



**INNOVANCE Free PS Ag Assay**

# **Advantages in free protein S antigen testing**

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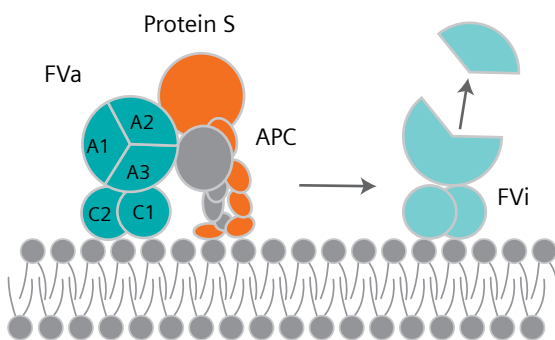
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# Protein S: Function and clinical impact

Protein S (PS) is a vitamin K-dependent plasma protein that is mainly synthesized by the liver. Within the hemostatic cascade, protein S exhibits multiple essential anticoagulant functions to control and limit procoagulant activity. Protein S acts as a nonenzymatic cofactor of two major natural anticoagulants: activated protein C (APC) and tissue factor pathway inhibitor (TFPI).

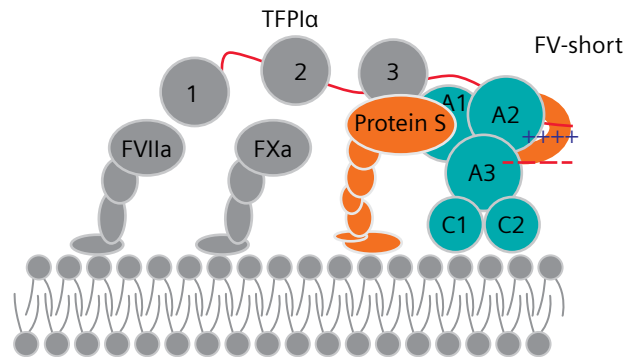
## Cofactor of activated protein C (APC)

In the presence of calcium, protein S forms a complex with APC that binds to phospholipid surfaces and accelerates the APC-catalyzed proteolytic inactivation of factors Va and VIIIa.



## Cofactor of tissue factor pathway inhibitor (TFPI)

Protein S enhances the formation of the FXa-TFPI complex and the subsequent inhibition of the TF-FVIIa complex.



Protein S is also involved in several additional nonanticoagulant functions, such as angiogenesis, apoptosis, and inflammation.<sup>1,2</sup>

Protein S circulates in human blood with a half-life of about 2 days at a plasma concentration of about 25 mg/L (350 nM), of which about 40% is free and 60% is in complex with C4b-binding protein (C4BP), a regulator of the classical complement pathway. However, only the free, noncomplexed protein S fraction exhibits anticoagulant activity.

### Subtypes of congenital protein S deficiency

Protein S Subtype	PS Activity	Free PS Antigen	Total PS Antigen	Frequency
Type I (quantitative deficiency)	↓	↓	↓	~80%
Type II (dysfunctional protein)	↓	N	N	<5%
Type III (disturbed distribution)	↓	↓	N	15–20%

N: normal, ↑: increased, ↓: decreased

Protein S deficiency can be hereditary or acquired; the latter is frequently observed due to hepatic disease, anticoagulation therapy with vitamin K antagonists, or estrogen intake, as well as in the late stages of pregnancy or inflammatory diseases.<sup>3</sup>

Protein S deficiency is a risk factor for thromboembolic diseases, although this is more clearly observed in thrombophilic families than in the general population. Free protein S levels <41% were associated with a hazard ratio (HR) of 5.6 for a first venous thrombotic event and 3.0 for a recurrent event in a pooled analysis of five large thrombophilia family cohort studies, whereas low protein S was not a thrombosis risk factor in the general population.<sup>4</sup>

Hereditary protein S deficiency can be detected in 1–13% of patients with idiopathic deep vein thrombosis (DVT), especially in younger patients less than 40 years of age. The protein S deficiency can be caused by a general deficiency of protein S (type I, quantitative deficiency, frequent); a qualitative, dysfunctional deficiency (type II, “nonactive” protein, rare); or a reduction of free, functional protein S but normal total protein S level (type III, disturbed distribution, less frequent).<sup>3–5</sup> Type I deficiency was found to be a considerably stronger risk factor than type III deficiency;<sup>5</sup> the relevance of type II is not clear due to the low number of affected patients.

