

Intended use and applications

Status: IVD, CE mark.

Intended use: Quantitative determination of Protein C (PC) in human citrated plasma by clotting assay. Automatic or Manual method.
Congenital or acquired Protein C deficiency is a risk factor for venous thrombosis.

Reagents and principle

REAGENTS:

R1: Optimized **PC Deficient plasma**, lyophilized with an heparin neutralizing agent. (3*1ml)
R2: PC specific **Activator (Protac®)** containing phospholipids, in an optimized concentration. (3*1ml)

PRINCIPLE:

The diluted assayed plasma is mixed with PC deficient plasma (R1). Then, the Activator Reagent (R2) is added. Clotting is initiated by the addition of Calcium (Ca²⁺), and clotting time (CT) is recorded. PC being the limiting factor, there is a direct linear relationship, on a bilogarithmic graph paper, between the PC concentration and the corresponding clotting time.

Characteristics and advantages

- Simple and easy to perform method, with a total assay time of 8 min.
- Easy to use with **automated instruments** or basic equipment. Fully automatable on any laboratory coagulation instrument with a mechanical or a photometric clot detection.
- Tests per kit: ~3*20 (automate) or 3*10 (manual method)
- **Calibrators and controls** validated against the International Standard for human (PC), plasma (NIBSC; SSC/ISTH lot 3).
- **Extended dynamic range : 25 to 200% PC** in human citrated plasma
- Assayed dilution 1:10 with imidazole buffer
- Specific, sensitive,
- Detection threshold ~10% PC (eg. **PC deficient plasma measured 8%PC**)
- Stability after reconstitution : 24 hours at 2-8°C, 8 hours at RT (18-25°C), 1 month frozen.
- Safe, optimized, **standardized**: highly purified Protac®; inter lots correlation r2 = 0.93.
- Contains an heparin neutralizing agent so that it can be performed in patients with heparin therapy (up to 1 IU/ml), or dicoumarol treated (PC activity is then decreased). In presence of an abnormally prolonged CT, confirming the diagnosis with another method is recommended.
- Caution: avoid any evaporation or contamination of reagents during use.

Calibration curves (example for lot 01001)

Intra and inter-assay data (KC10)

INTRA-ASSAY : The 2 samples are tested 10 fold in the same series.

INTER-ASSAY : The 2 samples are tested 9 fold, in 9 independent series, each one being newly calibrated.

Mean measured value (IU/ml or % PC), SD and CV% are reported:

Samples	PC concentration %	Intra-Assay CV% (N=10)	Inter-Assay CV% (N=9)
Sample 1	87%	6.7	8.4
Sample 2	63%	7.3	5.7

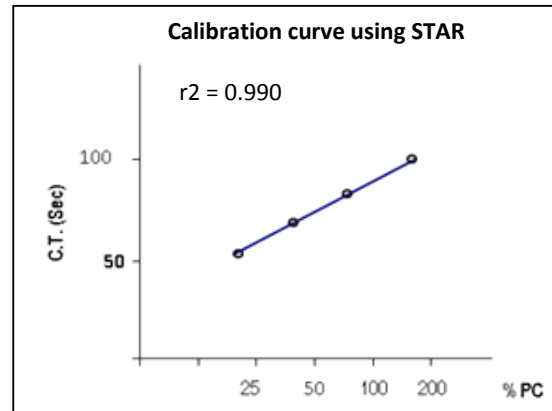
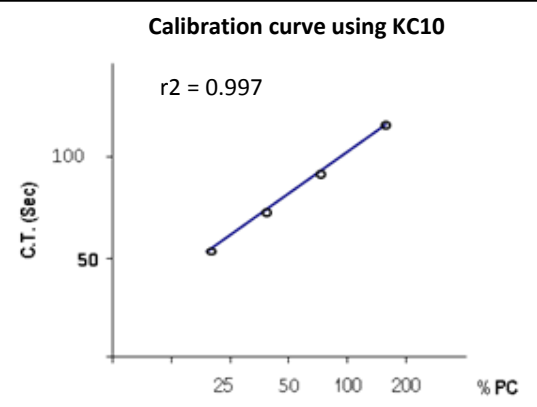
Conclusion:

In this evaluation using the KC10 instrument, CVs (on measured concentrations) are 5-8%.

