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Protein S deficiencies

Why free PS antigen assay is the first choice

There are different types of assays for Protein S on the market. Over the past decades, in many hospital laboratories, the older clot-based Protein S activity assay has been replaced with the newer Free Protein S immunoassays.[1] The major, pertinent societies and journals also insist that free Protein S antigen assays should be the first choice for screening of Protein S deficiencies.[2, 3, 4] Here is a short summary to explain why.

Official Recommendations

Protein S activity assays should be avoided and only used for special cases. Instead, free Protein S antigen assays are the primary choice.[2, 3] A review in the official journal of the International Society for Laboratory Hematology, summarizes the recommendations for Protein S testing very concisely[4]:

> - The initial or screening assay should be a free PS antigen assay.
> - The initial or screening assay should not be a PS activity assay.
> - If the free PS antigen assay is abnormal, then PS activity and total PS antigen assays should be performed to determine the deficiency type (Type I, Type II, or Type III).
> - For the determination of PS deficiency, compare the patient's value to age-appropriate reference interval and gender-specific reference intervals depending on the PS assay.
> - PS assays should not be performed during pregnancy, hormone therapy, vitamin K antagonist (VKA) therapy, or DOAC therapy.
>
> - ISTH

1 The External Quality Control for Assays and Test, managed by the ECAT Foundation, gives some insight into which assays laboratories use in Europe. For instance, in Survey 2021-M3 306 of the 395 participating labs (77%) reported results for FPS using latex immunoassays, whereas fewer labs reported results in total PS (13%) or PS-activity (43%).

2 Baker *et al.* Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. *British Journal of Haematology* 191:347–362 (2020)

3 Marlar *et al.* Recommendations for clinical laboratory testing for protein S deficiency: Communication from the SSC committee plasma coagulation inhibitors of the ISTH. *J Thromb Haemost.* 19:68–74 (2021)

4 Marlar & Gausman. Laboratory testing issues for protein C, protein S, and antithrombin. *Int. Jnl. Lab. Hem.* 36, 289–295 (2014)

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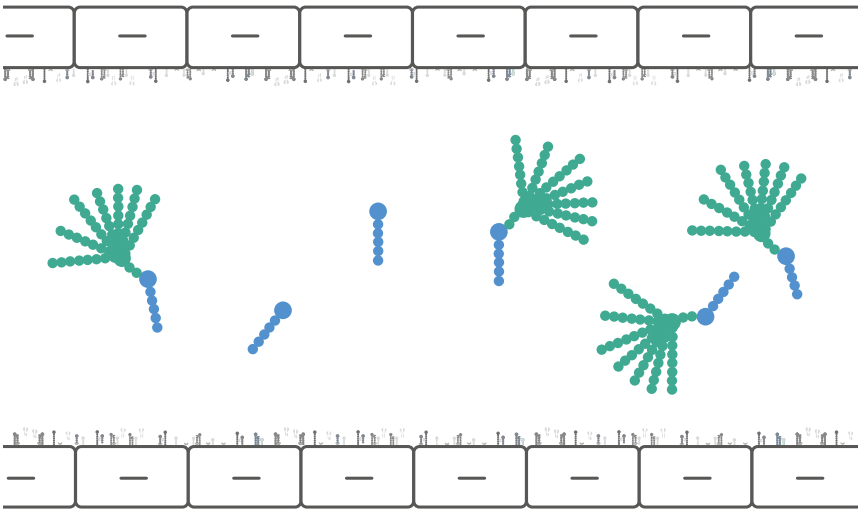
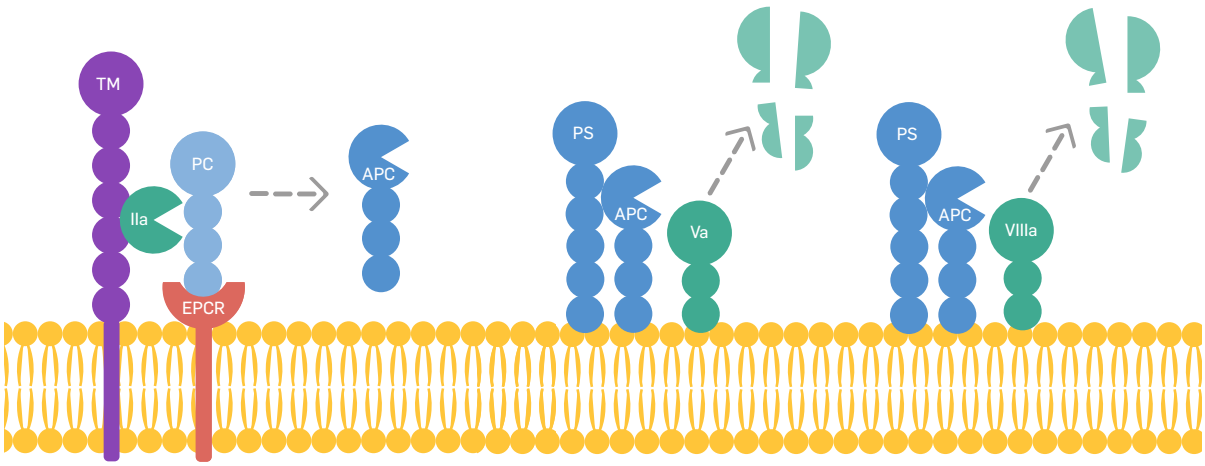
Physiological function of Protein S

