

An Evaluation of American Diagnostica's ACTICLOT® Protein C Resistance Assay. Clearly Distinguishes Between Homozygous, Heterozygous and Non Carriers of the Factor V Leiden Gene, Reducing the Need for Molecular (PCR) Testing.

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Summary

A total of 38 patient samples were tested with the ACTICLOT Protein C Resistance (aPCR) assay and with a second aPCR assay, referred to as kit A. Each sample selected had already been tested for activated protein C resistance and genetically tested for the presence of the factor V Leiden (FVL) mutation. The aPCR assay was performed on a KC10 and the results were compared against the FVL genotype.

20 of the 38 samples were aPC resistant for reasons other than the presence of the FVL mutation. Results showed that American Diagnostica's ACTICLOT Protein C Resistance assay clearly distinguished between patients with the FVL gene mutation and those without. This improved sensitivity and specificity could potentially decrease the number of samples referred for expensive PCR testing.

Terms of reference

The object of this exercise was to evaluate alternative methods for aPCR testing at Addenbrooke's hospital. Two aPCR kits were selected for evaluation, American Diagnostica's ACTICLOT Protein C Resistance assay and an alternative kit referred to as kit A.

Clear differentiation between samples with or without the FVL gene is important in an aPCR assay to exclude the mutation, without the need to do expensive genetic testing. The samples chosen had been previously tested for aPCR and genetically tested for the FVL mutation. A total of 38 samples were selected. 8 previously tested normal for aPCR and the FVL mutation; 7 heterozygote for FVL; 3 homozygotes and 20 that were aPC resistant without the FVL mutation.

Introduction

Protein C, when activated, is a naturally occurring inhibitor of factor Va and VIIIa. In FVL, a single point mutation occurs in position 506 of the factor V gene, replacing an arginine with a glutamine residue. This mutation renders factor Va resistant to cleavage by activated protein C. This in turn results in an increased half life of factor Va and therefore increases the risk of thrombosis. This is the most common inherited form of thrombophilia. The aPCR assay is a screening test for the FVL mutation.

The purpose of this evaluation was to determine if the ACTICLOT Protein C Resistance assay would provide an alternative to the method used currently.

Principle of Test

The ACTICLOT Protein C Resistance assay is a functional clotting assay that is based on a factor V dependent prothrombin activator derived from snake venom. Two equal aliquots of plasma are required and diluted with factor V deficient plasma. Next, factor V activator with activated protein C (RVV-V + aPC) is added to one diluted aliquot, while factor V activator alone (RVV-V) is added to the second diluted aliquot. These are then incubated for 3 minutes at 37°C. Clotting is initiated by the addition of a factor V dependent prothrombin activator in the absence of calcium (PTA). The time for the clot to form is recorded. The ratio of the clot time in the presence of aPC to the clot time in the absence of aPC is used to determine aPC resistance.

The principle of kit A is based on the prolongation of the clotting time of the tested plasma in the presence of aPC in a calcified medium. Factor X is snake venom activated therefore eliminating the influence of all coagulation factors further up the pathway. The results are reported in seconds.

Materials and Methods

Specimens

Frozen plasma samples

Reagents ACTICLOT aPCR assay

3 vials of RVV-V + aPC Reagent (lyophilised)

3 vials of RVV-V Reagent (lyophilised)

3 vials of PTA Reagent (lyophilised)

3 vials of Dilution Plasma (lyophilised)

Laboratory controls were used.

Tests were performed as per the manufacturer's instructions.

Reagents Kit A

4 vials of FV deficient plasma enriched with phospholipids

4 vials venom derived FX activator.

4 vials of activated protein C in a calcium medium

Negative and positive controls are provided.

This test was performed using the laboratory's existing aPCR method.