Several studies have shown an association between hereditary protein S deficiency and pregnancy-associated VTE as well as adverse pregnancy outcomes, such as fetal loss, fetal growth retardation, preeclampsia, or placental abruption; however, these studies were of small size and included heterogeneous populations, so the data are not sufficient to demonstrate a definite causal link.<sup>7</sup>

Protein S is a key regulator of the coagulation cascade.

# Current recommendations for protein S testing

Thrombophilia testing in general should be performed only when the result will have an impact on further patient management. A family history of VTE or young age when presenting with VTE might trigger testing for thrombophilia, as well as repeated pregnancy complications. Recent reviews of how the presence of hereditary thrombophilia affects further management and therapy are provided by Crowley and Hunt<sup>8</sup> and the ACOG Practice Bulletin #197.<sup>9</sup>

Current recommendations consider the free protein S antigen (free PS Ag) assay to be the preferred initial test for protein S evaluation.<sup>8-12</sup> Using free PS Ag as a surrogate assessment of the PS activity (as only the free portion of PS exhibits functional activity), the vast majority (95-99%) of PS deficiencies can be detected.<sup>11</sup>

The minor disadvantage of free PS Ag is that the rare type II defect may not be detected; however, protein S activity assays are affected by a rate of up to 10–15% inaccurate results.<sup>11</sup>

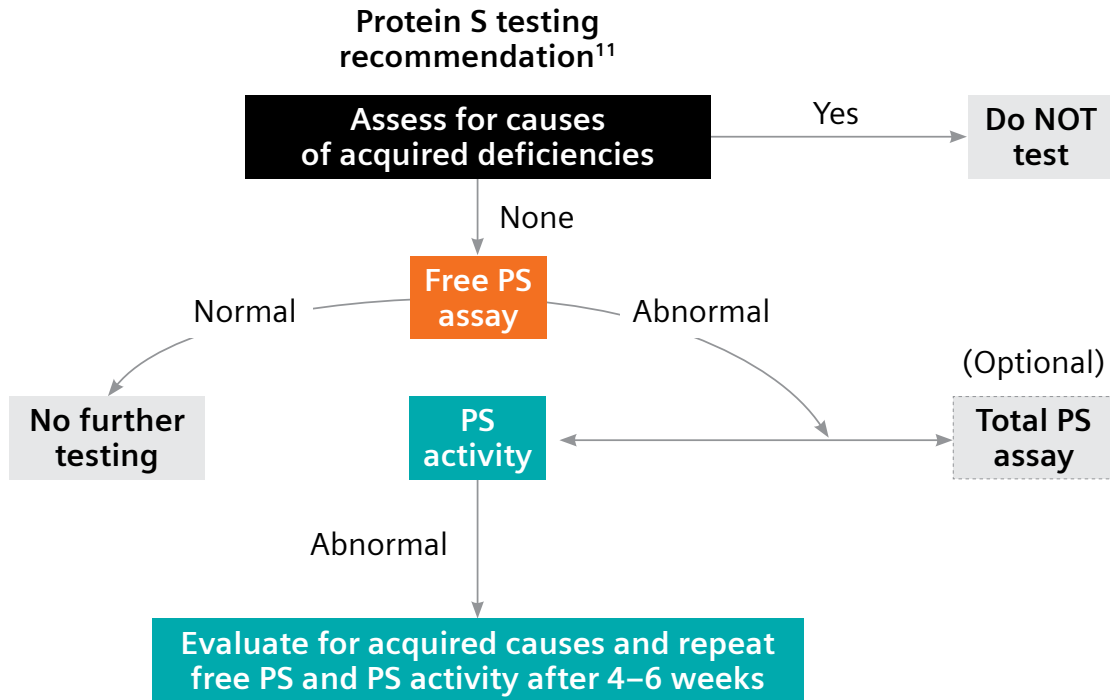
Protein S activity assays are affected by several frequent pre-analytical and analytical interferences that might produce erroneously low results, substantially reducing the specificity of protein S activity testing for diagnosis of PS deficiency.<sup>11, 12</sup>

