



INNOVANCE Free Protein S 항원 검사의 Sysmex CS-5100에서의 수행능 평가

Analytical Performance of INNOVANCE Free Protein S Antigen on Sysmex CS-5100

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Background: Protein S deficiency is a common cause of thrombophilia. Free protein S has been suggested as one of the best screening tests for this deficiency. We evaluated an immunoturbidimetric free protein S reagent, INNOVANCE Free Protein S Antigen (Free PS Ag; Siemens Healthcare Diagnostics, Germany), using a CS-5100 coagulation analyzer (Sysmex, Japan).

Methods: The performance of INNOVANCE Free PS Ag was evaluated according to the CLSI guidelines. Precision, linearity, and verification of reference intervals were examined. The INNOVANCE Free PS Ag was also compared by the STA-Liatest Free Protein S immunoturbidimetric assay (Diagnostica Stago, France).

Results: The repeatability and within-laboratory imprecision of INNOVANCE Free PS Ag were 0.8% CV and 2.0% CV at the normal level, and 1.3% CV and 2.3% CV at the abnormally low level, respectively. This assay showed linearity from 4.0% to 151.9% (correlation coefficient $r=1$, $P<0.0001$). Reference intervals for males and females were verified as acceptable. INNOVANCE Free PS Ag was comparable with STA-Liatest Free Protein S with a very high correlation ($r=0.935$, $P<0.0001$). The results for the INNOVANCE antigen were higher.

Conclusions: The INNOVANCE Free PS Ag on a Sysmex CS-5100 coagulation analyzer has excellent analytical performance and is comparable with the STA-Liatest Free Protein S assay.

Key Words: Protein S, Immunoturbidimetry, Performance

INTRODUCTION

Protein C and protein S together play important roles in blood anticoagulation by inhibiting factors Va and VIIIa. Quantitative or functional deficiency of protein S is associated with thrombophilia [1, 2]. The prevalence of inherited protein S deficiency in the nor-

mal White population is as low as 0.01% to 1.0% [3]. Among 222 Korean patients with unprovoked venous thromboembolisms (VTEs), the prevalence of hereditary protein S deficiency was 1.8% following antithrombin III deficiency (6.3%) and protein C deficiency (5.4%), resulting in a 15% rate of hereditary thrombophilia among patients with unprovoked VTE [4]. Although the clinical usefulness of thrombophilia testing has not yet been established [5, 6], there are a few conditions for which thrombophilia testing is indicated. These include symptomatic deep vein thrombosis, thrombophlebitis, pulmonary embolism, being a female relative of a patient with thrombosis, planning a pregnancy, or considering estrogen use [7].

Approximately 40% of the protein S that circulates freely in the blood presents as a cofactor of activated protein C. Sixty percent of protein S is complexed to C4b-binding protein and inactive. Inherited protein S deficiency is subclassified into three types according to the total protein S, free protein S, and protein S func-

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Received: February 22, 2018

Revision received: July 18, 2018

Accepted: July 25, 2018

This article is available from <http://www.labmedonline.org>

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tion. To differentiate these, free protein S is a better biomarker than total protein S [8].

Free protein S can be determined by clot-based protein S activity assay or free protein S antigen assay. Although protein S activity assay has high sensitivity (>90%), its clinical specificity is not high (40% to 70%). Thus, protein S activity can cause misdiagnosis of protein S deficiency [9]. In contrast, free protein S antigen assay shows more accurate results than the activity assay [9, 10]. Free protein S antigen can be determined immunologically using ELISA and latex particle agglutination, and using monoclonal antibodies that only recognize the unbound form of protein S.

Free protein S antigen is regarded as the basis of the initial test for detecting protein S deficiency. Thus, evaluation of the test's performance is crucial. The College of American Pathology (CAP) Proficiency Testing Program has graded free protein antigen S immunoassays as being intermediate for accuracy, showing an all-method bias of 8.8%, and precision, with an all-method CV of 17.3% (range of method-specific CVs, 3.4%–44.1%) [10].

In this study, we evaluated the performance of the INNOVANCE Free Protein S Antigen (Free PS Ag; Siemens Healthcare Diagnostics, Erlangen, Germany) on a Sysmex CS-5100 coagulation analyzer (Sysmex, Kobe, Japan), using the immunoturbidimetric principle.

