

Dabigatran (Pradaxa) Testing Only From Aniara!

Worth Its Weight In Reliability & Accuracy: A True Measure Of Drug Sample Concentration.

Assay designed for the quantitative measurement of Dabigatran and other direct Thrombin Inhibitors in plasma; contains highly purified human thrombin (same quality as HEMOCLOT Thrombin), and an optimized human pooled plasma, used as substrate. The assay allows specific measurement of Dabigatran or other Thrombin inhibitors by their direct antithrombin activity. It is insensitive to the Prothrombin and fibrinogen concentrations in the tested patient plasma specimen. According to the posology used, and to the patient's clinical context (antithrombin therapy, substitutive anticoagulant in presence of HIT or extra-corporeal circulation), the assay works with different plasma dilutions. Assay dynamic ranges are usually from 0 to 2 µg/mL (low range) for Dabigatran, Argatroban or from 0 to 5 µg/mL (high range), when used for Hirudin testing (high range).

For more information, please visit www.dabigatrantesting.com

Hemoclot Thrombin Inhibitors*

- ACK002K Hemoclot Thrombin Inhibitor Kit 3x10
- ACK002L Hemoclot Thrombin Inhibitor Kit 3x25

Dabigatran*

- A224701 Dabigatran Control Plasma
- A222801 Dabigatran Plasma Calibrator

Adaptations Available (More To Come)

- ACL 7000
- STA
- SYSMEX CA1 500
- BCS
- ACL Top
- STA-R

Direct Thrombin Inhibitors**

- A220202 BIOPHEN DTI (Direct Thrombin Inhibitors)

Introduction

In certain situations it may be clinically helpful to assess the anticoagulant activity in patients. The **Hemoclot Thrombin Inhibitors (HTI)** kit (improved thrombin time "like" clotting assay) is reliable and accurate for this purpose (1, 2), and needs to be calibrated with specific Dabigatran plasma calibrators at well defined concentrations.

Aim

Preparation and validation of freeze dried plasma calibrators and controls (2 lots) supplemented with dabigatran (active moiety of the prodrug) at defined concentrations for dedicated assays, covering the expected plasma concentrations in usual Pradaxa® applications.

Assay Principle

Plasma calibrators or diluted tested plasma (**dilution 1:8**) are mixed with a normal human plasma pool (**Reagent 1**). Clotting is then initiated by adding a constant and in excess amount of highly purified human α-thrombin (**Reagent 2**). The clotting time (CT) measured is directly related to the concentration of assayed Direct Thrombin Inhibitor (DTI) in plasma.

Materials and Methods

Preparation of Plasma Calibrators and controls:

Dabigatran was added to plasma at 3 concentrations (0.05, 0.25 and 0.50 µg/ml) for calibrators, and at 2 concentrations (0.10 and 0.30 µg/ml) for controls, and lyophilized.

Validation studies: These preparations were evaluated for:

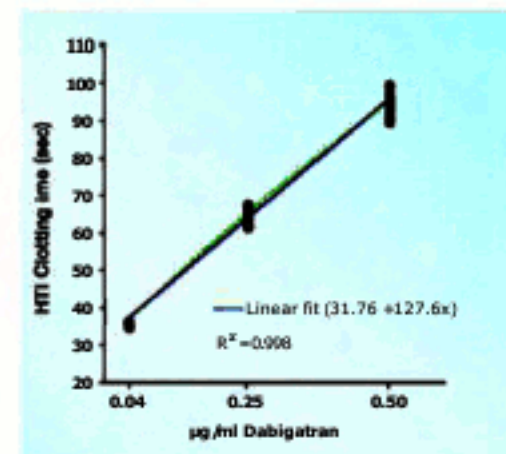
- Linearity of calibration curves
- Stability following reconstitution
- Dabigatran concentration established against dabigatran spiked in various plasma pools using HTI assay, or by HPLC-MS/MS method (reference method) kindly run by Boehringer Ingelheim.
- Basic plasma values (without dabigatran) and matrix effect (recovery study).
- Standardization from lot to lot and comparison with HPLC results.

Results

Calibration Curve

Calibration curve with Dabigatran Plasma

Calibrators on STAR (6 independent series in duplicate)



A linear dose response curve is obtained with the HTI assay using the STAR, and similar results can be generated with various laboratory coagulation instruments ($R^2 > 0.99$).

LLQ is <0.05 µg/ml.

