# Laboratory Testing for the Evaluation of Phenotypic Activated Protein C Resistance

Laure Morimont, PharmD[1,2] Nathalie Donis, MBS, PhD[1] Céline Bouvy, MSc, PhD[1]
François Mullier, PharmD, EuSpLM, PhD[3] Jean-Michel Dogné, PharmD, PhD[2]
Jonathan Douxfils, PharmD, PhD[1,2]

[1] Department of Research, Qualiblood Sa, Namur, Belgium
[2] Department of Pharmacy, Faculty of Medicine, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), University of Namur, Namur, Belgium
[3] Hematology Laboratory, Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Université Catholique de Louvain, CHU UCL Namur, Yvoir, Belgium

**Address for correspondence** Laure Morimont, PharmD, Rue du Séminaire 20a, 5000 Namur, Belgium (e-mail: laure.morimont@qualiblood.eu).

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## Abstract

Activated protein C (APC) resistance (APCR) is considered a risk factor of venous thromboembolism (VTE). The most common genetic disorder conferring APCR is a factor (F) V Leiden mutation, but many other factors are also implicated, such as other *F5* mutations (e.g., FV Hong-Kong and FV Cambridge), protein S deficiency, elevated factor VIII, exogenous hormone use, pregnancy and postpartum, depending on how APCR is defined. Considering the large population affected, the detection of this phenotype is crucial. Two types of tests are currently available: clotting time-based assays (with several versions) and thrombin generation-based assays with the endogenous thrombin potential (ETP)-based assay. The purpose of this review is therefore to discuss the performances of these tests and the cases in which it would be appropriate to use one over the other. Initially, as APCR was thought to be solely related to the FV Leiden mutation, the objective was to obtain a 100% specific assay. Clotting-time based assays were thus specifically designed to detect this inherited condition. Later on, an APCR condition without a FV Leiden mutation was identified and highlighted as an independent risk factor of VTE. Therefore, the development of a less specific assay was needed and a global coagulation test was proposed, known as the ETP-based APCR assay. In light of the above, these tests should not be used for the same purpose. Clotting time-based assays should only be recommended as a screening test for the detection of FV mutations prior to confirmation by genetic testing. On the other hand, the ETP-based APC resistance assay, in addition to being able to detect any type of APCR, could be proposed as a global screening test as it assesses the entire coagulation process.

**Keywords**

- activated protein C resistance
- factor V Leiden
- venous thromboembolism
- blood coagulation tests
- phenotype
- thrombin generation
- thrombophilia
- hypercoagulable state

The complex formed by activated protein C (APC) and protein S plays a crucial role in the endogenous anticoagulant pathway. This system provides an important control over the blood coagulation cascade by inactivating activated factor V (FVa) and activated factor VIII (FVIIIa).[1,2] The activation of protein C takes place on the surface of endothelial cells and involves thrombin, thrombomodulin (TM), and the endothelial protein C receptor (EPCR) (►**Fig. 1**).[3] Thrombin

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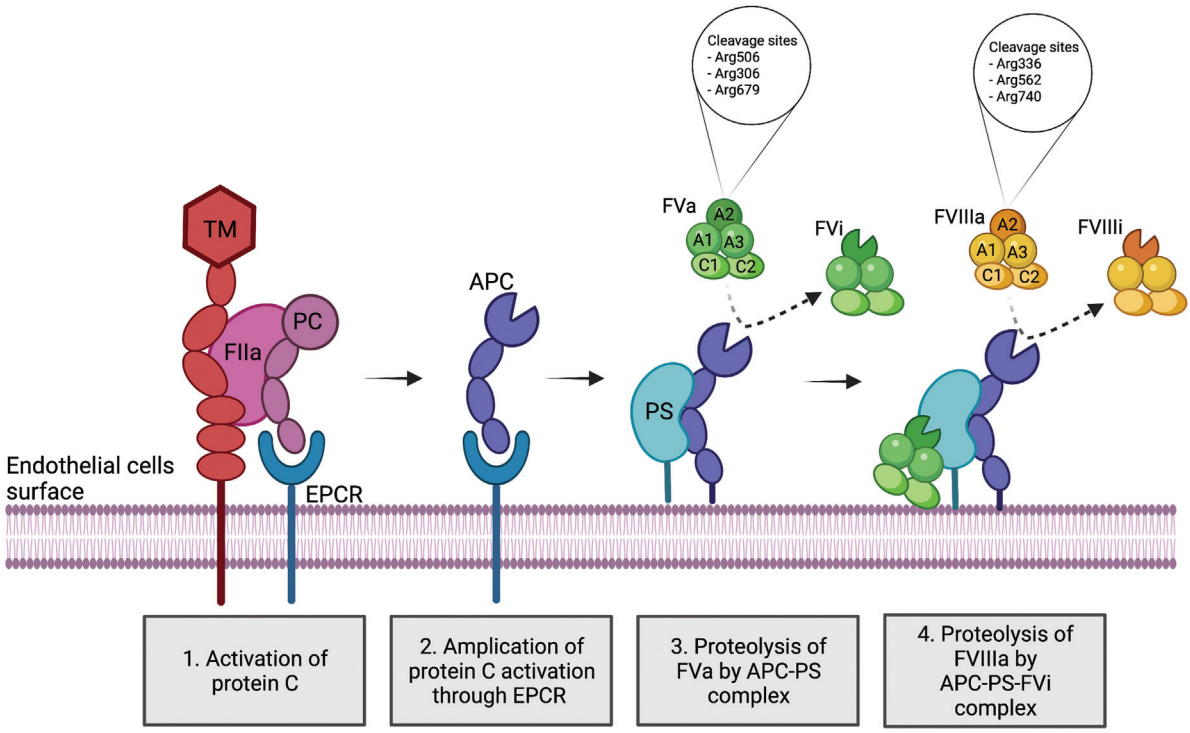


