ANIARA DIAGNOSTICA LLC

Please note that the uses described in the following page(s) have not been approved or cleared by FDA, with respect to the described assay or test.

In the US, the product is intended **For Research Use Only. Not for Use in Diagnostic Procedures.**

Aniara Diagnostica LLC • 7768 Service Center Drive • West Chester, OH 45069 • Phone (513) 770-1991
Fax (513) 573-9241 • Email info@aniara com • www.aniara.com

ANIARA
Manufactured By: HYPHEN BioMed

DIRECT THROMBIN INHIBITORS' ACTIVITY MEASUREMENT IN PLASMA

M. Peyrafitte, AM. Vissac, J. Amiral

INTRODUCTION

- Direct Thrombin Inhibitors (DTIs, such as Lepirudin®, Bivalirudin® or Argatroban®) have increasing prophylactic or curative applications in severe clinical situations at high risk context. New oral DTIs are being introduced as a safe substitute to dicumarol therapies, with curative, preventive or prophylactic applications (for example dabigatran).
- For some of the applications (mainly curative or in intensive care units), or for monitoring the kinetics course of drug activity in body, laboratory methods are required. These methods are useful for drug posology adjustment, for avoiding overdosage and for targeting the dose presenting the highest benefit/risk ratio. These assays must present the most limited interference from other plasma factors, and especially from the progressive activity of plasma Antithrombin (ATIII). Ecarin Clotting Time (ECT) and aPTT are useful methods, but have variable sensitivity or insufficient reliability, are sensitive to some plasma factors and cannot measure therapeutic DTI levels >1 µg/ml.
- Specialized calibrated clotting and chromogenic assays, fully automatable, with no matrix effect, accurate and sensitive in the low as well as in the high concentration range, were developed for quantitating various DTIs.

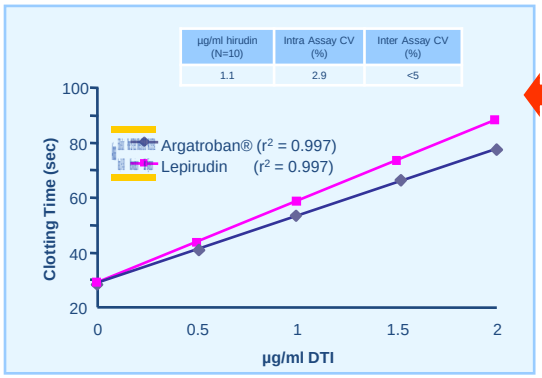
MATERIAL AND METHODS

- **Clotting assay ("Hemoclot Thrombin Inhibitors") :**
  This is an improved and sensitized thrombin time assay, adjusted to the therapeutic ranges of DTIs:
  - A "substrate" normal plasma pool (R1) is mixed with the diluted tested plasma (1:8 to 1:20).
  - Coagulation is initiated with (h)-α-thrombin (R2) containing calcium and clotting time (CT) is recorded. There is a direct dose response curve between DTI concentrations and CT.
- **Chromogenic assay ("Biophen DTIs"):**
  This is a kinetics chromogenic assay based on the inhibition of a constant and in excess amount of human thrombin:
  - Tested specimen (1:10 to 1:25) is incubated with a thrombin specific substrate (R1), and purified human thrombin (R2) is then added.
  - Color development (A405) is an inverse linear relationship of DTIs' concentration.

The assays were evaluated for their dose responses to various DTIs in plasma, as well as for accuracy, reproducibility, and comparison with a conventional aPTT assay.

Results

Clotting assay:
Dose response curves (low range) obtained with 2 different DTIs: Lepirudin® and Argatroban® (STA-R instrument).



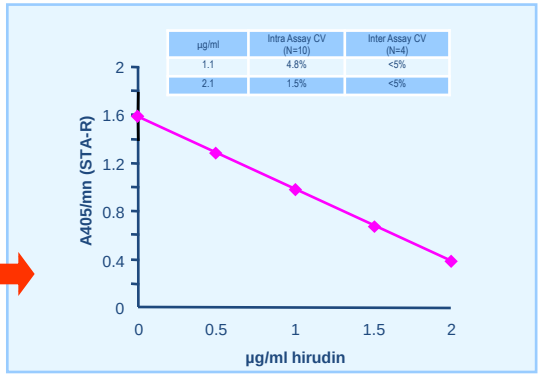
Excellent linearity in the usual therapeutic range.