## Potential assay interferences

	PS Activity Assay	Free PS Ag Assay
Pre-analytical interferences	Sensitivity to presence of residual platelets, or delay in testing (or freezing of sample) → <b>false-low results</b>	<b>None</b>
Analytical interferences	Depending on assay format, presence of FV Leiden or elevated FVIII → <b>false-low results</b>	Potentially interfering endogenous antibodies (e.g., Rheumatoid Factor [RF]) → <b>false-high results</b>
Medication	Direct Xa and thrombin inhibitors (DOACs), depending on assay format, and heparin → <b>false-low results</b>	<b>None</b>

## Clinical factors influencing PS activity and free PS antigen levels

Condition	Effect of Free, Active PS
Therapy with vitamin K antagonists	Reduction of PS synthesis
Acute and chronic inflammatory disease	Increase of C4b-binding protein and increased binding of protein S with reduction of free, active PS
Pregnancy	Decrease with duration of gestation
Estrogen intake (oral contraception, hormone replacement therapy)	Decrease of PS
Newborns, young children	Decreased PS compared to adults
Nephrotic syndrome	Decrease of PS due to renal loss

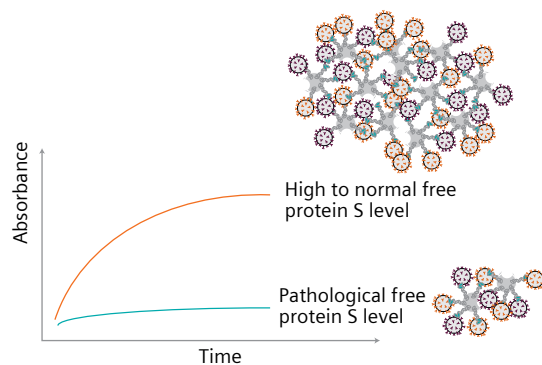
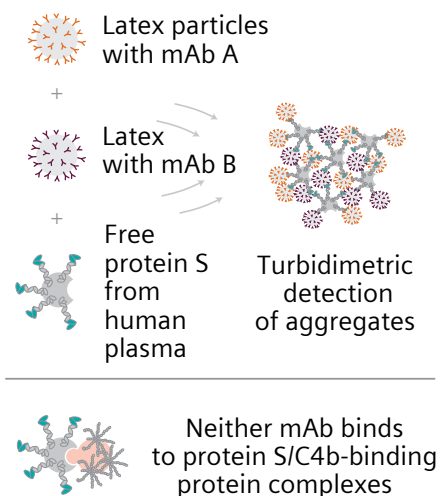


## INNOVANCE Free PS Ag Assay: Expand precision medicine through improved diagnostic accuracy

### Test principle, calibration, and traceability

The INNOVANCE® Free PS Ag Assay is based on monoclonal antibodies developed by Siemens Healthineers. The assay employs polystyrene particles covalently coated with two monoclonal

antibodies (mAb A and mAb B) that provide high specificity for free PS and do not bind to PS/C4b-binding protein complexes. As shown below, the turbidimetric assay measures the resulting antibody/free PS aggregates.



INNOVANCE Free PS Ag Assay results are provided in % of the norm. Assay calibration is performed with standard human plasma calibrated against the 2nd WHO International Standard for Protein S.