**Fig. 1** Protein C—protein S anticoagulant pathway. APC, activated protein C; Arg, arginine; EPCR, endothelial protein C receptor; FIIa, activated factor II (thrombin); FVa, activated factor V; FVi, inactivated factor V; FVIIIa, activated factor VIII; FVIIIi, inactivated factor VIII; PC, protein C; PS, protein S, TM, thrombomodulin.

promotes fibrin formation as well as platelet and endothelial cell activation but it also plays a role in the anticoagulant pathway by binding to TM to promote the activation of protein C. The EPCR then further amplifies the activation of protein C.[3] When APC is generated, it remains bound to the EPCR for a short time before associating with protein S on the surface of platelets or endothelium whereon it inhibits coagulation by degrading FVa and FVIIIa through proteolytic cleavage.[4] The proteolysis by APC occurs at amino acids position 306, 506, and 679 in FVa and 336, 562, and 740 in FVIIIa.[5] Nevertheless, the cleavage of FVIIIa is not only performed by the APC-protein S complex but also requires the inactivated form of FV as a cofactor.[2] This pathway plays a key role in controlling coagulation and its disruption can easily unbalance homeostasis, causing a hypercoagulable state associated with an increased risk of thrombosis.[6]

Functional defects in the protein C pathway, due to inherited or acquired conditions, define a plasma phenotype known as APC resistance (APCR), and is considered as a risk factor of venous thromboembolism (VTE).[5–8] The most common genetic condition conferring APCR is the FV R506Q, better known as FV Leiden mutation. A transition (guanine to adenine) at nucleotide 1691 in the gene coding for factor V results in the replacement of arginine (R) at position 506 by a glutamine (Q). This substitution makes the 506-position less sensitive to proteolysis by the complex formed by APC with protein S. This slows the inactivation rate of FVa by 10-fold, resulting in an increased thrombin generation and a hypercoagulable state.[9–12] This genetic risk factor for VTE is found in 20% of patients with a first VTE event and in 50% of familial thrombosis.[12] Heterozygosity for FV Leiden occurs in 3 to 8% of the general U.S. and European population, while homozygosity occurs at a frequency of approximately 0.02%.[13,14] Compared with healthy individuals, the risk of first VTE event is threefold higher in heterozygous carriers whereas it may reach a relative risk (RR) of 30 to 80 in homozygous carriers.[13,15]

Other less frequent mutations on the *F5* gene have also been identified and are FV Cambridge, FV Hong Kong, FV Bonn, FV Nara, FV Besançon, and FV Liverpool.[16–19] FV Cambridge and FV Hong Kong, affect the Arg306-cleavage site of FV. Arginine is replaced with threonine in FV Cambridge and with glycine in FV Hong Kong. Although FV Cambridge mutation is uncommon, the prevalence of FV Hong Kong is approximately 4% in Chinese population.[20] Nevertheless, as the anticoagulant APC-cofactor activity of FV is essentially related to the 506-cleavage site rather than the 306-cleavage site, these mutations cause a moderate APCR and the increased risk of thrombosis associated with these two mutations remains unclear.[18,20] Additional missense mutations such as the FV Besançon (Ala2986Asp), FV Liverpool (Ile358Thr), FV Nara (Trp1948Arg), or FV Bonn (Ala512Val) had also been associated with an increased risk of thrombosis as they seemed to impact either FV levels or the anticoagulant activity of inactivated FV, therefore hampering the inactivation of FVIIIa.[16,19,21,22] Finally, the FV HR2 haplotype, characterized by multiple linked missense or silent mutations is associated with a slight decrease

