHIBITORS IN PLASMA WITH A NEW CLOTTING METHOD, WITHOUT INTERFERENCE OF CLOTTING FACTOR LEVELS.

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### INTRODUCTION

Hirudin is now available as an emergency antithrombotic drug used for immediate treatment of life threatening thrombotic risk. However, its use is risky as its posology must be totally appropriate for the application concerned, as no antidote is available. Hyper-dosage may induce bleeding (especially in brain), whilst hypo-dosage is not enough efficient for fighting thrombosis. Furthermore, hirudin concentrations are usually assayed in patients with highly imbalanced haemostasis. Dosage must not be influenced by plasma levels of coagulation factors. We developed a new, physiological, clotting assay, based on the inhibition of highly purified human  $\alpha$ -thrombin by tested plasma (where hirudin is measured) diluted in a normal plasma pool. This assay can be performed within minutes and is very useful for adjusting hirudin posology. It can be used also for any anti-Thrombin specific drug and can be automated on laboratory instruments.

### MATERIALS

- Normal citrated pooled plasma, lyophilised, used as substrated for diluting assayed plasmas from Hirudin treated patients (R1).
- Highly purified and stabilised human Thrombin (with calcium), in the  $\alpha$  form (R2).
- Sodium chloride, used as diluent for tested plasmas.
- Hirudin calibrators: plasmas supplemented with a well defined hirudin concentration.

### PERFORMANCE CHARACTERISTICS

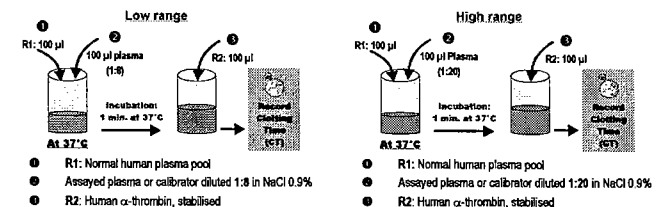
- Dynamic range: **Low:** 0 to 2  $\mu\text{g/ml}$   
**High:** 0 to 5  $\mu\text{g/ml}$
- No interference of Fibrinogen or Prothrombin concentrations
- Intra-assay CV: **0.2 to 2%**.
- Recovery of Hirudin spiked in plasma: **94 to 103%**.
- High stability of restored reagents.
- Use of highly purified  $\alpha$ -Thrombin, which makes the assay completely specific for Thrombin inhibitors.
- Sensitive to all the anti-Thrombin specific drugs (Hirudin, Melagatran, etc...).
- Good correlation with Ecarin Clotting Time, when Fibrinogen and Prothrombin concentrations are normal.

### CONCLUSIONS

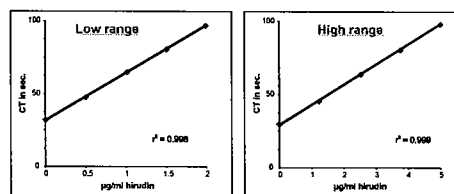
- ▶ This simple, reliable and reproducible assay is totally specific for measuring hirudin in plasma.
- ▶ It also allows measuring any new anti-Thrombin specific drug in plasma or in any biological fluid.
- ▶ There is no interference of clotting factor deficiencies especially those of Fibrinogen or Prothrombin, which are frequently present in patients treated with Hirudin.
- ▶ The assay can be performed within minutes and requires only a basic clotting equipment.
- ▶ It is automatisable on laboratory instruments.

### METHODS

Two assay ranges are proposed for low (0 to 2  $\mu\text{g/ml}$ ) and high (0 to 5  $\mu\text{g/ml}$ ) Hirudin concentrations.



### CALIBRATION CURVES



### ASSAY OF MELAGATRAN

