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.**

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Manufactured By: HYPHEN BioMed

HEMOCLOT Protein S technical file (#ACK041K)

Intended use and applications

Status: IVD, CE mark.

Intended use: In vitro method for the quantitative determination of Protein S in human citrated plasma using a clotting assay, with a manual or automated method..

Reagents and principle

REAGENTS:

R1: PS depleted plasma, lyophilised. 3 vials of 1ml
R2: Activation reagent, lyophilised. 3 vials of 1ml

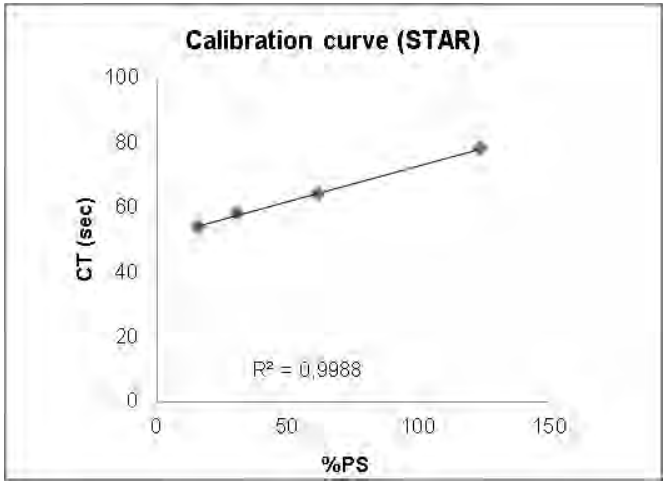
PRINCIPLE:

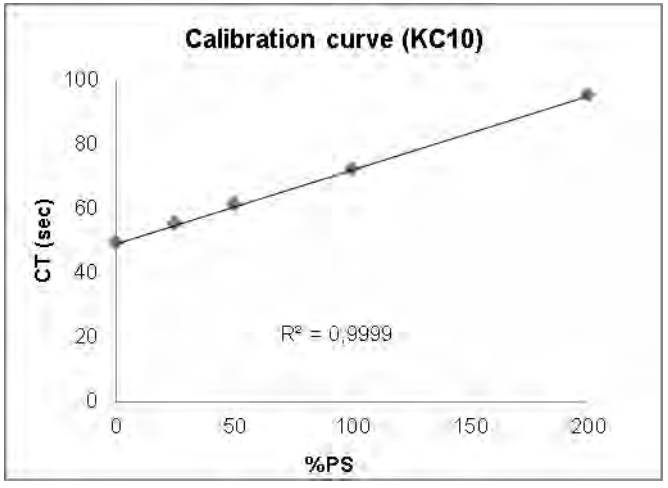
APTT like clotting assay, but triggered by factor IXa in the presence of phospholipids, calcium, and of a constant and in excess amount of Activated Protein C (APC). Assayed Protein S is then the limiting Factor.
In the first step, the diluted assayed plasma is mixed with Protein S deficient plasma (R1). Then, the activator reagent (R2), in a constant and optimised concentration, is added. Clotting is initiated by the addition of calcium (Ca 2+). Clotting time (sec) is then recorded. Protein S being the limiting factor, there is a direct relationship, between the Protein S concentration and the corresponding clotting time.

Characteristics and advantages

- Simple, rapid, with only 2 reagents and calcium: total assay time < 3 min..
- Easy to use with **automated instruments** or basic equipment .
- Tests per kit: ~2*35 (STAR) or 2*50 (microplate)
- **Associated calibrators and controls** validated against the International Standard for PS, plasma.
- **Wide linearity and dynamic range: Calibration up to 200% PS** in human citrated plasma; linear range 10-200% PS (or 7,5-150% depending on automate used).
- Assayed dilution 1:20 with imidazole buffer
- Detection threshold about 10% PS
- Specific, sensitive, reproducible: PS deficient plasma measured <5% PS; inter assay CV(CT)<5%
- Highly stable (at least 8h at RT (18-25°C), or 24h at 2-8°C, or freezing possibility).
- Safe, optimized, **standardized**: highly purified factors, with conformity certificate, and good inter lots consistency.
- No significant interference of FVIII:c in the assayed sample up to at least 200% FVIII:C. An heparin neutralizing substance is included in the reagent. Presence of LA or FVL mutation may interfere in the assay
- Caution: avoid any evaporation or contamination of regents during use.