 

in circulating FV levels due to an impaired secretion of FV. The increased risk of thrombosis associated with this condition is ambiguous but combined with a heterozygous FV Leiden mutation, the degree of APCR is similar to that observed in homozygous FV Leiden carriers, hence it is characterized as pseudo-homozygous APC resistance.[23–25]

As protein S and FVIII are also part of this regulatory pathway, protein S deficiency or high FVIII levels could lead to an APCR phenotype. Hereditary protein S deficiency is a relatively rare disorder with a prevalence of 0.03 to 0.13% in the general population (although more frequent in Japan and China) but the associated RR of VTE was estimated between 5.0 and 11.5 compared with wild-type individuals.[13,26] Regarding high FVIII levels (>150 IU/dL), a risk ratio of VTE of 4.8 was determined compared with normal individuals (FVIII levels< 100 IU/dL).[27,28]

In addition, the hormonal status of women is also a condition which confers a resistance to APC. Pregnancy and postpartum periods, as well as administration of exogenous hormones, such as combined hormonal contraceptives (CHCs) or hormone replacement therapies (HRTs) during menopause, expose women to hormonal changes, and are associated with an increased risk of VTE. Indeed, compared with non-pregnant non-user, a fivefold increased risk of VTE is reported during pregnancy, and depending on the estro-progestative association, the RR varies between 1.3 and 5.6 in women using CHCs or HRTs.[29–36]

Pregnancy and the use of CHCs or HRTs cause changes in plasma levels of almost all proteins involved in coagulation and fibrinolysis.[37] These changes might be considered as relatively modest when measured separately but they could have a supra-additive effect leading to a pro-coagulable state responsible for this increased risk of VTE.[38] Overall, rises in coagulation factors II, V, VII, VIII, IX, X, XI, XII, and von Willebrand factor (VWF), as well as fibrinogen levels are observed. On the other hand, antithrombin, free protein S, and tissue factor pathway inhibitor (TFPI) levels, three proteins contributing to the anticoagulant system, are decreased.[39–42] As for fibrinolysis, there is an increase in plasminogen levels but a decrease in tissue plasminogen activator antigens and plasminogen activator inhibitor-1 levels.[37] These hormonal changes, both during pregnancy and following the use of hormonal therapy, are also associated with APCR.[38,43] This phenomenon has been first described in 1995 by Henkens et al[44] and Olivieri et al[45] and is now largely documented. Afterward, APCR became an important biomarker to evaluate the increased risk of VTE associated with CHCs, leading, in 2005, to the Committee for Medicinal Products for Human Use of the European Medicines Agency stating that APCR should be investigated during the development of new steroid contraceptives in women.[46]

Given the countless number of people with APCR, whether acquired or inherited, its detection is of great interest. Over the years, numerous assays have been developed and some of them might have the potential to be used as a screening tool with the aim to identify a hypercoagulable state, yet without determining the underlying cause. The purpose of this review is therefore to discuss the performances of these tests and the cases in which it would be appropriate to use one over the other.

## Laboratory Testing for APC Resistance

### Clotting Time-Based Assay

The original assay for screening APCR was described by Dahlbäck et al in 1993 and consisted of an activated partial thromboplastin time (aPTT)-based method to determine the sensitivity of a patient's plasma after addition of exogenous APC.[47] The aPTT assay is based on the principle that in citrated plasma, the addition of phospholipids (PLs), activator of FXII (e.g., micronized silica or ellagic acid), and calcium chloride triggers the formation of a stable clot. The time between activation and clot formation is recorded in seconds and represents the aPTT.[48]

When exogenous APC is added, the aPTT is prolonged, but in plasma from patients with APCR, this prolongation is less pronounced.[47] Indeed, in case of a FV Leiden mutation, the anticoagulant APC-protein S pathway is less effective, resulting in a shorter clotting time compared with a normal plasma in the presence of exogenous APC. Results are usually expressed as a ratio between the aPTT (+APC) and the aPTT (−APC). Values >2.0 are expected in normal populations while a FV Leiden mutation typically gives a ratio <2.0. Nevertheless, each laboratory must verify its own cutoff since it may differ according to the kit used, as recently reported in several external quality control surveys.[49–51] Results may also be reported as normalized ratio, which is the ratio of the patient's APC ratio divided by the ratio of a normal pooled plasma (NPP) run on the same day; however, normalization against NPP does not improve the diagnostic performance, and unknown FV status in the donor population affects the accuracy.[52] These external quality control surveys not only reported important discrepancies between the different kits in the market but also within a particular kit, revealing that improvements have to be done for the proper screening of FV Leiden-induced APC resistance.[47,50,51] In addition, other FV mutations, e.g., FV Hong-Kong and Cambridge, may lead to intermediate APC resistance ratios compared with wild-type FV and FV Leiden.[18]

