New, highly sensitive and specific multiplatform latex automated turbidimetric assay for measurement of Free Protein S in plasma

V. VILLEDIEU[1], M. PEYRAFITTE[1], G. RENAUD[1], C. DUNOIS[1] and J. AMIRAL[2]

1 HYPHEN BioMed, Neuville-sur -Oise, France.

2 SH Consulting, Franconville, France.

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CONTACT INFORMATION: www.hyphen-biomed.com

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INTRODUCTION

Clinical laboratories increasingly need robust, highly stable routine coagulation assays. Laboratory practice for Protein S testing remains variable and time consuming (ELISA), poorly reproducible (automated assays) or sensitive to interferences from coagulation factors or anticoagulant substances (clotting methods). A new ready to use, fully automated multiplatform latex immunoturbidimetric assay was developed for rapid measurement of Free Protein S antigen (FPS:Ag) in citrated human plasma, on any automated laboratory instrument.

AIM

The aim of the present study is to evaluate performances of a new immunoturbidimetric latex FPS:Ag assay that was adapted on different coagulation analyzers and to compare tests results with those obtained by reference methods on various patient plasmas.

METHOD

Free Protein S assays

The LIAPHEN™ Free Protein S is a multiplatform automated immunoturbidimetric method, based on antigen-antibody reaction: FPS antigen of the tested sample reacts with latex particles sensitized with two complementary mouse monoclonal anti-Free PS antibodies, leading to latex particles agglutination. This agglutination can be directly detected by a change of absorbance which is directly proportional to the amount of FPS:Ag.
Comparison study is performed using Innovance Free PS Ag (Siemens Healthineers, Marburg, Germany) on CS-5100 (Sysmex, Kobe, Japan) and STA® LIATEST Free Protein S on STA-R® Max (Stago, Asnières, France).

Plasma Samples

Lyophilized controls are used for performance study, frozen Free PS deficient plasmas are spiked with purified Free PS for linearity and hook effect analysis. Citrated plasma samples covering the whole FPS measuring range and used for comparison study are from healthy volunteers or patients with high and low levels of FPS:Ag. Plasma samples were stored at −70 °C until use.

RESULTS

Both normal and abnormal controls demonstrated excellent inter-assay and intra-assay precisions for all analyzers. The coefficient of variation (CV%), is from 1.2 to 6.5% (Table 1).

	Mean (FPS:Ag %)	CV% intra-series				CV% inter-series		
		CS-5100	STA-R® Max	ACL TOP®	BCS® XP	CS-5100	STA-R® Max	ACL TOP®
QC1	98	1.8	2.8	3.4	4.9	1.9	3.4	2.4
QC2	29	2.5	3.5	6.5	4.5	1.2	3.6	4.4

Table 1: Performance of LIAPHEN™ Free Protein S reagent on various analyzers using lyophilized control plasmas. Intra-series (n=40), inter-series (n=30, 10 runs, 5 days) .

The specificity is verified with FPS deficient plasma (0.6%) and reference interval in normal plasmas is from 60 to 137% (n=120, CS-5100).
Interference study is performed using 2 levels of controls and no interference is observed up to values indicated in Table 2.

	Hemoglobin	Bilirubin	Intralipids	Heparins*	DOACs**	Fibrinogen	Platelets
CS-5100	1000 mg/dL	60 mg/dL	1000 mg/dL	10 UI/mL	400 ng/mL	12 g/L	490x10^3 U/µL
STA-R® Max	1000 mg/dL	75 mg/dL	1000 mg/dL	10 UI/mL	400 ng/mL	12 g/L	474x10^3 U/µL
ACL TOP®	1000 mg/dL	75 mg/dL	1000 mg/dL	2 UI/mL	400 ng/mL	12 g/L	474x10^3 U/µL

Table 2: Interferences in LIAPHEN™ Free protein S on various analyzers using spiked lyophilized control plasmas. *Heparins tested are UFH and LMWH, ** DOACs tested are Dabigatran, Rivaroxaban, Apixaban and Edoxaban.

Dynamic range is from 6 to 300% on CS-5100 and from 10 to 200% on STA-R®, ACL TOP® and BCS® XP with automated redilution. No hook effect up to 600% on CS-5100 and to 400% on others analyzers (Figure 1).

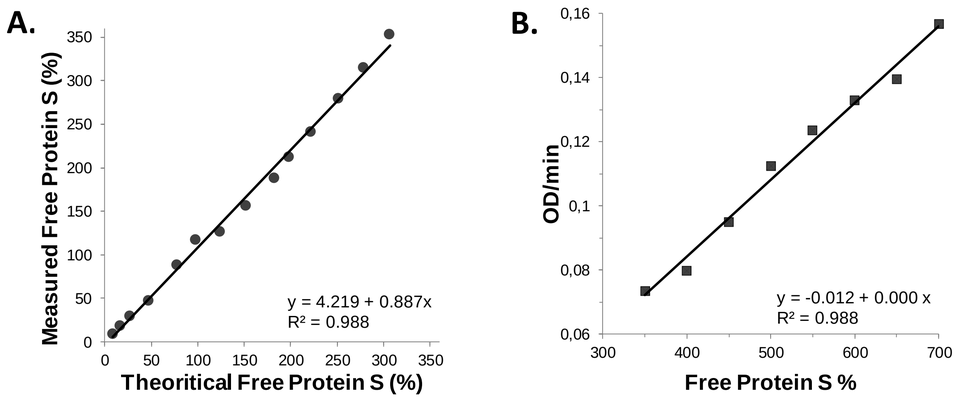

Figure 1: Linearity of LIAPHEN™ Free protein S reagent (A) and Hook effect analysis (B) on CS-5100. Tests are performed using plasmas spiked with purified Free PS.