MATERIALS AND METHODS

1. Reagents and test analyzer

The performance of the INNOVANCE Free PS Ag was evaluated by the immunoturbidimetric detection of aggregates generated by the selective binding to polystyrene particles coated with two different monoclonal free protein S antibodies. Among the several automated coagulation analyzers the manufacturer suggested, we used the Sysmex CS-5100.

2. Samples

To evaluate precision and linearity, we used QC materials. To verify the reference interval and comparability, we used clinical samples. Normal control samples were acquired from a health examination center. Patients' samples were collected from 47 patients for whom free protein S antigen testing was ordered.

Residual samples from the patients were used for the performance evaluation. The patients' samples were drawn in 3.2% so-

dium citrate vacuum tubes, centrifuged to obtain platelet-depleted plasma, and stored them at -70°C before the experiments.

3. Precision

We evaluated the repeatability with the two levels of QC materials according to the CLSI EP15-A3 [11] by testing 20 replicates in one run. We assessed within-laboratory imprecision with the same QC materials tested at one run per day in four replicates for 7 days.

4. Linearity

We prepared five concentrations of samples according to the CLSI EP6-A [12]. The samples were mixed at differing volume ratios of low (L) to high (H): 0.75L+0.25H, 0.50L+0.50H, 0.25L+0.75H. Low (4.0%) and high (151.9%) samples were prepared using normal QC plasma. The manufacturer gave an analytical measurement range of 10% to 150%; to determine the linearity of the assay, we tested five points twice.

5. Verifying the reference interval

The reference intervals suggested by the manufacture were verified according to CLSI EP28-A3C [13]. Forty reference individuals were recruited from our health examination center. The 20 males and 20 females had normal complete blood counts, prothrombin times, and activated partial thromboplastin times.

6. Method comparison

To ensure the comparability of the INNOVANCE Free PS Ag, we compared the results of the samples from 47 patients with the results from STA-Liatest Free Protein S (Diagnostica Stago, Taverny, France) on STA-R Max (Diagnostica Stago) according to CLSI EP09-A3 [14] and using Pearson's correlation coefficient (*r*). STA-Liatest Free Protein S is an immunoturbidimetric method of measuring free protein S antigen.

7. Statistical analyses

Statistical analyses were performed using Analyse-it, v. 4.92.4 (Analyse-it Software, Ltd, Leeds, UK). Precision of the assays was determined by means, standard deviations, and CVs. Assay linearity was determined by simple linear regression. Comparison between INNOVANCE Free PS Ag and STA-Liatest Free Protein S was performed by Deming regression analysis and correlation between the two assays was assessed by Pearson's correlation coef-

ficient. We interpreted the correlations as high=0.68 to 1.0 and very high=0.90 or greater [15], with $P<0.05$ indicating statistical significance.

RESULTS

1. Precision

The repeatability CVs were 0.8% (95% confidence interval [CI]: 0.7% to 1.3%) and 1.3% (95% CI: 0.3% to 0.6%) for the normal and abnormal control plasma, respectively. The within-laboratory CVs were 2.0% (95% CI: 1.4% to 4.1%) and 2.3% (95% CI: 1.7% to 4.6%) for the normal and abnormal QC materials (Table 1). The manufacturer suggests that within-laboratory CV of an analytical system on the same lot of control plasma be $<10\%$.

2. Linearity

From the linear regression analysis, the assay was fit with a linear equation: $y=1.001x-0.8942$, $r=1.0$ ($P<0.0001$) (Fig. 1). The assay was linear from 4.0% to 151.9%, which covered the manufacturer's analytical measurement range.

3. Reference interval

The reference interval in males is from 72.8% to 131.4% with

the Sysmex CS-5100. One test result was out of the reference interval. The reference interval in females is from 64.7% to 115.3% with the CS-5100. The normal female controls in this study were within that interval except for one example.