The very first aPTT-based APC resistance assay was subject to multiple variables, hampering the interpretation of the result. Anticoagulant drugs (e.g., vitamin K antagonists, heparins, direct FXa inhibitors and direct thrombin inhibitors) and factor deficiencies tended to falsely increase the APC ratio while protein S deficiency, lupus anticoagulant, pregnancy, and hormonal therapy (e.g., HRTs, CHCs) tended to decrease it, leading to acquired APCR.[53] To avoid these interferences and focus on the detection of FV Leiden, modifications were implemented such as the addition of a heparin neutralizer (e.g., polybrene) or even the dilution of patient's plasma with a « FV deficient » plasma, in order to become insensitive to abnormal coagulation factors levels other than FV. The normalization against NPP was also. Other alternative methods trigger the coagulation cascade directly

 

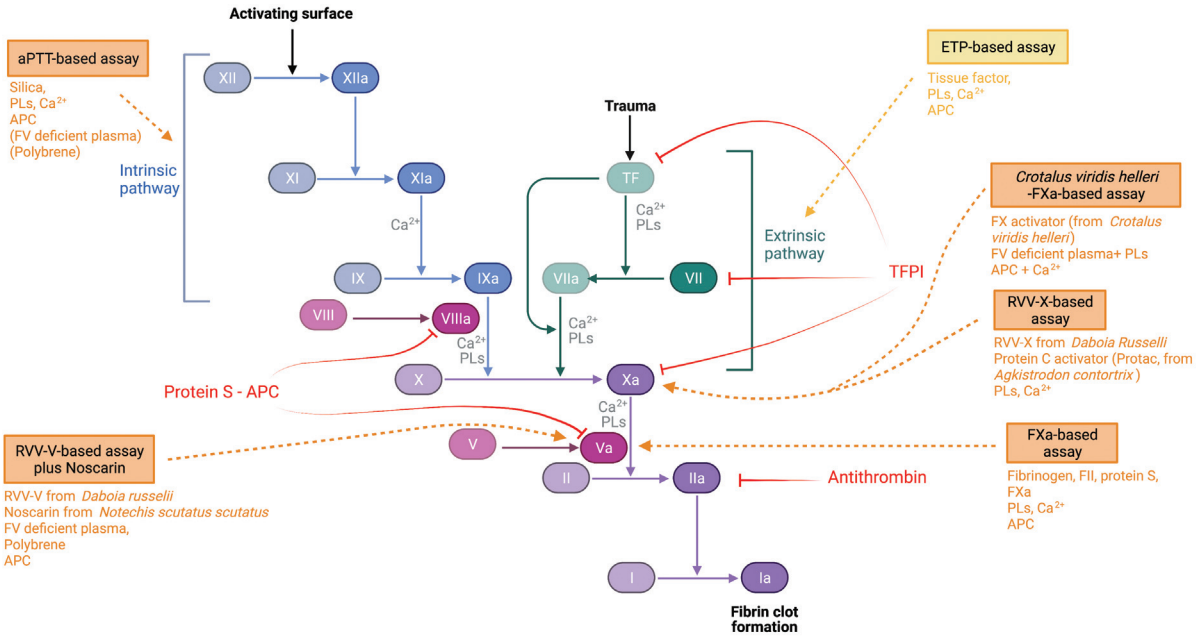


**Fig. 2** Simplified overview of the coagulation cascade and activated protein C (APC) resistance assays. APC, activated protein C; aPTT, activated partial thromboplastin time; ETP, endogenous thrombin potential; PLs, phospholipids, TF, tissue factor; TFPI, tissue factor pathway inhibitor.

through the activation of FX, by using either snake venom from *Crotalus viridis helleri* or from Russel Viper Venom (RVV-X, a snake venom extracted from *Daboia russelii*) or through the activation of FV by using RVV-V (also from *Daboia russelii*) plus Noscarin, an FV-dependent prothrombin activator extracted from *Notechis scutatus scutatus*.[54] This latter assay is sometimes referred as a prothrombin-based APCR assay,[55–57] and it does not require the presence of calcium ions and PLs, therefore allowing to eliminate the influence of lupus anticoagulant.[55] Beside these snake venom-based assays, the FXa-based assay is a test for which the patient's plasma is diluted in a proprietary reagent containing FII, fibrinogen, protein S, and APC. Purified FXa, PLs, and calcium are then added to initiate the coagulation.[49,53,58] In parallel, a calibration curve derived from dilutions of heterozygous FV Leiden plasma pool converts clotting times to percentages. The prolongation of the clotting time is an inverse relationship to the FV Leiden concentration present in the tested specimen. Normal FV presence does not affect this test. The expected value in a FV Leiden heterozygote stands between 25 and 75% and for a homozygous, it is above 75%.[49,53,58] The activation pathways and clotting mixtures of these clotting-time based assays are shown in ►**Fig. 2** and the commercially available kits are listed in ►**Table 1**.