Intra and inter assay CV on obtained clotting times are <5%.

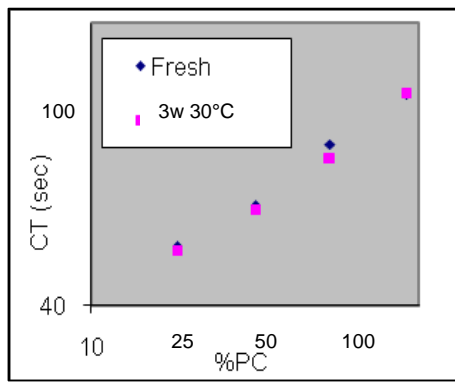
Form AH147
08-2010

D.750.30/ CK/031K /1 - (Date: Jul 2010) Page 1/4



"Heath stress study" (accelerated ageing)

Lyophilized reagents are stored for 3 weeks at 30°C, and then compared freshly restored with the same one stored at 2-8°C, on a calibration curve and controls (here using KC10).

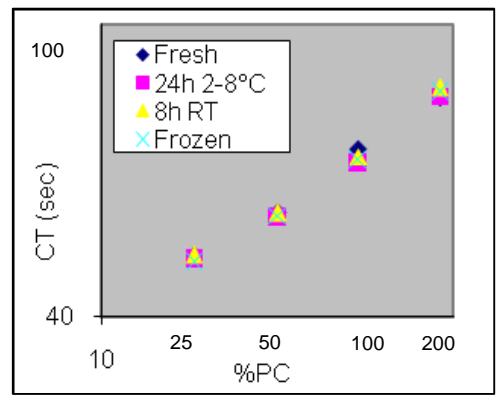


Storage:	Fresh	30°C
	Measured %PC	
Control 1	67	75
Control 2	55	53

Conclusion: The calibration curves are not affected by the storage at 30°C for 3 weeks, and controls are well recovered. Performances are well preserved during the storage at 30°C, which allows shipping the reagents at RT.

Stability after reconstitution (2-8°C, RT(18-25°C), freeze-thaw cycle) (STAR):

Reconstituted reagents are stored for different periods at various temperatures, and then compared, on a calibration curve and controls, with the same ones stored at 2-8°C and freshly restored.



Storage:	Fresh	24 hours 2-8°C	8 hours RT	Frozen-thawed
	Measured %PC			
Control 1	68	72	68	69
Control 2	46	45	44	43

Conclusion: The calibration curves are not affected. Excellent recovery for controls. Performances are well preserved after 8h at RT, 24h at 2-8°C, or freeze thawing.

Recovery studies

As an example, a lyophilized plasma pool (considered at 100%PC) and a PC deficient plasma were mixed at various levels, to verify consistent recovery, and line of identity:

Expected %PC	Measured %PC	Recovery %
150	159	106%
100	110	110%
75	77	103%
50	48	96%
10	10	(100%)
0	6	Not applicable

Conclusion: it showed satisfying results.

Heparin influence

An heparin neutralizing substance is present in reagent R1. Various individual normal plasmas (or lyophilized plasmas pools) were spiked with concentrations of UFH or LMWH, in the range 0 to 2 IU/ml, to evaluate the influence of the presence of heparin in the test:

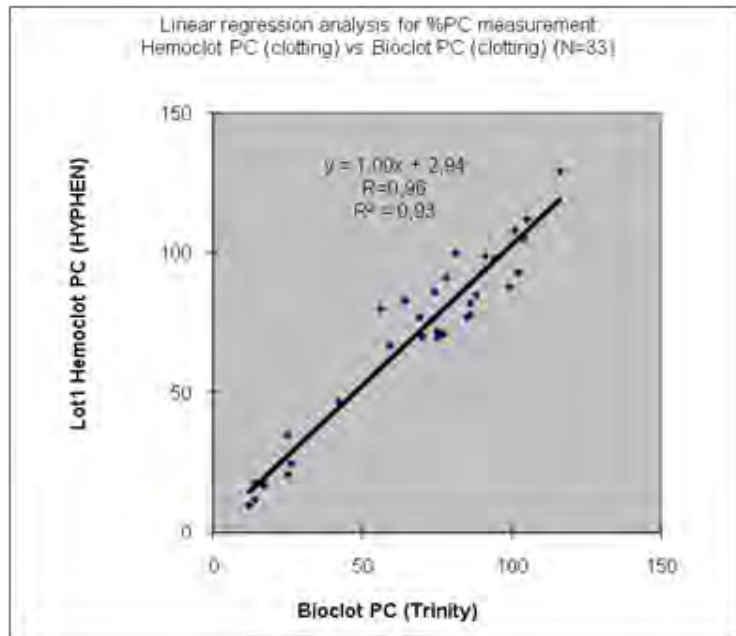
Heparin (IU/ml)	PN1 +UFH	PN2 +UFH	PN3 +UFH	Cal Pool1 +UFH	Cal Pool2 +LMWH
0	137	92	86	85	99
1	135	80	78	81	/
2	132	63	70	82	103

Conclusion: results remain globally unchanged up to at least 1 IU/ml heparin added to plasma.

Form AH147
08-2010

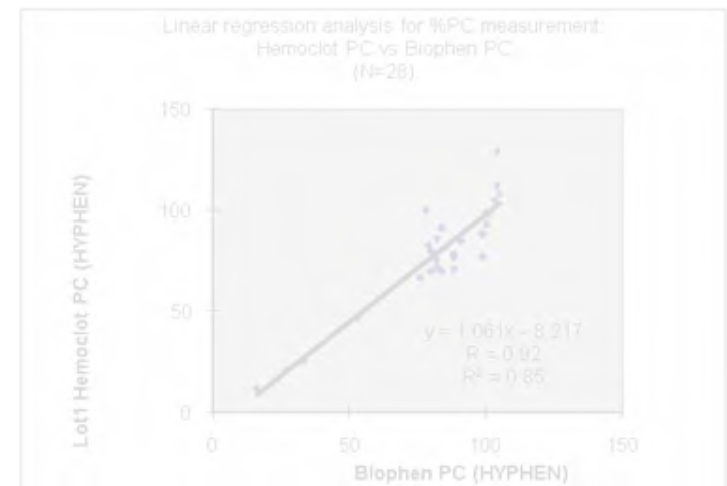
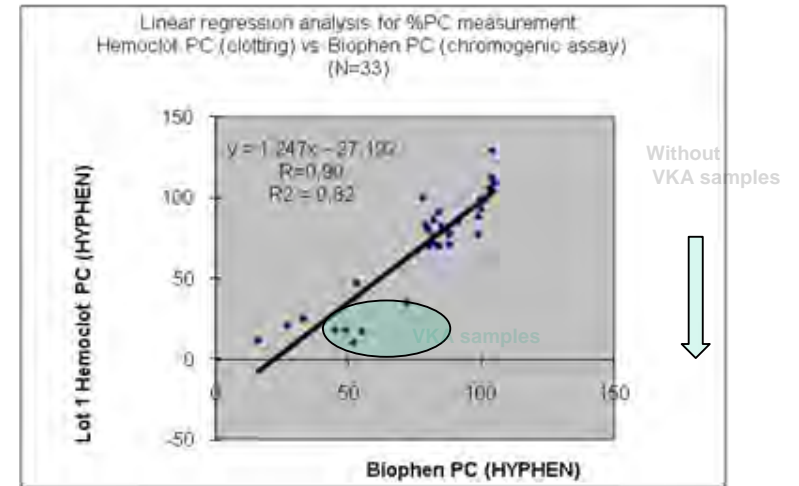
Performance comparison with Bioclot PC (clotting assay, TRINITY) and Biophen PC (chromogenic assay, HYPHEN BioMed).

Internal comparison for PC% measurement using Hemoclot PC (lot 1 = 82502) or Bioclot PC (lot 08/08; Trinity) devices, and the STAR instrument, on N=33 samples (4 normal plasma pools, 10 normal plasmas from French blood bank, 7 « recovery samples » covering the dynamic range made by spiking normal plasma pool or purified PC into a PC deficient plasma, N=4 heparinized samples, N=5 VKA/dicumarol treated samples, N=3 samples addressed for thrombosis risk check up left from analysis).