Calibration curves (example for lot 00802-1/01403-2, calibrated using a normal frozen plasma pool)





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Example of recovery

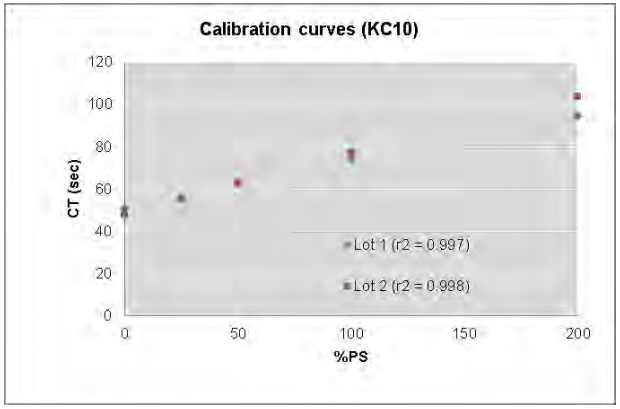
A normal frozen human plasma pool (lot A1071) was mixed at various levels in PS depleted plasma (lot 00301-1 measured <5% PS), to verify consistent recovery:

Expected PS%	Measured PS%	% Recovery
100	107	107
80	87	109
60	70	117
40	42	105
20	17	85
0	0	na

Conclusion: Good recovery

Inter lots

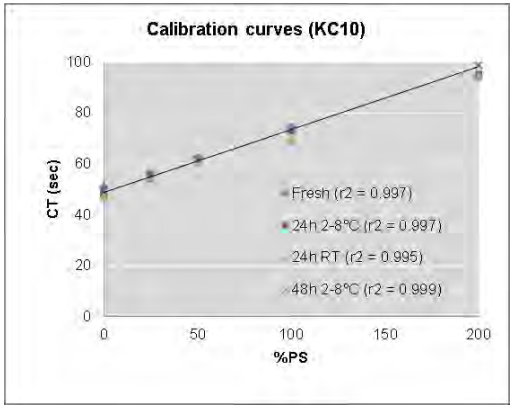
Internal inter-lots comparison for PS% calibration curves (reference frozen plasma pool) using Hemoclot PS (Lot 1: 00802-1/01403-2 and lot 2: 02301-1/02301-2) and the KC10 instrument:



Conclusion: good consistency of reactivity from lot to lot.

Stability studies (2-8°C, RT(18-25°C), freezing, "Health stress" at 30°C):

Reagents are stored for various periods at 2-8°C or RT (18-25°C or freezed thawed after reconstitution. Lyophilised reagents are also heated at 30°C for 3 weeks (« heat stress », accelerated ageing), and the tested freshly reconstituted on freshly prepared calibration curve , in parallel with freshly restored for the same lot stored at 2-8°C:



Conclusion: Reconstituted reagents are stable for at least 24h at 2-8°C or 8h at RT (18-25°C). Performances of calibration curve are also expected well preserved after freezing cycle, or storage at 30°C for 3 weeks. This allows shipping the reagents at RT for a short period without damage.

Inter-assay (KC10 and STAR, lot 00802-1/01403-2):

Freshly prepared independant calibration curves made from normal frozen human plasma pool are tested, using STAR or KC10 instrument. Mean measured CT (sec), SD and CV% are reported:

%PS **(STAR)**	Mean CT (sec) (N=5)	SD	CV%
16	53,1	0,8	1,5
31	56,7	0,8	1,5
62	63,2	1,0	1,6
124	78,0	1,6	2,0
r2	0,998		

%PS (**KC10**)	Mean CT (sec) (N=4)	SD	CV%
25	54,2	1,8	3,2
50	60,3	2,2	3,7
100	71,1	2,6	3,6
200	98	4,3	4,4
r2	0,997		

Conclusion: Here using STAR and KC10, inter-assay CV% (on CT) are respectively < 2% and <5%

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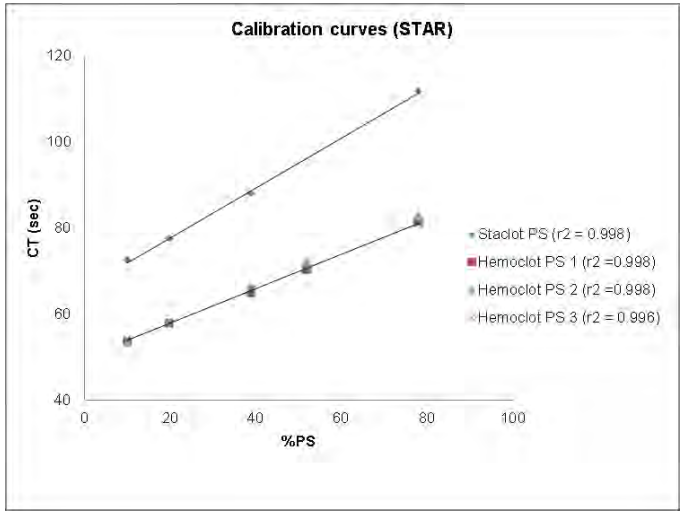
Preliminary evaluation vs Staclot PS:

The normal and abnormal quality control systems of both devices, as well as HYPHEN calibrator, were crossed over the 2 kits and determined against calibrator curves established with the SSC/ISTH lot 3 standard.
The STAR method was used for each kit.
At least 3 independant series (ie a total of at least 9 different vials) were analyzed with Hemoclot PS (HBM, lot 00802-1/01403-2), while Staclot PS (Diagnostica Stago) used was lot 105373.
The results were as follows:



