CVD StripA[ssay]

INTENDED USE

The *ViennaL*ab CVD StripA[ssay] provides materials for the isolation of DNA from human whole blood, the *in vitro* amplification of relevant gene sequences, and the subsequent detection of twelve mutations associated with cardiovascular disease by reverse-hybridization.

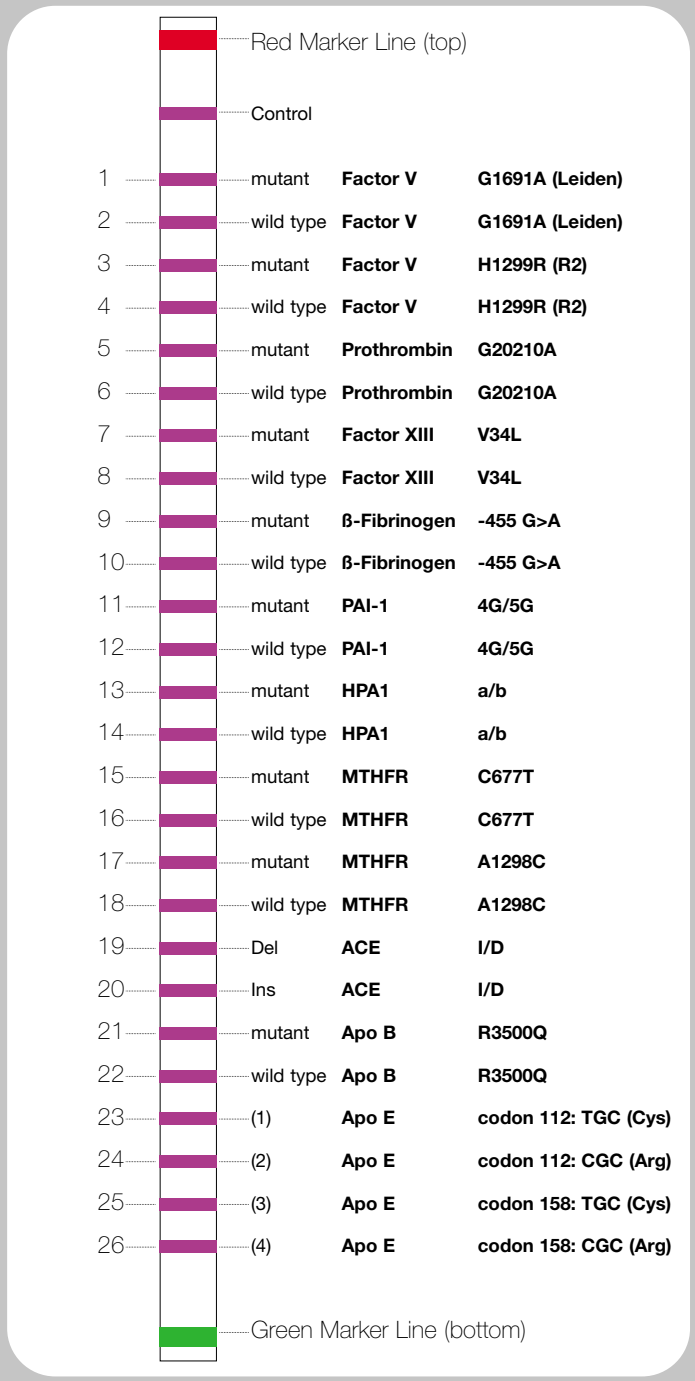
INTRODUCTION

A number of genetic and environmental risk factors have been found or suspected to predispose to cardiovascular disease (CVD), the term collectively used for disorders of the heart and blood vessels. Among the environmental components associated with CVD are physical activity, diet, alcohol and drug consumption, smoking and stress. Genetic susceptibility may be caused by mutations and polymorphisms in a variety of genes mainly involved in blood coagulation, regulation of blood pressure, and metabolism of lipids, glucose, homocysteine or iron.
Among the candidate markers for inherited CVD risk are variations in the genes for blood coagulation factors V (FV), II (prothrombin), and XIII (FXIII), ß-fibrinogen (FGB), platelet glycoprotein IIIa (GPIIIa), plasminogen activator inhibitor-1 (PAI-1), 5,10-methylenetetrahydrofolate reductase (MTHFR), angiotensin-converting enzyme (ACE), as well as apolipoproteins B (Apo B) and E (Apo E).

PRINCIPLES OF THE ASSAY

The CVD StripA[ssay] is based on the reverse-hybridization principle, and includes three successive steps: DNA is isolated from anticoagulated blood by a rapid and convenient procedure. Then, relevant gene sequences are simultaneously *in vitro* amplified and biotin-labelled in a single («multiplex») amplification reaction. Finally, the amplification products are selectively hybridized to a test strip, which contains allele-specific (wild type and mutant) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.
The assay covers the following 12 mutations: FV R506Q (Leiden), FV H1299R (R2), Prothrombin G20210A, Factor XIII V34L, ß-Fibrinogen -455 G-A, PAI-1 4G/5G, GPIIIa L33P (HPA-1), MTHFR C677T, MTHFR A1298C, ACE I/D, Apo B R3500Q, Apo E2/E3/E4.

