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NEW ASSAYS FOR MEASURING DIRECT THROMBIN INHIBITORS IN PLASMA

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INTRODUCTION

- Direct Thrombin Inhibitors (DTIs) have increasing and promising curative, preventive or prophylactic applications in severe clinical situations at high risk context, and are candidates for substituting to long term oral anticoagulant therapies with vitamin K antagonists.
- Laboratory methods are required for adjustment of drug efficacy and for avoiding overdosage. They must present the most limited impact to other plasma factors (eg. Antithrombin, Prothrombin, Fibrinogen).
- Ecarin Clotting Time (ECT) and aPTT are useful but too sensitive, insufficiently reliable at high DTI therapeutic levels, and patient coagulation factors may interference.
- Specialized calibrated clotting and chromogenic assays, fully automatable, with no matrix effect, accurate and sensitive at low and high concentration ranges, were developed for quantitating various DTIs.

METHODS

- Clotting assay ("Hemoclot Thrombin Inhibitors"):

Sensitized thrombin time, using a "substrate" normal plasma pool (R1) mixed with the diluted test plasma (1:8 to 1:20). Clotting time (CT) is recorded after addition of (h)-α-thrombin (R2) containing calcium.

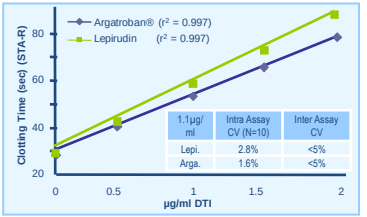
- Chromogenic kinetics assay ("Biophen DTI"):

Tested specimen (1:10 to 1:30) is incubated with thrombin substrate (R1), and (h)-α-thrombin (R2) is added. Measured A405 is inversely proportional to DTI concentration.

- Aim:
  - To evaluate dose response curves to various DTIs in plasma;
  - To establish accuracy, reproducibility;
  - To compare with a conventional aPTT assay.
- Direct Thrombin Inhibitors tested:
  - Lepirudin (Refludan®)
  - Argatroban®

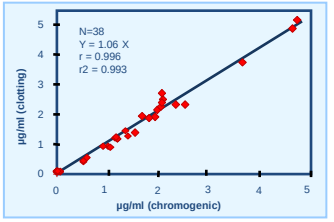
RESULTS

Clotting assay: dose response curves with 2 DTIs



➢Excellent linearity in the usual therapeutic range for any DTI (Lepirudin, Argatroban® …).

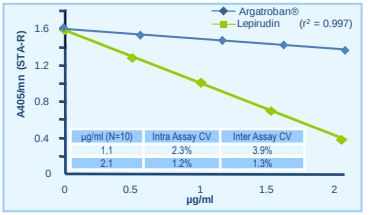
Linear regression analysis for measured Lepirudin with both methods (various levels added to normal plasmas)



➢Excellent correlation and possible extended dynamic range up to 5µg/ml (useful eg . in ECC).

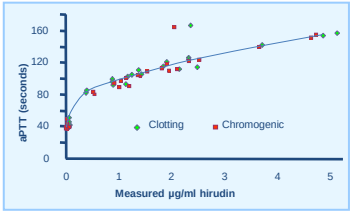
NB: For Argatroban®, the clotting assay offers a much higher sensitivity than the chromogenic assay, which is then not appropriate within the normal therapeutic range.

Chromogenic assay: dose response curve with 2 DTIs



➢Excellent performance with Hirudin and analogues, but not suitable for the Argatroban® usual therapeutic range (with low inhibitory potency of thrombin chromogenic activity).

Measured aPTT on Lepirudin spiked plasma samples (clotting or chromogenic assays), normals or Argatroban® treated patients (clotting only).



Mean [Range]	µg/ml Argatroban (clotting assay)	ratio M/T (aPTT reagent 1)	ratio M/T (aPTT reagent 2)
Normals (N=20)	0 [<0.01]	1.0 [0.9-1.2]	1.0 [0.9-1.1]
Treated patients (N=33)	1. 0 [0-3.2]	2.5 [1.0-4.9]	3.2 [1.2-6.1]

➢aPTT (>1 µg/ml) lacks of linearity and reliability (clotting times too prolonged).

CONCLUSIONS

- Clotting and chromogenic assays **simple and rapid, standardized, calibrated** with the DTIs used. Specific calibrators and controls available for Hirudin and Argatroban®.
- Clotting method (1:8 dilution): excellent linearity, sensitivity, and accuracy over the usual therapeutic range, from **0.1 to 2.0 µg/ml** (possibly 0.25 to 5.0 µg/ml) for **Lepirudin** and **Argatroban®**, and we predict that it could also be used with **Bivalirudin®** or new oral DTIs such as **dabigatran etexilate**.
- Reflects the patient "true anti-IIa potential".
- Well correlated methods (r^2>0.99), consistent with aPTT results.
- **Safe, highly stable, and reliable** tools, **easily performed with basic equipment or major coagulation analyzers**, for measuring DTIs' activity in plasma with **no matrix effect** or in purified milieu. Especially useful for monitoring DTIs in emergency or in curative applications, and in analytical and pre-clinical studies for emerging DTIs, for which methods are requested by both users and authorities.

GENERAL REFERENCES

1- Greinacher A; Warkentin T, « The direct thrombin inhibitor hirudin »; *Thromb Haemost* 2008; 99:819-829.

2- « Landmarks in Anti-Thrombin drug development: the Argatroban study »; *Seminars in Thrombosis and Hemostasis*; Vol 34, Suppl 1, Oct 2008.

3. Stangier J, "Clinical Pharmacokinetics and Pharmacodynamics of the Oral Direct Thrombin Inhibitor Dabigatran Etexilate", *Clin Pharmacokinet* 2008; 47(5): 285-295

4. Hacquard M, de Maistre E, Lecompte T. "Lepirudin: is the approved dosing schedule too high?" *J Thromb Haemost* 2005; 3:2593–6.