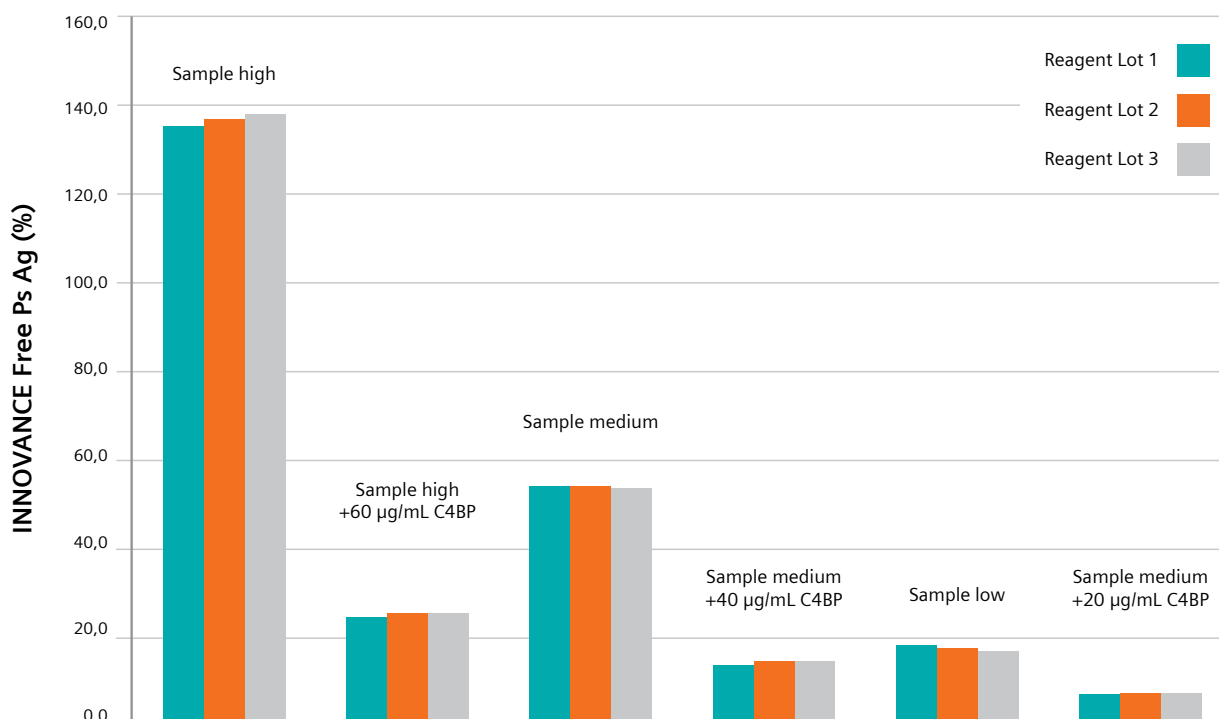
Traceability of the INNOVANCE Free PS Ag Assay to the current international standard has been demonstrated by testing three standard human plasma lots with three different reagent lots on three individual instruments per instrument type. Recovery in relation to the assigned value of the 2nd WHO International Standard for Protein S was between 103.6% and 102.9% respectively.

### High precision, linearity, antigen-excess security, and specificity for free PS ensure reliable results

The INNOVANCE Free PS Ag Assay shows excellent linearity and precision over the entire calibrated range of 10–150% of norm, with 1.01–5.29% repeatability and 1.14–5.48% within-device CVs as well as excellent lot-to-lot consistency.\*

No high-dose hook effect (prozone effect) is observed up to a tested concentration of 588% of norm free protein S, ensuring reliable results even in the presence of highly increased free protein S levels. Furthermore, the assay is robust against potential interference by lipidemia, bilirubinemia, and hemolysis and is not influenced by the presence of rheumatoid factors (up to 2500 IU/mL) or platelets (up to  $19.9 \times 10^7/\text{mL}$  in fresh or frozen plasma). Addition of a blocking reagent in the assay minimizes interference from heterophilic antibodies (e.g., human anti-mouse antibodies [HAMA]).

The INNOVANCE Free PS Ag Assay is highly specific by design, detecting only the free form of protein S without cross-reactivity to PS/C4BP complexes (total PS), as shown in the data below. A molar excess of purified C4BP in relation to the free protein S concentration was added to the patient sample, resulting in an at least 50% decrease of free PS Ag measured.



\*Date represented for Sysmex CS-5100 System.

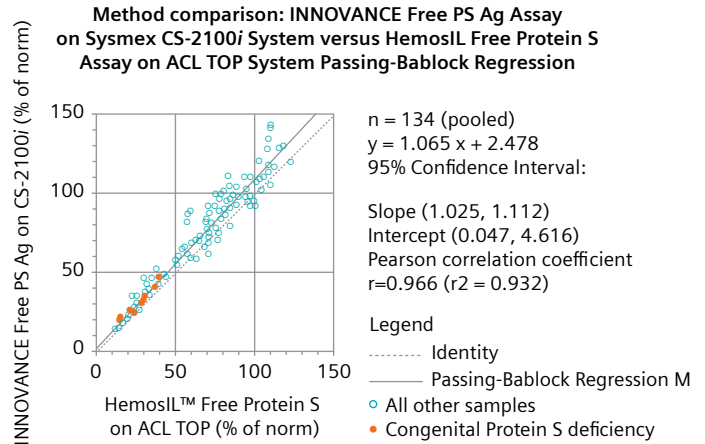
## Method comparison

INNOVANCE Free PS Ag Assay results correlate well when the assay is run on different coagulation analyzers, such as the Sysmex® CS-5100, Sysmex CS-2500, and BCS® XP Systems:

