Thrombin Inhibitors : general information

- Increasing curative or preventive applications of DTIs in severe clinical situations at high risk context (eg VTE, orthopedic (total hip or knee replacement, …), which are candidates for substituting to long term oral anticoagulant therapies with VKA. Laboratory methods are required for drug efficacy adjustment and avoiding overdosage, with the most limited impact to other plasma factors : ECT, TT and aPTT are used but too sensitive and less reliable at high DTI therapeutic levels, and patient coagulation factors may interfere.

- Hirudin (Refludan®; Bayer HealthCare Pharmaceuticals) : Intravenous injection, individual dosing, and frequent laboratory monitoring required. Irreversible binding to thrombin. More effective than heparin but associated with increased bleeding.

- Other DTIs: Bivalirudin (Angiox®; The Medicines Company, eg for PCI) and Argatroban (Argatra®; Mitsubishi Pharma, eg for HIT): Intravenous administration, more favorable safety profile than hirudin, reversible binding to thrombin (inhibit both free and clot-bound thrombin), individual dosing and laboratory monitoring still required.

- Dabigatran Etexilate(Pradaxa®, Boehringer Ingelheim): oral fixed-dose "once or twice daily" as a prodrug of its active moiety dabigatran, specific and reversible DTI inhibiting both free and clot-bound thrombin, no food interference, limited drug interaction, mainly renal excretion, no antidote available (possible dialysis), no need for monitoring claimed except in case of suspicion of excess of anticoagulant activity.

Thrombin Inhibitors : key publications

- Mackman N , Becker RC. DVT: A New Era in Anticoagulant Therapy. Arterioscler Thromb Vasc Biol 2010; 30(3): 369-371.
- Verheugt FWA. The new oral anticoagulants. Neth Heart J. 2010; 18(6): 314-318.
- Ufer M. Comparative efficacy and safety of the novel oral anticoagulants dabigatran, rivaroxaban and apixaban in preclinical and clinical development. Thromb Haemost 2010; 103: 572-585.
- Becattini C, et al. New anticoagulants for the prevention of venous thromboembolism. Drug Des Devel Ther 2010; 4:49-60.
- Saugel B et al. Argatroban therapy for heparin-induced thrombocytopenia is ICU patients with multiple organ dysfunction syndrome: a retrospective study. Critical care 2010; 14(R90): 1-7.
- **Ryn JV et al. Dabigatran etexilate – a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. Thromb Haemost 2010; 103: 1116-1127.**
- Laux V et al. Direct Inhibitors of coagulation proteins – the end of the heparin and low-molecular-weight heparin era for anticoagulant therapy? Thromb Haemost 2009; 102: 892-899.
- Fareed J, et al . Survival of heparins, oral anticoagulants, and aspirin after the year 2010. Semin Thromb Hemost 2008; 34: 58-73.
- Blann AD, Khoo CW. The prevention and treatment of venous thromboembolism with LMWHs and new anticoagulants. Vasc Health Risk Management 2009; 5: 693-704.
- Bounameaux H. The novel anticoagulants: entering a new era. Swiss Med Wkly 2009; 139 (5-6): 60-64.
- Harenberg J, Wehling M. Current and Future Prospects for Anticoagulant Therapy: Inhibitors of Factor Xa and Factor IIa. Semin Thromb Hemostasis 2008; 34 (120): 39-57.
- Francis CW New issues in oral anticoagulants. Am Soc Hematol 2008; 259-265.
- Stangier J, "Clinical Pharmacokinetics and Pharmacodynamics of the Oral Direct Thrombin Inhibitor Dabigatran Etexilate", *Clin Pharmacokinet* 2008; 47(5): 285-295
- Blech S et al. The metabolism and disposition of the oral direct Thrombin Inhibitor, Dabigatran, in humans. Am Soc Pharmacol Experiment Ther. 2008; 36(2): 386-399.
- « Landmarks in Anti-Thrombin drug development: the Argatroban study »; *Seminars in Thrombosis and Hemostasis*; Vol 34, Suppl 1, Oct 2008.
- Greinacher A; Warkentin T, « The direct thrombin inhibitor hirudin *»; Thromb Haemost* 2008; 99:819-829.
- Hacquard M et al. "Lepirudin: is the approved dosing schedule too high?" *J Thromb Haemost* 2005; 3:2593–6.