These modified assays have been developed to detect the phenotype associated with a FV Leiden mutation specifically, although other FV mutation may lead to APCR as well, and which may be variably identified in APCR assays, depending on assay sensitivities and the assay cutoffs used.[18] Compared with genetic analysis using polymerase chain reaction technology, these functional tests are much less expensive and present a shorter turnaround time. Furthermore, as these clotting time-based assays show a sensitivity and a specificity of almost 100% for the detection of FV Leiden mutation, it could make sense to abstain from genetic testing.[55] However, their high specificity may be considered as a limitation as they may not be able to detect any resistance to APC, induced by either intrinsic or extrinsic factors (e.g., elevated FVIII

**Table 1** Commercially available clotting time-based assays for APC resistance evaluation

| Assay name | Commercial kit | Laboratory |
|---|---|---|
| aPTT-based assay | HemosIL Factor V Leiden (APC Resistance V) | Werfen |
| | Chromogenix Coatest APC Resistance | Werfen |
| | Chromogenix Coatest APC Resistance – V | Werfen |
| RVV-X-based assay | ProC Ac R Assay | Siemens |
| *Crotalus viridis helleri*-FXa based assay | STA-Staclot APC R | Stago |
| RVV-V plus Noscarin | Pefakit-APC-R Factor Leiden | Pentafarm |
| | Acticlot Protein C resistance | Sekisui |
| FX-based assay | HemoClot Quanti-V Leiden | HYPHEN BioMed |

 

levels, protein S deficiency, pregnancy and postpartum, or even the use of CHC and HRT), or other *F5* mutations.[24,59–61] For this reason, Nicolaes et al developed in 1997 a new method to assess APCR resistance based on a global coagulation test instead of clotting time-based assay, known as the endogenous thrombin potential (ETP)-based APC resistance assay.[62]

### Thrombin Generation-Based Assay

The ETP-based APC resistance assay was developed to assess acquired APCR in pregnant women, women taking oral CHCs or HRTs, and in subjects with protein S deficiency, or carrying either an FV Leiden or a G20210A prothrombin mutation.[63]

This test is a variant of the thrombin generation assay, a global coagulation test enabling a continuous overview of clotting compared with the standard coagulation assays like aPTT, which retrieve only a clotting time result and represent approximately 5% of thrombin generation. The ETP-based APCR assay is based on the measurement of thrombin generation in presence and in absence of a defined amount of exogenous APC.[64] The activation of coagulation occurs via the extrinsic pathway, following the addition of PLs, tissue factor (TF), and calcium (►**Fig. 2**). In the absence of APC, the resulting thrombin generation curve reflects all the pro- and anticoagulant reactions that regulate both thrombin formation and inhibition. In the presence of APC, thrombin generation is significantly decreased in a normal plasma sample (i.e., approximately 90%, see below). The end point of the test, which is the total amount of thrombin that has been generated over time, is quantitated by the ETP which corresponds to the area under the thrombin generation curve (►**Fig. 3**).[38]

The amount of APC introduced in the test, to obtain a good sensitivity and to limit the inherent variability of the assay, targets a decrease of 90% of the ETP of a healthy pooled plasma, i.e., a pool of plasma constituted of men and women of childbearing age not using a hormonal contraceptive therapy. In other words, the ETP retrieved in presence of APC, represents only 10% of the baseline ETP (in absence of APC).[64]

Results are expressed as a ratio, the normalized APC sensitivity ratio (nAPCsr), computed as the ratio of the ETP measured in presence and absence of APC in the tested plasma divided by the same ratio of the reference plasma (e.g., a healthy pooled plasma) (Eq. 1). As the denominator value is close to 0.1, this allows to obtain a scale ranging from 0 to 10. Importantly, conversely to the aPTT-based assay, the higher the nAPCsr, the more resistant the patient is to APC.

$$\mathrm{nAPCsr} = \frac{SampleplasmaETP\ (+APC) \,/\, SampleplasmaETP\ (-APC)}{ReferenceplasmaETP\ (+APC) \,/\, ReferenceplasmaETP\ (-APC)} \quad \text{(Eq. 1)}$$

The test was initially performed on a thrombin generation system based on the cleavage of a chromogenic substrate specific for thrombin.[62] Because of technical and methodological difficulties limiting this technique[65] and because of the technological advance brought by the calibrated automated thrombogram (CAT) developed by Hemker et al in the early 2000s,[66] the ETP-based APC resistance assay was adjusted on a fluorometric technique.[65,67] However, the inherent limitations of the CAT system, such as the lack of standardization of the reagents, the absence of quality controls, and reference plasma and the batch-to-batch variation, were also applicable for this assay.[68–71] Indeed, besides the differences imposed by a chromogenic or a fluorogenic assay, with the use of defibrinated plasma or platelet-poor plasma, respectively, differences in the source and concentration of TF, APC, and PL vesicles, led to variable sensitivity of the assays toward the APCR.[38,72] This hampered study-to-study comparisons which *in fine* impeded the proper evaluation of APC resistance induced by hormonal therapies or the evaluation of prothrombotic states. For this reason, this test has long been put aside.