Conclusion:
Results obtained using Hemoclot PC and Bioclot PC (clotting assays) are globally consistent.
Low levels of PC are well detected.

Internal comparison for PC% measurement (STAR) using Hemoclot PC (Lot1=82502) or Biophen PC (lot 82302, HYPHEN), on N=33 samples (same as previously).

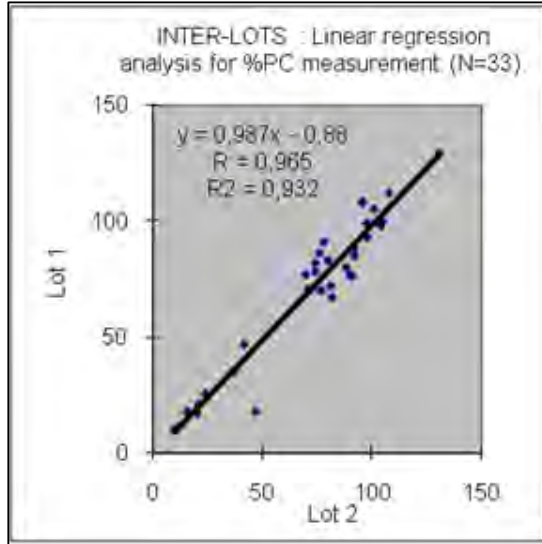


Conclusion:
Results obtained using Hemoclot PC (clotting assay) and Biophen PC (chromogenic assay) are globally consistent.
Low levels of PC are well detected.
For the 5 VKA samples tested, higher results are noted when using the chromogenic assay.

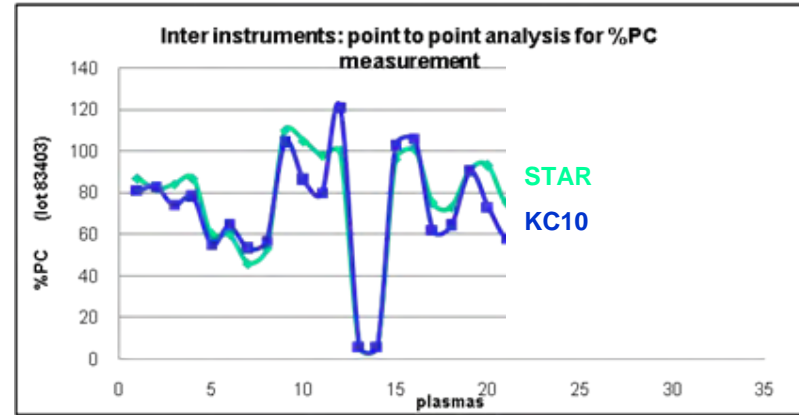
Inter lots and inter-instruments

1. Internal inter-lots comparison for PC% measurement using Hemoclot PC lot 1=82502 vs lot 2 =83403, using the STAR instrument, on N=33 samples (4 normal plasma pools, 10 normal plasmas from French blood bank, 7 « recovery samples » covering the dynamic range made by spiking normal plasma pool or purified PC into a PC deficient plasma, N=4 heparinized samples, N=5 VKA/dicumarol treated samples, N=3 samples addressed for thrombosis risk check up left from analysis).
2. Internal inter-instruments (KC10 vs STAR) evaluation on measured PC% using Hemoclot PC (lot 83403).

1. Inter lots:



2. Inter instruments: STAR vs KC10



Conclusion: Results are consistent from lot to lot, and inter-instruments, over the dynamic range. A low PC level is detected whatever the method is. Normal plasmas are mainly measured >60%PC, a PC deficient plasma is measured <10%, VKA treated samples are measured at a lower level as expected (and confirmed lower than the level measured by chromogenic assay).

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. Biophen PC (#A221202/A221205) (CE, 510(k))
3. Zymutest PC (#ARK027A) (CE)
4. Buffers: Calcium chloride 0.025M (#AAR001A/K); Imidazole buffer (#AAR021A/K/L) or alternatively Owren koller type buffer (#AAR003A/K). (CE)