4. Comparability

The results for the INNOVANCE Free PS Ag on the Sysmex CS-5100 varied from 33.5% to 131.6%. The INNOVANCE Free PS Ag was comparable to the STA-Liatest Free Protein S, reflected as $INNOVANCE=1.14 \times STA-Liatest+7.995$ (Fig. 2). The INNOVANCE

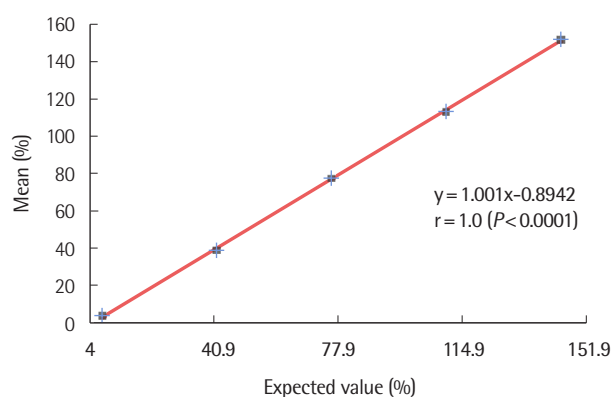


Fig. 1. Linearity of INNOVANCE Free Protein S Antigen on Sysmex CS-5100.

Table 1. Precision of INNOVANCE Free Protein S Antigen on Sysmex CS-5100

Control	Mean (%)	SD	CV (%)		Manufacturer's claim (CV, %)	
			Repeatability	Within-laboratory	Repeatability	Within-laboratory
Normal	89.5	1.78	0.8	2.0	0.8	1.2*
Abnormal	32.1	0.73	1.3	2.3	1.0	1.6*

*According to the manufacturer's insert, acceptable variability of the within-laboratory CV is $<10\%$.

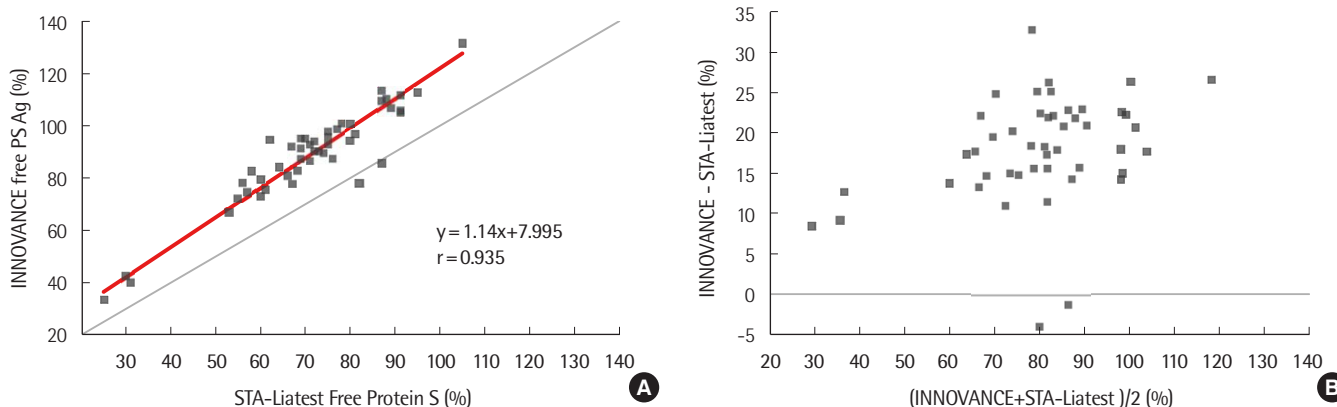


Fig. 2. Comparison of free protein S between INNOVANCE Free Protein S Antigen on Sysmex CS-5100 and STA-Liatest Free Protein S on STA-R Max. (A) Comparison with Deming regression. (B) Difference plot.

antigen showed higher results than did the STA-Liatest Free Protein S. Pearson's r for the results of STA-Liatest Free Protein S was 0.935 (95% CI: 0.885 to 0.963, $P < 0.0001$), which was a very high correlation. Among the eight specimens that were below the reference interval with STA-Liatest Free Protein S, three were below the reference interval with the INNOVANCE Free PS Ag.

DISCUSSION

In this study, the performance of the INNOVANCE Free PS Ag reagent on a Sysmex CS-5100 coagulation analyzer was good. The repeatability and within-laboratory precisions were excellent for the normal and abnormal QC materials. The linearity of the assay was also excellent, from 4.0% to 151.9%, with a Pearson's correlation of 1.0 ($P < 0.0001$). The reference intervals were acceptable for both males and females. The collective results indicated that the INNOVANCE Free PS Ag was comparable with the STA-Liatest Free Protein S.