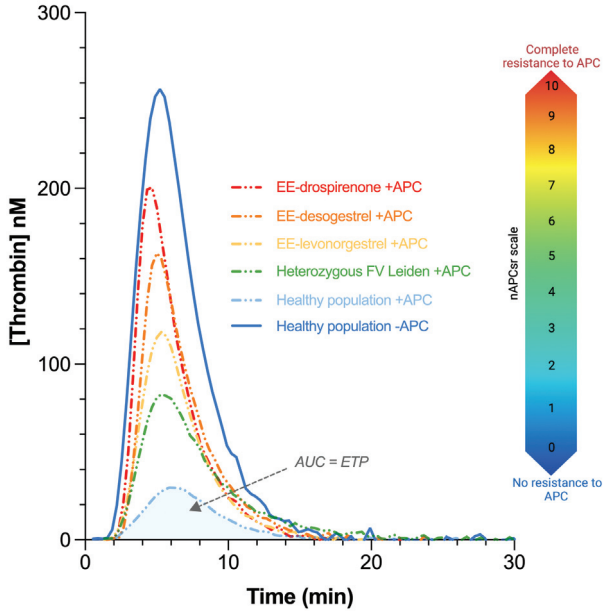


**Fig. 3** Thrombin generation curves in absence (*continuous lines*) and in presence of APC (*dotted lines*) of healthy donors (*blue*), of a woman carrier of a heterozygous FV Leiden (*green*) and of women using combined oral contraceptives containing either ethinylestradiol (EE) with levonorgestrel (*yellow*), with desogestrel (*orange*) or with drospirenone (*red*). APC, activated protein C; AUC, area under the curve; EE, ethinylestradiol; ETP, endogenous thrombin potential; nAPCsr, normalized APC sensitivity ratio.

In 2019, Douxfils et al proposed a standardized methodology that met all the standard requirements imposed on clinical biology laboratory tests in terms of analytical performances (i.e., Food and Drug Administration "Guidance for Industry: Bioanalytical Method Validation"[73] and International Council for Harmonization Q2 [R1] "Validation of Analytical Procedures: Text and Methodology"[74]). The ETP-based APCR was validated on the CAT device using commercially available reagents to ensure batch-to-batch traceability, recovery, and reproducibility of the method over time.[64] This enabled the reduction of inter-laboratory variability and allowed laboratory-to-laboratory and study-to-study comparison and evaluation.[75] Ultimately, this validation provides

 

pharmaceutical industries, regulatory bodies, and physicians with a reproducible sensitive and validated assay that could be proposed as a gold standard for the assessment of all types of APCR.

This test, being performed in standardized conditions, quantifies the degree of APCR through the nAPCsr scale ranging from 0 to 10. As shown in ►**Fig. 3**, APC resistance, characterized by a higher thrombin generation curve in presence of APC, is observed in carriers of a FV Leiden mutation as well as in women using CHCs. Besides, significant differences can be observed depending on the estrogen–progestogen association (e.g., levonorgestrel-containing products vs. desogestrel- or drospirenone-containing products) of CHCs. In addition, the presence of both FV Leiden mutation and CHC leads to a supra-additive effect which is reflected by higher nAPCsr values compared with CHC use or FV Leiden mutation alone (►**Fig. 4**).

However, this test has some limitations. Like the aPTT-based APC resistance assay, the ETP-based assay is sensitive to anticoagulants. The addition of polybrene in the triggering reagent allows to neutralize heparin in plasma samples, which allows to fully restore thrombin generation in the presence of concentrations of unfractionated heparin and low molecular weight heparin (e.g., enoxaparin) up to 1.0 and 1.2 IU/mL, respectively.[76] Nonetheless, this has not yet been investigated in the presence of exogenous APC and deserves further investigations. Regarding the use of direct oral anticoagulants (DOACs), there are three commercially available DOAC removing agents: DOAC Stop, DOAC Remove, and DP-Filter. However, these charcoal-based agents showed a slight procoagulant effect on thrombin generation at the medium TF concentration used in the ETP-based APC resistance assay. Indeed, in absence of anticoagulant, they induced a higher peak, a higher mean velocity rate index, and a lower time-to-peak compared with a non-treated plasma and this impact had been associated with a decrease in TFPI levels of plasma samples.[77,78] As a result, diminished TFPI levels could impact the ETP-based APC resistance assay as this physiological anticoagulant protein plays an important role.[61] Indeed, TFPI inhibits FXa and subsequently TF and FVIIa by forming an inactive FXa-TFPI-TF-FVIIa quaternary complex. If TFPI levels decrease, FXa levels increase, therefore protecting FVa from inactivation by APC and reducing sensitivity for APC.[61]