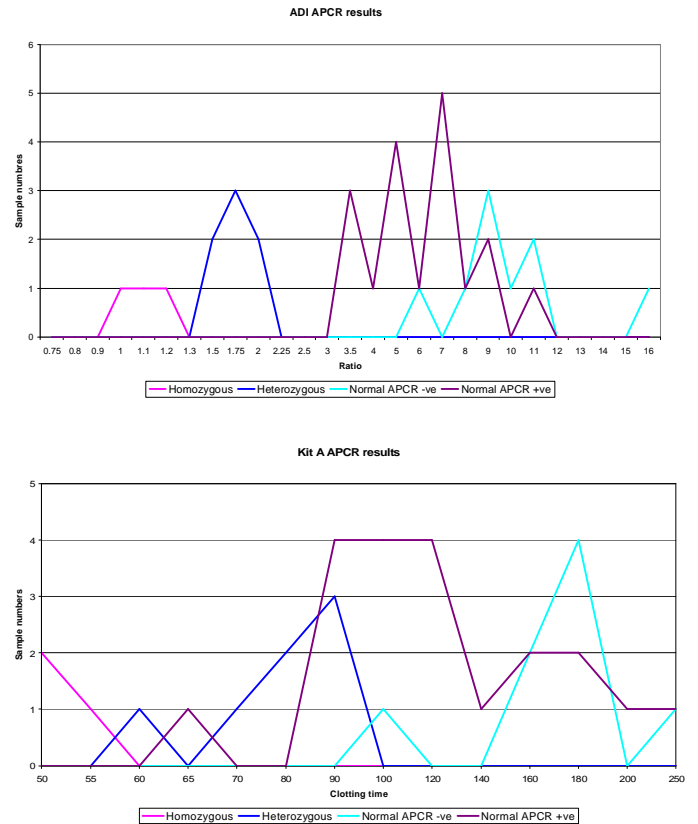
Results

Patient	FVL status	ACTICLOT ratio	Kit A
Control 4	Normal	9.97	153
Control 5	Heterozygous	1.96	35.6
1	Normal	10.26	141.6
2	Normal	8.28	97.9
3	Normal	15.48	162.9
4	Normal	8.27	143.2
5	Normal	10.63	171.6
6	Normal	5.38	256.3
7	Normal	7.78	168.9
8	Normal	8.15	168.7
AVERAGE		9.27	144.6
9	Heterozygous	1.92	67.0
10	Heterozygous	1.53	78.2
11	Heterozygous	1.42	76.3
12	Heterozygous	1.41	86.3
13	Heterozygous	1.56	86.5
14	Heterozygous	1.64	80.8
15	Heterozygous	1.56	55.5
AVERAGE		1.58	75.8
16	Homozygous	0.99	54.6
17	Heterozygous	1.10	58.3
18	Homozygous	1.01	53.6
AVERAGE		1.03	55.5

Results of known aPC resistant samples with no detectable FVL mutation.

Patient	FVL normal	ACTICLOT ratio	Kit A
19	aPC resistant	6.33	187.7
20		6.81	112.9
21		10.14	137.1
22		8.90	114.5
23		7.09	142.3
24		4.48	86.0
25		6.42	87.4
26		5.51	95.8
27		4.84	97.0
28		4.70	246.4
29		4.00	104.8
30		4.37	82.4
31		3.99	143.6
32		4.50	92.6
33		3.45	90.6
34		6.73	171.6
35		6.26	106.9
36		3.47	87.6
37		8.03	63.8
38		3.48	166.5
AVERAGE		5.67	120.9

Figure 1



Discussion

From the results obtained with the ACTICLOT Protein C Resistance assay it can be seen clearly that there is no overlap between the ratios obtained from patients with the FVL mutation and those without. Even individuals who had previously been shown to be aPC resistant for other reasons were clearly separated from the heterozygous group. This can be explained by looking closely at the reagents used. The use of factor V deficient plasma and heparin inhibitors have been used widely in the past to improve the sensitivity and specificity of the test, but the use of a factor V dependent prothrombin activator makes the assay independent of FVIII activity. The absence of phospholipids also eliminates any interference from lupus anticoagulants; this is of particular interest due to the high prevalence of lupus anticoagulant in the thrombophilic population.

The results obtained with kit A did not show the clear differentiation between groups as demonstrated by the ACTICLOT Protein C Resistance assay, as shown in figure 1. This may have been for technical reasons, as the manufacturer of kit A did not provide a protocol for performing the test on a KC10, but could also be because of the differences in the constituents of the kits and the

different principles used (reference 5). The advantage of the ACTICLOT Protein C Resistance assay over kit A is that clotting is initiated by the addition of a factor V dependent prothrombin activator. This is not calcium or phospholipid dependent and is therefore not affected by lupus anticoagulants.

Conclusion

ACTICLOT Protein C Resistance assay is a phenotypic assay with the sensitivity to distinguish between homozygote and heterozygote carriers of FVL without using molecular (PCR) methods.

It shows improved sensitivity and specificity when compared to APTT based techniques and potentially decreases the number of samples referred for expensive PCR testing. ACTICLOT Protein C Resistance assay is reported not to be affected by abnormal PT, PTT, protein C, protein S, lupus anticoagulant, FVIII, heparins, fibrinogen or antithrombin plasma levels.

References

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