Extended stability is tested for reagent and on board (SOB) (Table 3).

	Stability			
	CS-5100	STA-R® Max	ACL TOP®	BCS® XP
SOB	10 days	7 days(1)	7 days	
2-8°C	4 months			
18-25°C	2 weeks			

Table 3: Stability of LIAPHEN™ Free Protein S on various analyzers using spiking lyophilized plasmas. (1) STA-R® Max with reducer.

Correlation is determined using clinical samples, statistical analysis demonstrated an excellent correlation between immuno-turbidimetric methods (r = 0.990 and r = 0.972) and between analyzers (r = 0.994 and r = 0.991), results obtained are reliable and statistically equivalent at those obtained with reference methods (Figure 2).

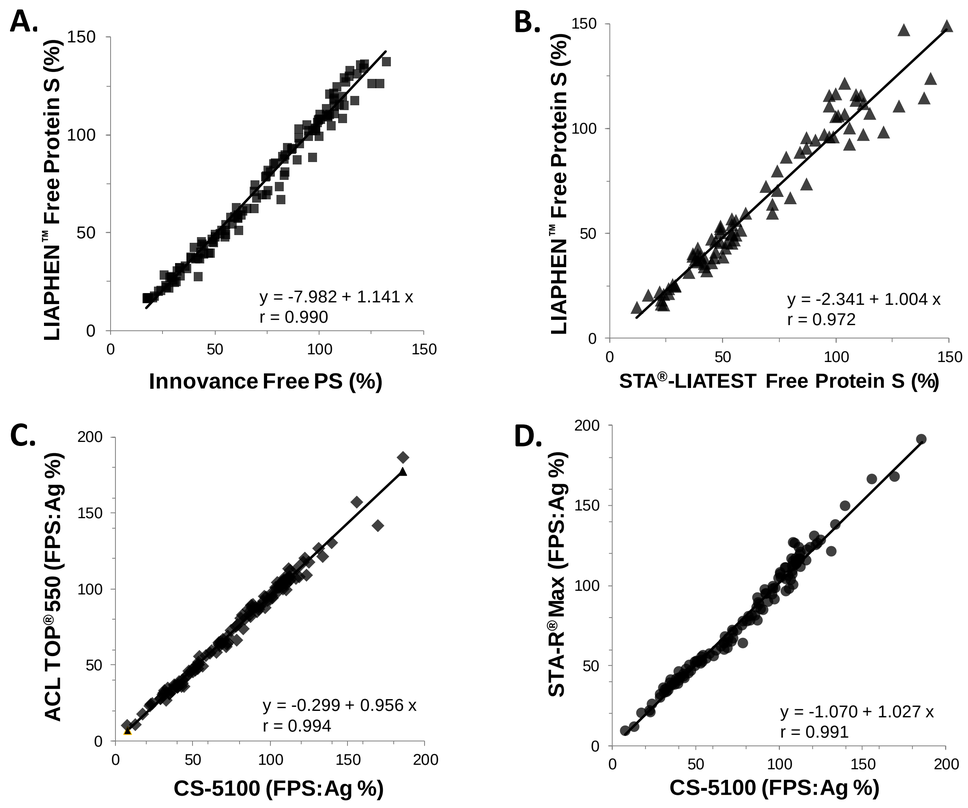

Figure 2: Correlation results of relevant samples assessed in this study and shown by regression analysis, using reference method or analyzer. (A) Correlation of LIAPHEN™ Free Protein S and Innovance Free PS Ag (n = 131), (B) Correlation of LIAPHEN™ Free Protein S and STA® LIATEST Free Protein S (n = 83), (C) Correlation of LIAPHEN™ Free Protein S on CS-5100 and ACL TOP® (n = 125), and (D) Correlation of LIAPHEN™ Free Protein S on CS-5100 and STA-R® Max (n=125). Correlation of LIAPHEN™ Free Protein S on CS-5100 and BCS®XP (n=53), y = 10.270 + 0.833x and r = 0.983 (data not shown).

CONCLUSIONS

LIAPHEN™ Free PS is a fully automatable, simple, standardized and highly sensitive assay, safe and effective, to rapidly assess Free PS concentrations in human citrated plasma. With low CVs, long stability and limited interferences, it provides a good alternative to current Free PS reagents. This polyvalent multiplatform assay can be used on all available instruments.

REFERENCES

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