According to the recent communication from the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, a current barrier to its use, which could be eliminated in the future, is the unavailability of this technique in clinical routine.[53] Indeed, the validated methodology has recently been transferred on an automated thrombin generation platform, the ST Genesia system. Data revealed good reproducibility (standard deviations of 2.0 and 3.5% for within- and between-run reproducibility respectively), appropriate sensitivity toward hormonal therapies (significant differences between healthy individuals and women using various CHCs), and comparable results to those obtained with the validated methodology on the CAT system (Pearson correlation coefficient [95% confidence interval] of 0.9497 [0.9168–0.9697], based on 60 plasma samples).[79]

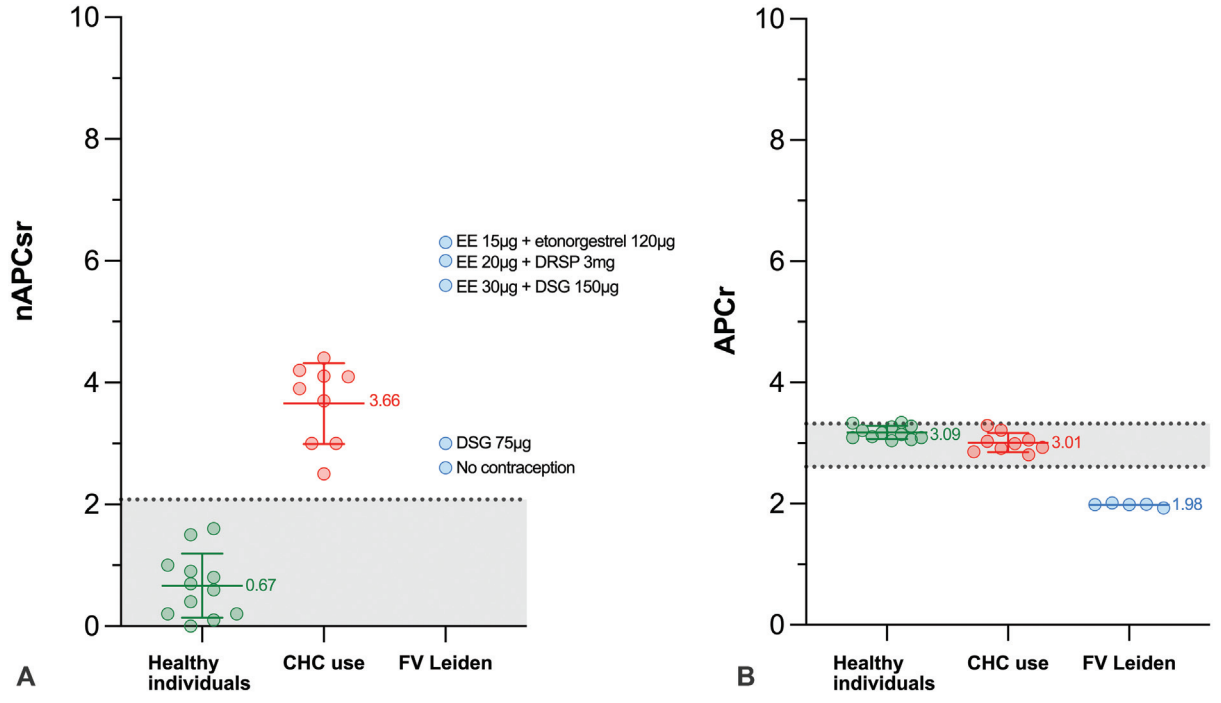


**Fig. 4** Assessment of APC resistance of healthy individuals ($n = 12$), carriers of heterozygous FV Leiden mutation ($n = 5$), and women using combined hormonal contraceptives ($n = 9$), using (**A**) the ETP-based or (**B**) the aPTT-based (with predilution) APC resistance assays. Gray areas on both graphs represent normal ranges. APCr, activated protein C ratio; CHC, combined hormonal contraceptive; EE, ethinylestradiol; DRSP, drospirenone; DSG, desogestrel; nAPCsr, normalized activated protein C sensitivity ratio.