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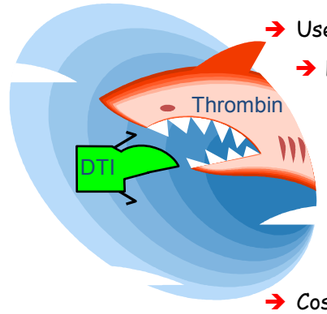
Manufactured By: HYPHEN BioMed

HEMOCLOT THROMBIN INHIBITORS

Technical File

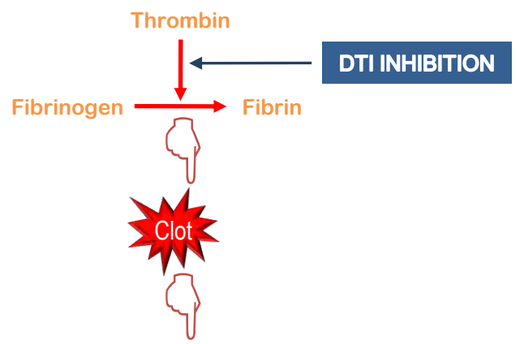
"TRUE" anti-thrombin activity of Direct Thrombin Inhibitors in plasma



- Use of highly purified human thrombin (α form)
- Linear Dose-response curve
- No interference of prothrombin or fibrinogen concentrations
- Evaluation of the « true » direct anti-thrombin activity of the DTI
- Reproducible and consistent from run to run, and lot to lot
- Cost effective

Highly sensitive assay of choice for Hirudin, Lepirudin, Bivalirudin, Argatroban, Dabigatran, Angiox®…

Clotting assay
For all DTIs



HEMOCLOT Thrombin Inhibitors (DTI)
Ref ACK002K/ACK002L

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

HEMOCLOT THROMBIN INHIBITORS (#ACK002K/L) INTENDED USE AND PRINCIPLE

IVD (CE mark) : as an aid for the quantitative measurement of hirudin and other DTIs in plasma (eg dabigatran when required, in case of suspicion of excess of anticoagulant activity, or Argatroban®) with a clotting method .

The diluted tested plasma is mixed with a normal human plasma pool (R1). Clotting is then initiated by adding a constant and in excess amount of highly purified human α-thrombin (R2). The clotting time (CT) measured is directly related to the concentration of assayed DTI in plasma.

R1: normal plasma pool, lyophilised
R2 : purified human calcium thrombin, lyophilised.

CHARACTERISTICS AND ADVANTAGES

- **Rapid (**< 3 min), **Fully automatable** or easy to use with basic equipment

  (~ 3x10 (ACK002K) or 3x25 (ACK002L) tests/kit

- **Standardized calibrators and controls for Hirudin (Lepirudin) , Argatroban®, Dabigatran**
- **Covers usual levels in treated patients' plasma:**

  Up to 2µg/ml Hirudin or Argatroban , or 0.50 µg/ml Dabigatran (low range, 1:8 dilution)

  Extended range up to 5 µg/ml Hirudin (eg ECC) (high range, 1:20 dilution)

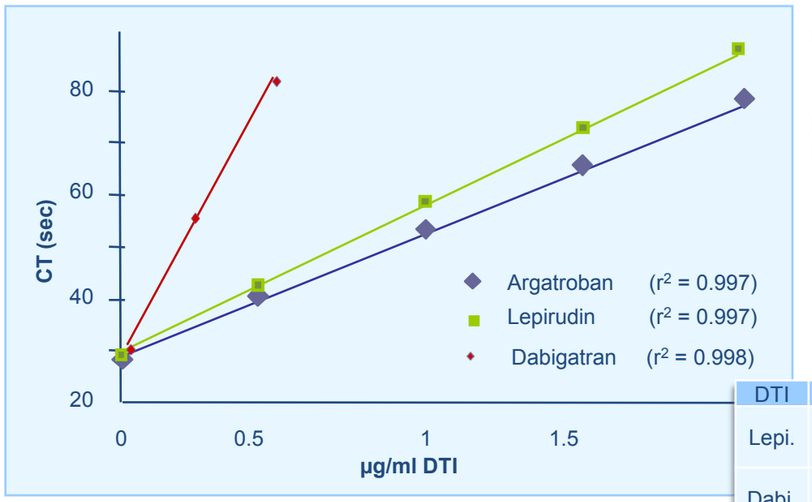
Possible research use with other DTIs, by specifically adjusting the calibration and protocol, or by alternative expression of inhibition as "hirudin equivalent".