Sysmex CS-5100 System vs. BCS XP System:  
 $y = 0.945x + 4.132$ ;  $r = 0.994$ ;  $n = 346$

Sysmex CS-5100 vs. Sysmex CS-2500 System:  
 $y = 0.978x + 0.992$ ;  $r = 0.998$ ;  $n = 321$

In addition, INNOVANCE Free PS Ag Assay results correlate well with those of other assays used for free PS Ag determination, as shown on the right:



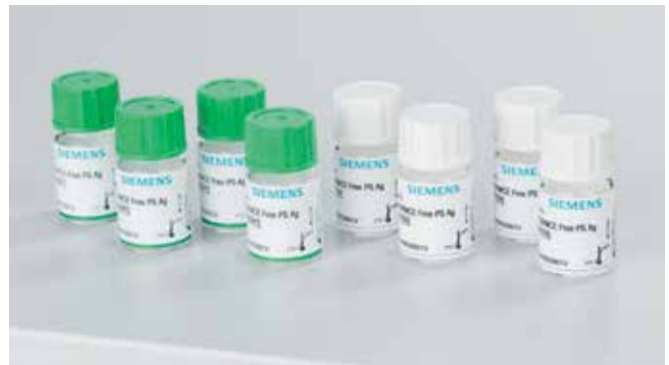
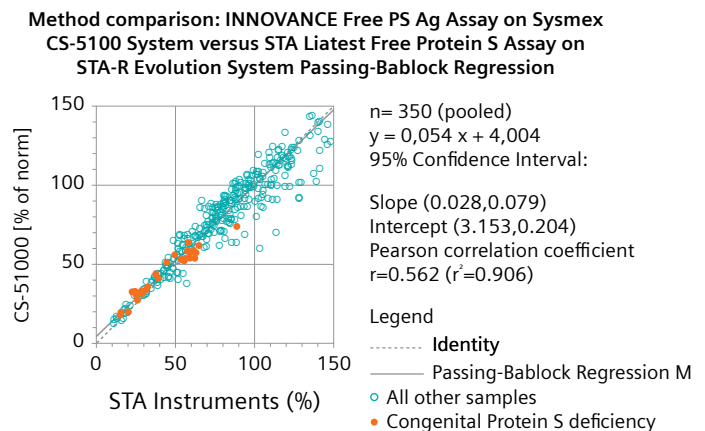
## Excellent stability for economical use

The INNOVANCE Free PS Ag Assay provides onboard reagent stability of up to 72 hours and stability of up to 8 weeks once opened. This excellent stability reduces waste and cost, allowing even the smallest labs to economically test for free protein S.

## Convenient, flexible, economical testing

The INNOVANCE Free PS Ag Assay is easy to use, reducing operator training requirements and hands-on time in the laboratory. The convenient liquid reagents require no mixing or preparation and load quickly and easily onto coagulation analyzers, providing simple handling and integration into your laboratory's normal operations.

For further convenience, flexibility, and economy, the assay is packaged in a simple, two-component kit consisting of reagent and buffer vials. One kit provides approximately 152 free protein S measurements, which are available in four reagent sets with approximately 38 tests per set. The assay uses readily available standard calibrator (standard human plasma) and controls (control plasma N and control plasma P). The INNOVANCE Free PS Ag Assay further enhances efficiency with stable calibration and open reagent stability while providing specific results.



INNOVANCE Free PS Ag Assay

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An estimated 5 million patients globally benefit every day from our innovative technologies and services in the areas of diagnostic and therapeutic imaging, laboratory diagnostics, and molecular medicine, as well as digital health and enterprise services.

We are a leading medical technology company with over 120 years of experience and 18,000 patents globally. Through the dedication of more than 50,000 colleagues in 75 countries, we will continue to innovate and shape the future of healthcare.

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