The authors of one retrospective study warned of inappropriate thrombophilia testing in inpatient hospital settings resulting in 42.7% (777 out of 1,817) of total orders in 2 years [16]. The diagnosis of inherited thrombophilia is difficult when coagulation factors change owing to systemic causes, such as acute thrombosis, anticoagulant treatment, pregnancy, oral contraceptive use, liver disease, or kidney disease.

One should consider acquired causes of protein S deficiency, biological variations, pre-analytical aspects, and test performance to detect hereditary protein S deficiency. In one study of biological variations in hemostasis variables in 40 healthy individuals, within-subject and analytical variations of protein S antigen were reported as 7.6% and 3.3%, respectively [17].

Since protein S activity assays rely on the activated protein C-dependent clotting pathway, there are multiple interferences regarding coagulation factor changes, particularly the presence of lupus anticoagulant. Protein S activity can be spuriously low, even in up to 10% to 15% of normal plasma samples [8]. Although free protein S antigen tests do not measure the protein S activity directly, low rates of falsely low or inaccurate values and high differentiation power of protein S deficiency makes free protein S antigen as a reliable marker [8].

In this study, the INNOVANCE Free PS Ag showed higher average values than did the STA-Liatest Free Protein S. When the refer-

ence intervals suggested by the manufacturers were compared, the reference values of the INNOVANCE Free PS Ag were also higher than those of STA-Liatest Free Protein S in both males and females. Both assays of free protein S utilize latex microparticles coated with two different monoclonal antibodies specific for free protein S. One possible explanation for the discrepancy between the two assays is that free protein S assays lack of reference method or standard and they use different monoclonal antibodies with different specificity. As reported in the CAP proficiency testing program, free protein S assays showed intermediate inter-assay variability [10]. There were five discrepant male results between two assays, and these five results were within the lower 10% of the lower reference limit for STA-Liatest Free Protein S (from 57% to 61% compared with reference intervals of 62% to 154%), whereas INNOVANCE detected normal values within the upper 10% of the lower reference limit (from 73.3% to 79.5% with reference intervals of 72.8% to 131.4%) except for one value of 82.8%. Those five results are not clinically meaningful considering that the clinical cut-off for hereditary protein S deficiency is far below the reference interval [18, 19].

In conclusion, the INNOVANCE Free Protein S Antigen reagent on the Sysmex CS-5100 coagulation analyzer showed excellent analytical performance and is comparable with STA-Liatest Free Protein S.

요 약

배경: 단백질S결핍증은 혈전성향증의 공통 원인 중 하나이며, 유리 단백질S 검사가 단백질S결핍증의 최선의 선별 검사의 하나로 제시되었다. 이 연구에서는 유리 단백질S를 면역비탁법으로 측정하는 시약인 INNOVANCE Free Protein S Antigen (Free PS Ag; Siemens Healthcare Diagnostics, Germany)을 Sysmex CS-5100 응고 장비(Sysmex, Japan)에 적용하여 검사 수행능 평가를 진행하였다.

방법: CLSI 가이드라인에 따라 INNOVANCE Free PS Ag의 성능을 평가하였으며, 시약의 정밀도, 직선성, 그리고 참고구간의 검증을 평가하였다. 또한 INNOVANCE Free PS Ag 시약을 다른 면역비탁법 검사인 STA-Liatest Free Protein S (Diagnostica Stago, France)와 비교 분석하였다.

결과: INNOVANCE Free PS Ag의 반복정밀도와 검사실 내 비정밀도는 정상농도범위의 정도관리물질에서 변이계수(CV) 0.8%와 2.0%의 결과를 보였고, 비정상농도범위의 정도관리물질에서 변이계수 1.3%와 2.3%의 결과를 각각 나타내었다. 시약은 4.0%에서

151.9% 구간에서 직선성(상관계수 $r=1$, $P<0.0001$)을 유지하였다. 제조사가 제시한 참고구간은 남녀 각각에서 검증되었다. INNOVANCE Free PS Ag 시약은 STA-Liatest Free Protein S와의 동등성이 확인되었고, 매우 높은 상관관계($r=0.935$, $P<0.0001$)를 보였으나 INNOVANCE 시약의 결과가 높게 측정되었다.

결론: INNOVANCE Free Protein S Antigen을 Sysmex CS-5100 응고장비에 적용하였을 때 우수한 검사 수행능을 보여주었고, STA-Liatest Free Protein S 시약과 동등한 검사로 생각된다.

AUTHORS' DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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