Can be used for any DTI (Lepirudin®, Bivalirudin®, Argatroban®, …)

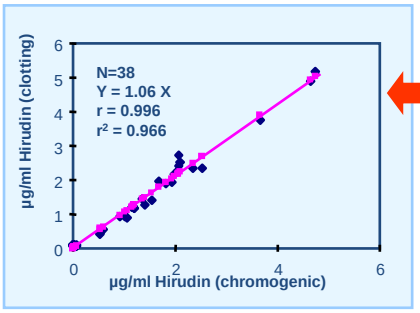
Excellent performances with Hirudin and analogues, but not enough sensitive for Argatroban® in the usual therapeutic range (chromogenic assays are not suitable for therapeutic concentrations of Argatroban®).

Kinetics and dynamic range easily adjusted to various DTIs.

Chromogenic assay:
Linear dose response curve for Hirudin (STA-R)



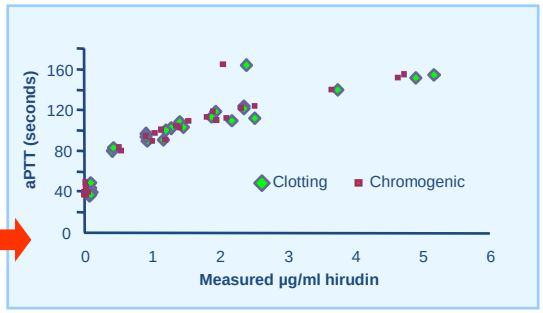
Linear regression analysis for hirudin spiked into normal plasma at various concentrations measured either with the clotting or the chromogenic method



Excellent correlation and possibility to extend the dynamic range up to 5µg/ml, which can be useful in some clinical applications such as ECC.

aPTT lacks of accuracy and reliability above 1 µg/ml of hirudin in plasma, as clotting times are then too prolonged.

Measured aPTT on plasma samples spiked with hirudin (concentration determined by clotting or chromogenic assay)



DISCUSSION

- Simple and standardized assays, easily automatable, using safe (purified human factors) and highly stable reagents (48 to 72h at 2-8°C, or frozen).
- No matrix effect (diluted plasma samples and short reaction time) and possibility to measure DTIs activity in purified milieu.
- Specific calibrations with the DTI used are required, and available for some of them (Hirudin, Argatroban®), for improved robustness and standardization.
- Excellent linearity, sensitivity, accuracy over the usual therapeutic range:

  0.1 to 2.0 µg/ml Hirudin (possibly 0.25 to 5.0 µg/ml) for both methods.

  0.1 to 2.0 µg/ml of Argatroban® for the clotting assay.
- Both methods are well correlated (r2 = 0.96), and consistent with aPTT results, which are expected to be less accurate at high levels of DTIs.

CONCLUSIONS

- Both clotting and chromogenic methods are **calibrated** with the various DTIs used. A quality control system (calibrators and controls) is available.
- They offer **safe, reliable** and **rapid** tools, **easily automated** on the major coagulation analyzers, for measuring DTIs' activity in plasma (or purified milieu).
- They introduce appropriate tools for monitoring the specific DTI activity in curative applications, as well as in analytical and pre-clinical studies for emerging DTIs.
- The clotting method can be used with the standard assay dilution (1:8) for Lepirudin®, Bivalirudin® and Argatroban®, and we predict that it could also be used with new oral DTIs such as dabigatran etexilate.
- These methods can be easily performed in any laboratory where the basic coagulation equipment is available.

GTH Vienna (Austria) Feb 4-7, 2009

Form AH94
02-2009

ANIARA
7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 **Toll Free:** 866.783.3797 **Fax:** 513.573.9241 **Email:** info@aniara.com
www.aniara.com