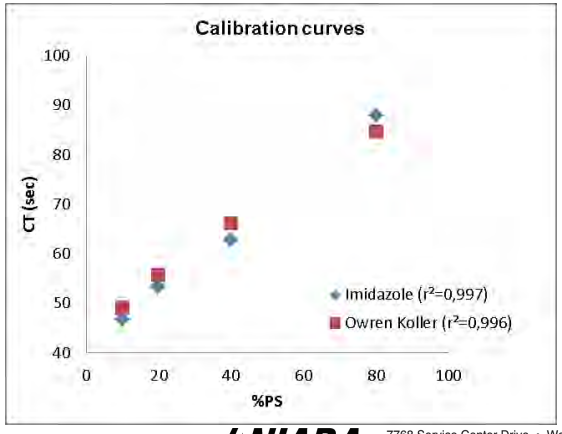
Measured % PS	Staclot PS	Hemoclot PS (3 independent series)		
Stago SYST N 104564 [70-96%]	86			
Stago SYST P 104564 [29-43%]	34	na		
Stago SYST N 105386 [78-108%]		93	102	96
Stago SYST P 105386 [28-40%]	na	30	35	34
HBM Normal Control	70	70	74	68
HBM Abnormal Control	38	31	28	31
HBM Calibrator	57	62	64	61

Conclusion: Results are well consistent between the 2 kits.

Preliminary evaluation vs Staclot PS:

The influence of using Imidazole vs Owren Koller type buffer was verified on reactivity obtained for a calibration curve.

Results were as follows:



Conclusion:

Good consistency in reactivity, both buffers can be used provided the same buffer is used for calibration and all tested points.

Each laboratory should verify expected performances and acceptance ranges in its exact working conditions.

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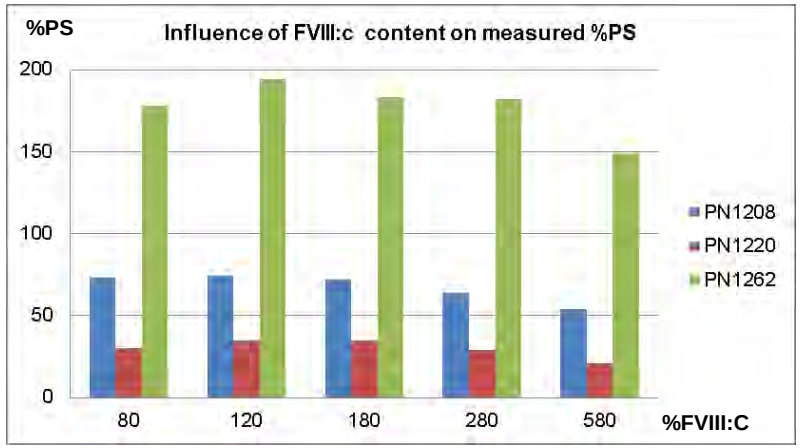
Reference Interval (Normal Range) and Interferences

- PS concentration is usually expected between 60-140% in healthy adults. These data are confirmed by an internal study on individual normal plasmas (Precision Biologics), which yielded Min-Max values of 62-121%PS.
- Potential interference of various factors was tested on pathological samples (Lupus Anticoagulant, LA) or patients carrying R5606Q mutation (FV Leiden, resistant to the action of Activated Protein C), or of samples with increased FVIII:C content. Results for measured PS% were as follows:

Lupus samples	Mean PS%
N=8	<10%

FVL samples (actPCr)	PS%
1	33
2	65
3	44
4	71
5	44
6	32
7	13
8	77
9	29
10	48



Conclusion: Presence of LA or FVL may interfere in the assay and least to underestimation of measured PS% (this has to be further confirmed considering the clinical context and by comparison to measured free PS by immunological method). There is no interference of FVIII:C content up to at least 200% FVIII:C in the test sample. An heparin neutralizing substance being included in the reagent, there is no expected interference for Heparin treated patients in the usual therapeutic range.

QMS, Quality Control system and referential

This reagent is designed, manufactured, controlled and followed according to HYPHEN BioMed quality management system (based on ISO 9001 and ISO 13485) , and according to the 98/79/CE directive from European parliament and council (October 27th, 1998) related to IVD medical devices.
A complete lot master file is recorded and validated in full compliance with Manufacturing and Quality Control SOP for each lot, as well as complete stability studies .
Homogeneous performance from lot to lot is ensured by using Internal Reference Standards for PC (plasma), accurately validated against the corresponding NIBSC International Standard, as well as by using various quality controls covering the dynamic range.

Related products

1. Biophen Plasma Calibrator, Normal and Abnormal Control Plasmas (#A222101/A223201/A223301) (CE)
2. Zymutest Free ProteinS (#ARK015A)(CE)
3. Zymutest Total Protein S (#ARK021A) (CE)

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