 

## In Practice When Should These Tests Be Used?

APCR testing is not indicated in unselected patients presenting with VTE and even less for prevention of thrombotic events. Indeed, the evaluation of APCR is only recommended for situations in which the test result may give an indication of the recurrence risk or influence the anticoagulant treatment. Typically, it concerns patients suffering from VTE before the age of 40 and patients who are from apparent thrombosis-prone families (>2 other symptomatic family members).[80]

As the FV Leiden mutation is more common than acquired APCR and other *F5* mutations, the current recommendations call in first place, the use of clotting-time based assay with plasma predilution.[53] However, APCR without FV Leiden mutation represents an independent risk factor of VTE,[7,8] so there is a value in having a less specific assay, able to detect any APCR phenotype. The ETP-based APCR assay is capable of doing so, which makes it less specific and therefore not able to identify the underlying cause of APCR.

Thus, both test types certainly assess APCR, but they should not be used for the same purpose. Moreover, inconsistent results, as shown in ►**Fig. 4**, are typically observed between these two assays. Clotting time-based assays should only be recommended as a substitute of genetic testing for FV Leiden mutation and not for the evaluation of APCR during, for example, the development of steroid contraceptives in women, although it is mentioned in the EMA guidelines (EMEA/CPMP/EWP/519/98 Rev.1).[46] In contrast, the ETP-based APCR assay has demonstrated, for over 20 years, its ability to detect any APCR phenotype, and especially, those associated with female hormonal changes, although it was neither validated nor standardized until recently.[64] This test not only detects the presence of acquired APCR but it also correlates with the RR of VTE associated with the different CHC formulations available in the market.[81] Despite the fact that CHCs are used by over 150 million women worldwide,[82] no clear risk minimization strategy to manage the risk of VTE has been implemented. Overall, the absolute risk of VTE remains low (i.e., 5 to 12 for 10,000 women a year). However, given the large number of CHC users, 22,000 thrombosis related to CHC use would occur each year in Europe.[83] The worldwide use of HRT is certainly lower, but the annual incidence is higher with three to four cases of VTE per 1,000 women.[36,84,85]

As the field of personalized medicines is currently expanding, a biomarker capable of reflecting the "coagulability status" of individuals would be of great interest. Indeed, VTE is a multifactorial disease whose occurrence depends on the interaction between gene defects and environmental factors.[86] As a result, exposure to high-risk situations such a surgery, trauma, immobilization, pregnancy, or hormonal therapy may trigger a thrombotic event in individuals either in absence or presence of genetic mutations. This suggests that the evaluation of thrombophilia should be based on phenotypic expression rather than only focusing on genotypic expression.

The ETP-based APC resistance assay, reflecting all the pro- and anticoagulant reactions might be a potential candidate. Indeed, the typical information obtained by thrombin generation investigation (i.e., without exogenous APC added) is available, providing much more information than the APC resistance itself. As it enables a more global assessment of coagulation process, this assay is also sensitive toward other factors of thrombogenicity like the prothrombin G20210A mutation, antithrombin, and protein S deficiencies or high FVIII levels.[87–90]

A method able to assess the entire coagulation process is valuable, as this can better reflect bleeding and thrombotic risks as compared with clotting time-based assays.[91] Indeed, the end point of clotting-time based assays occurs after the formation of only 5% of total thrombin, which means that recorded clotting times only correspond to the initiation phase of the coagulation process.[91]

## Conclusion

A global screening test could be the key to detect prothrombotic phenotypes associated with an increased risk of VTE. The ETP-based APCR assay, by considering the entire coagulation process and assessing thrombin generation in two different conditions (with and without exogenous APC), allows the identification of a large panel of prothrombotic states. Currently, the evaluation of a complete thrombophilia panel requires multiple coagulation tests, which can make the interpretation of the results expensive and challenging. Indeed, changes in coagulation factors levels may not exceed their respective normal ranges, when assessed individually. On the other hand, the increased thrombogenicity, resulting from the additive effect of these changes could be captured by the ETP-based APC resistance assay. This represents an interesting approach that needs further clinical validation to assess and score a hypercoagulable state that would help the clinician in decision-making.

**Conflict of Interest**

Dr. Bouvy reports other from QUALIblood, during the conduct of the study; Dr. Douxfils reports other from Qualiblood, during the conduct of the study; personal fees from Stago Diagnostica, personal fees from Daiichi-Sankyo, personal fees from Portola, personal fees from Roche, personal fees from Roche Diagnostics, personal fees from DOASENSE, personal fees from Mithra Pharmaceuticals, personal fees from Estetra, personal fees from Werfen, personal fees from Technoclone, outside the submitted work; Dr. Morimont reports other from QUALIblood, during the conduct of the study; and Dr. Donis reports other from QUALIblood, during the conduct of the study. The remaining authors do not report any conflicts of interests.

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