- **Reproducible, sensitive, no impact of other plasma factors, highly stable**

  (24h2-8 C, 8hRT,frozen)

- Purified (h)Thrombin tested for viral safety.
- **Reflects the "true anti-IIa" potential:** presence of heparin or other anti-IIa substances may interfere in the assay and prolong CT, to avoid any underestimation of an existing hypocoagulability.

DOSE RESPONSE CURVES TO 3 DTIs. AND REPRODUCIBILITY (STAR)



Untreated patients <0.05 µg/ml

Excellent linearity in the usual therapeutic range for the tested DTI s

DTI	µg/ml	Intra Assay CV	Inter Assay CV
Lepi.	1.15	2.8% (N=10)	5.0% (N=6)
Dabi.	0.12	2.2% (N=20)	5.3% (N=20)
Arga.	1.25	2.3% (N=10)	2.2% (N=5)

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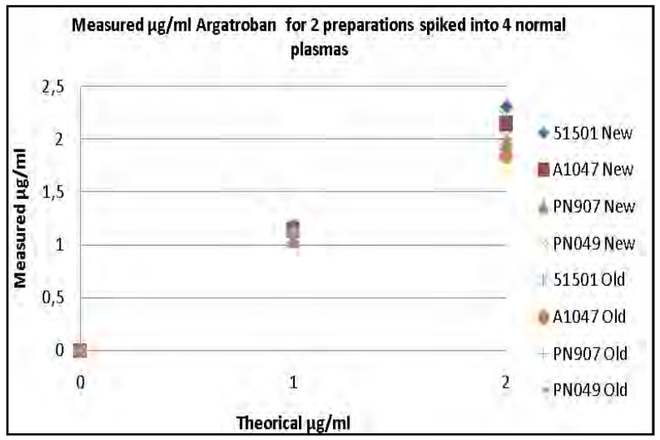
EXAMPLE OF RECOVERY RESULTS

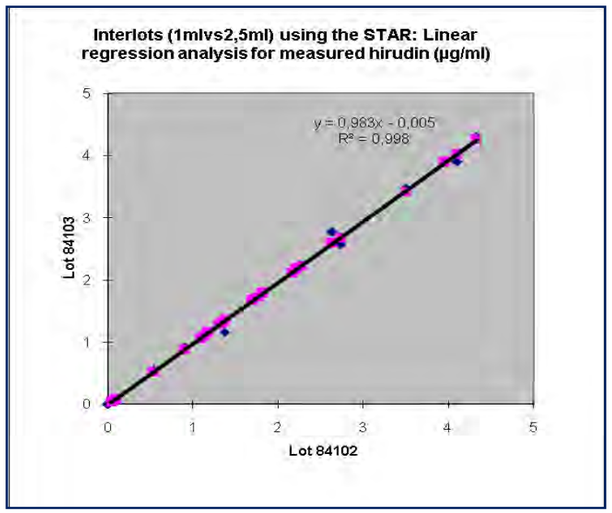


Dabigatran µg/ml:	In plasma		In TBS-BSA buffer	
	C1	C2	C3	C4
Spiked	0.10	0.25	0.10	0.50
Recovered	0.08	0.26	0.10	0.47

Good recovery, with no significant effect of other coagulation factors deficiency in the tested samples (eg AT, FII, Fibrinogen...).

Measured µg/ml in deficient plasmas spiked with **Lepirudin**	+0µg/ml	+1µg/ml
Def. II	0.04	1.13
Def. V	0.06	1.04
Def. X	0.05	1.01
Def. AT	0.05	1.10
Fibrinogen low	0.05	1.07

INTER LOTS CORRELATION ON STAR



(N=37) Lot	84103	84102
Mean µg/ml	1,21	1,23
Median	1,05	1,09
Sum	44,68	45,66
Min - Max	0 – 4.29	0 – 4.33

Excellent consistency of results from lot to lot.

RELATED PRODUCTS

1. **Hirudin** Standard Low (#ASC020K) or High (#ASC020L), Hirudin Plasma Control (#ASC025K).
2. **Argatroban** Plasma Calibrator (#ASC030K) and Control Plasma (# ASC035K)
3. **Dabigatran** Plasma Calibrator (#A222801) and Control Plasma (#A224801).
4. **Biophen DTI** (#A220202)(chromogenic assay, not suitable for Argatroban®)

Form AH83
01-2011