References: Lane, D.A., Grant, P.J. (2000), Blood 95, 1517-1532. Sykes, T.C.F., Fegan, C., Mosquera, D. (2000), J. Clin. Pathol: Mol. Pathol. 53, 300-306. Franco, R.F., Reitsma, P.H. (2001), Br. J. Haematol. 115, 491-506. Reiner, A.P., Siscovick, D.S., Rosendaal, F.R. (2001), Thromb. Haemost. 85, 584-595.



CVD StripAssay

TEST RESULTS:

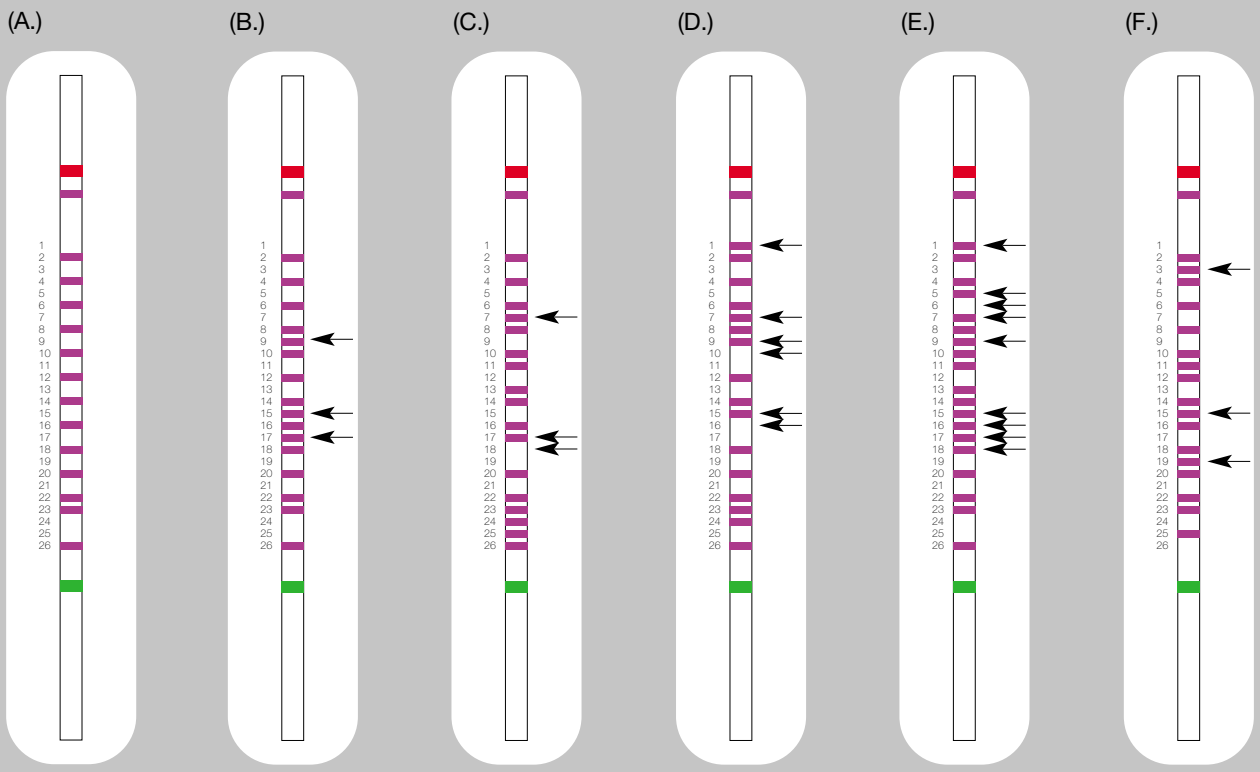
For each polymorphic position, one of three possible staining patterns may be obtained:

(1.)wild type probe only: *normal genotype*
(2.)wild type and mutant probe: *heterozygous genotype*
(3.)mutant probe only: *homozygous mutant genotype*

For the three apo E isoforms E2, E3 and E4 the following staining patterns are obtained:

E2 (112: Cys, 158: Cys) lines (1) + (3)
E3 (112: Cys, 158: Arg) lines (1) + (4)
E4 (112: Arg, 158: Arg) lines (2) + (4)

EXAMPLES:



	FV L	FV R2	PTH	FXIII	FGB	PAI-1	HPA1	M 677	M 1298	ACE	Apo B	Apo E
(A.)	norm.	norm.	norm.	norm.	norm.	4G/4G	a/a	norm.	norm.	I/I	norm.	E3/3
(B.)	norm.	norm.	norm.	norm.	**het.**	4G/4G	a/a	**het.**	**het.**	I/I	norm.	E3/3
(C.)	norm.	norm.	norm.	**het.**	norm.	5G/5G	a/b	norm.	hom.	I/I	norm.	E2/4
(D.)	**het.**	norm.	norm.	**het.**	hom.	4G/4G	a/a	hom.	norm.	I/I	norm.	E3/4
(E.)	**het.**	norm.	hom.	**het.**	**het.**	5G/5G	a/b	**het.**	**het.**	I/I	norm.	E3/3
(F.)	norm.	**het.**	norm.	norm.	norm.	4G/5G	a/a	**het.**	norm.	**I/D**	norm.	E2/2

VIENNA LAB Laboratoriumstechnik GmbH
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ViennaLab
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www.viennalab.com

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