To the Editor: 

Resistance to activated protein C (APC), caused by a mutated coagulation factor V (factor V Leiden), has been reported as a predisposing factor in a large fraction of patients with venous thromboembolism. Diagnosis of APC resistance is currently based upon measuring the ratio of activated partial thromboplastin times (APTTs) in the presence and absence of exogenous APC. However, determination of this APC resistance ratio is not reliable in patients receiving anticoagulant therapy. In their recent review of inherited thrombophilia in Blood, De Stefano et al cite unpublished observations indicating that predilution of the test plasma with factor V deficient plasma may increase the sensitivity and specificity of the APTT-based APC resistance assay for detection of mutant factor V. We have developed a modified APC resistance assay which not only includes factor V deficient plasma as a consistent supply of vitamin K-dependent coagulation factors and inhibitors, but also an additional dilution step to reduce the influence of anticoagulants such as heparin or lupus anticoagulants in the patient plasma samples. Details of this factor Va inactivation assay have been reported previously. Briefly, 50 µL of the plasma samples, prediluted 1:20 with barbital acetate buffer, are incubated for 7 minutes at 37°C in the presence of 50 µL factor V deficient plasma (Immuno, Heidelberg, Germany) and 50 µL APTT reagent (Platelin LS, Organon Technika, Eppelheim, Germany). After addition of 50 µL of a 0.025 mol/L CaCl₂ solution containing 70 nmol/L APC (Chromogenix, Mölndal, Sweden), clotting times are determined and expressed as the percentage of factor Va inactivation employing a standard curve generated by using normal pool plasma and 0.025 mol/L CaCl₂ solution containing 70 nmol/L, 35 nmol/L, 14 nmol/L, 7 nmol/L, and 0 nmol/L APC, corresponding to 100%, 50%, 20%, 10%, and 0% factor Va inactivation, respectively. Here we will summarize our current experience with this modified functional assay for the detection of factor V Leiden.

Samples from 197 patients with thrombotic disorders (including patients receiving heparin or oral anticoagulants) were evaluated for the presence of the factor V Leiden mutation using the polymerase chain reaction followed by digestion of amplification products with MnlI and detection of a restriction fragment length polymorphism as described by Bertina et al. In all test plasmas APC resistance was analyzed using a commercially available kit to determine the APC resistance ratio (Coatest APC Resistance; Chromogenix) and our factor Va inactivation assay (Fig 1). Median APC resistance ratios in patients with wildtype factor V (n = 122, ●), heterozygous factor V Leiden (n = 65, ○), and homozygous factor V Leiden (n = 10, ▲) were 2.62, 1.95, and 1.4, respectively. The corresponding values for factor Va inactivation were 118%, 34%, and 5%. APC resistance ratios were falsely negative (above the cutoff level of 2.0) in 30 heterozygous patients and 1 homozygous individual. The resulting low sensitivity (59%) of this assay for detection of factor V Leiden was expected because anticoagulated patients had been included. In 17 patients with wildtype factor V, APC resistance ratios were lower than 2.0 and thus falsely positive (specificity 86%). In contrast, sensitivity and specificity of the factor Va inactivation assay (cutoff 60%) for detection of factor V Leiden were 98.7% and 100%, respectively. The falsely negative result in the heterozygous individual with normal factor Va inactivation was caused by Factor V deficiency, readily detected by basic clotting tests. Factor Va
inactivation levels were below 15% in the 10 homozygous patients, but in none of the heterozygotes. In conclusion, the results show a high sensitivity and specificity (overall accuracy 99.5%) of the factor Va inactivation assay for detection of factor V Leiden in unselected patients with thrombotic disorders, including patients receiving anticoagulant therapy. Furthermore, the factor Va inactivation assay may allow a reliable differentiation between patients with hetero- and homozygous factor V Leiden. The absence of any patients without factor V Leiden but with abnormal factor Va inactivation, strongly supports the notion that the factor V Leiden mutation is responsible for the vast majority of factor V-associated cases of an abnormal anticoagulant response to APC.

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REFERENCES
High sensitivity and specificity of a coagulation assay for the
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