Protein S is a vitamin K-dependent glycoprotein which has an important role in the regulation of blood coagulation. The normal Protein S concentration in human plasma is around 25 mg/L, and, typically, about two-thirds of this is tightly bound to C4BPβ+.



Schematic and very simplified drawing of a blood vessel, excluding most blood components other than those discussed here. In normal human plasma, roughly two-thirds of total Protein S (Blue) is tightly bound to C4BPβ+ (Green). The remaining one-third is denoted "free" Protein S (FPS).

In normal human plasma, the fraction of total Protein S which is not bound to C4BPβ+, denoted "free" Protein S (FPS), can interact with activated Protein C (APC) as a cofactor and thereby stimulating the inactivation of coagulation factors Va and VIIIa5. Thus, free Protein S has an anticoagulant function and patients with low levels have an increased risk of thrombosis. This makes it important to monitor free Protein S when investigating cases of venous thromboembolism (VTE).



When thrombin (IIa) binds to thrombomodulin (TM) it loses its procoagulant properties and instead cleaves Protein C (PC) to form activated Protein C (APC). Because the endothelial protein C receptor (EPCR), as well as TM, are anchored to the cell membrane, the process takes place primarily near the blood vessels' endothelial cells. The main activity of APC (either still bound to EPCR or directly attached to the membrane surface through its N-terminal Gla-domain) is to cut, and thereby inactivate, coagulation factors Va and VIIIa; this activity is greatly enhanced when Protein S is bound to APC as a cofactor. Protein S bound to C4BPβ+, however, cannot participate in this process, and hence only "free" Protein S (FPS) has anticoagulant activity.

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Classification of Protein S Deficiencies – Which Assays to Use

Protein S deficiency is classified into three different types. Type I patients have decreased free and total protein S, type II denoting functional deficiency with normal protein S level, and type III being characterized by low free protein S but normal concentration of total protein S.

Classification	Total Protein S	Free Protein S	Protein S activity
Type I	Low	Low	Low
Type II	Normal	Normal	Low
Type III	Normal	Low	Low

Since the Protein S activity is low in all three types, it might seem natural to use a Protein S activity assay for the initial screening. However, and as clearly explained in recent guidelines from the International Society on Thrombosis and Haemostasis[2], as well as from the British Society for Haematology[3], immunological assays for free Protein S should be used for the initial screening of Protein S deficiencies. The main reason for this is that Protein S activity assays are not reliable. Protein S activity assays are associated with false results due to several possible interferences from e.g., Lupus Anticoagulant, Factor VIII, Factor V Leiden, DOACs, Vitamin K antagonist, and Heparin.

In addition, both the intra-lab and inter-lab precision for the Protein S activity assays are poor; inter-lab CVs are often over 30% for the activity assay, but usually below 10% for FPS latex immunoassays[1]. This could be due to the short open-vial stability of the activity reagent as well as the non-user-friendly format of this assay.

These problems with many possible interferences, as well as the poor precision, makes the Protein S activity assays all too unreliable for routine use. The official recommendations are clear that they should be used only in special cases, and that free Protein S assays should be the initial test when Protein S deficiencies are suspected.