The lyophilized system is highly stable after reconstitution ($\Delta C < 0.02 \mu\text{g/ml}$):

- 7 days at 2-8°C
- 48 hours at RT (18-25°C)
- At least 2 months frozen at -20°C or below

Performances maintained in overheating studies (3 weeks at 30°C), and in real time follow up at 2-8 C (data not shown).

Basic values and recovery (matrix effect):

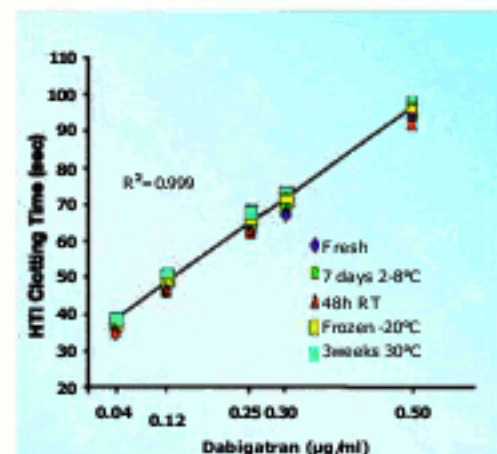
N=56 normals (untreated patients) were tested for basic clotting time, and **N=20 normals** spiked with two Dabigatran concentrations to evaluate matrix effect

Normals (untreated, N=56)	
Mean CT	30 sec
SD	0.3 sec
Dab. Conc.	<0.02 µg/ml

Basic clotting times are very homogeneous,

Stability

Stability of lyophilized calibrators and controls



and dabigatran well recovered with no matrix effect

Dabigatran added at (N=20):	+0.10 µg/ml	+0.25 µg/ml
Mean (µg/ml)	0.10	0.24
SD (µg/ml)	<0.01	0.01
Min-Max (µg/ml)	0.09-0.11	0.22-0.26
Recovery	100%	96%

Standardization of Dabigatran concentration and precision:

Dabigatran concentrations of prototype lots of lyophilized calibrators and controls were measured over multiple runs using HTI assay, or HPLC reference method:

Results for Lot 1	HTI assay Conc. (µg/ml) ± SD (N=16)	HPLC MS/MS Conc. (µg/ml) ± SD (N=12)
(Cal. 0)	0	0
Cal.1	0.11 ± 0.01	0.10 ± 0.01
Cal.2	0.28 ± 0.02	0.24 ± 0.01
Cal.3	0.53 ± 0.03	0.48 ± 0.01
QC Low	0.14 ± 0.01	0.12 ± 0.01
QC High	0.34 ± 0.02	0.29 ± 0.01

When measured using HTI assay against a fresh reference curve, lyophilized reagents were 2 to 5 % lower than frozen plasma before lyophilization. Tested by HPLC, the concentrations were 10 to 15 % lower. This could be induced by the slightly higher volume obtained with the reconstituted lyophilized plasma.

Within run (intervals, N=20) SD was <0.01 µg/ml, and inter assay SD (N>10) was in the range 0.01-0.03 µg/ml for determined concentrations using HTI kit, validating excellent intra lot homogeneity, and robustness of the assay.

Both lots were evaluated and concentrations assigned by HPLC as the reference method, and homogeneous results were obtained with the HTI assay.

Lot 1 was then used as the reference material, using the concentrations measured by HPLC, and used for measuring lot 2.

Inter lots homogeneity and consistency with HPLC results:

When tested with HTI assay calibrated with lot 1 (STAR, considering HPLC reference values), concentrations measured for lot 2 fully matched with those obtained by HPLC:

	Dabigatran	Expected Conc. µg/ml	Measured Conc. µg/ml	
			(HTI assay)	(HPLC)
Lot 2	Cal 1	0.05	0.04	0.04
	Cal 2	0.25	0.26	0.25
	Cal 3	0.50	0.48	0.50
	Ctl Low	0.10	0.13	0.12
	Ctl High	0.30	0.31	0.30
Lot 1	Ctl Low	0.10	0.12	0.12
	Ctl High	0.30	0.29	0.29

Measured values are consistent with HPLC results, and homogeneous from lot to lot Similar results were obtained with a chromogenic assay (anti-IIa 2 stages, Biophen® DTI) (data not shown).

Conclusions

- » Lyophilized dabigatran plasmas:
 - can be used to calibrate and control the quality of both clotting and chromogenic assays.
 - can serve as primary standards based on the values assigned by HPLC-MS/MS.
- » When required, the plasma of patients treated with Pradaxa® can be assessed with the described rapid, fully automatable and simple standardized system.

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