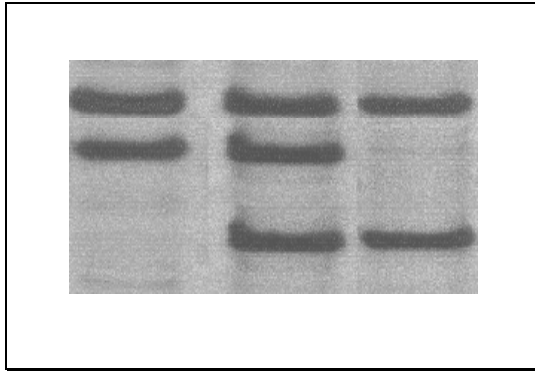




Factor V Leiden

Detection system of the G1691A mutation in the Factor V gene



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Kit Utility

The kit analyzes the presence of a polymorphism in the position 1691 (G1691A) in the gene that encodes for the coagulation Factor V.

Principle of the Assay

The analysis requires a PCR amplification of one fragment of the X exon in the gene that encodes for the human coagulation Factor V. The presence or absence of a base substitution is later detected by RFLP.

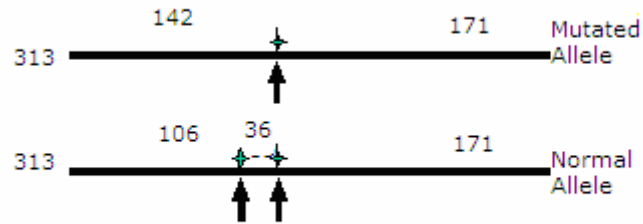
The restriction enzyme recognizes two restriction sites in the amplified fragment of a mutated individual. When a normal allele is amplified, one of these restriction sites disappears while the other one remains. This strategy has the advantage of providing a digestion control site in the amplified sequence.

After an adequate digestion, the amplification product should not be present. After digestion it is possible to define a mutated band and a normal band in the electrophoretic pattern.

Introduction

Factor V is a 300 Kd glycoprotein, which is encoded by gene F5, located in the long arm of chromosome 1 (locus 1q23). This gene is formed by 25 exons. The mutation known as Leiden is a substitution of one G for an A in nucleotide 1691 (G1691A) in exon 10, which generates the substitution of one arginine for a glutamine (R506Q or Q506 allele) in one of the protein's APC (activated protein C) cleavage sites. This amino acidic substitution destroys the cleavage site, preventing the degradation of Factor V, thus functionally resulting in a resistance to APC. Therefore the activated Factor V loses its anticoagulant effect.

Experimental strategy



Kit Presentation

Color that identifies the kit: Yellow

The ATGen kit for the detection of the Leiden mutation includes:

- 1 FV Leiden Reaction Mix tube (yellow solution).
- 1 FV Leiden Restriction Enzyme tube.
- 1 FV Leiden DNA Positive Control, containing heterozygous control DNA (once defrosted it is recommended to keep it at 4 °C).
- 1 FV Leiden Taq DNA polymerase tube.
- 1 FV Leiden Molecular Weight tube. It contains 4 bands corresponding to the amplification product and the three possible digestion bands. This tube should be kept in the post-amplification zone if possible.

The kit must be kept at -20 °C.

Our kits are available in two sizes: 20 and 50 reactions.

Necessary materials not provided with the test

- DNA-free PCR tubes
- Suitable pipettes
- Filtered pipette tips
- Gloves and robe
- Thermocycler
- Cuvette for vertical electrophoresis
- Acrylamide, electrophoresis buffer and loading buffer
- Gel coloring system with silver nitrate.
- Vortex
- Bio-hazard disposable container

Precautions

1. Only for in-vitro use.
2. All samples, reagents and controls should be considered potentially infectious.
3. Do not use alter the expiration date indicated on the package.



Storage and Stability

The kit must be stored at -20°C in order to assure its optimal performance through the expiration date indicated on the package.

Specimen Characteristics

The sample must be a DNA solution with a 150-200 ng / µl concentration, apt for PCR amplification.

ATGen recommends obtaining the DNA from blood specimens by using ADN Facil kit.

Protocol

Defrost the reaction mix and shake it vigorously by vortexing.

If possible, perform all manipulations in cold room.

Preparation of the amplification mix:

- 1. Add 18 µl of reaction mix per sample to be tested.
- 2. Add to the reaction mix, 1 µl of DNA Taq polymerase per sample to be tested.
- 3. Homogenize by moderate vortexing or pipetting.

It is recommended to prepare a single amplification mix containing the necessary quantities of reaction mix and DNA Taq polymerase, according to the number of samples to be analyzed.

It is necessary to add two reactions, one for the positive control and one for the negative control.

Amplification:

- 4. Aliquot the amplification mix, dispensing 18 µl in properly labeled PCR tubes.
- 5. Add 2 µl of sample to each tube.

The samples must contain between 100 and 200 ng of DNA (We recommend to use ATGen´s ADN Facil Kit for the DNA extraction).

- 6. Add 2 µl of FV Leiden control DNA into the positive control tube and 2 µl of the water previously used to dissolve the sample DNA in the negative control tube.

- 7. Run the program for FV.

Program: 33 cycles at 94 °C/0:30', 53 °C/0:30', 72 °C/0:30'; an initial denaturizing step: 3 minutes at 94 °C and a final extension step: 3 minutes at 72°C.

- 8. Place the tubes into the thermocycler when it reaches 94°C.

Once the program has ended and in the case you are not going to immediately perform the next step, keep the tubes at 4°C until the digestion stage.

Optionally, the amplification can be tested by electrophoresis by loading 5 µl of the reaction product and using a 6% acrylamide gel.

The expected size of the amplification product is 313 bp.

Digestion:

- 9. Once the program has ended, allow the temperature to drop until the tubes reach room temperature and then add 1 µl of restriction enzyme to every amplification tube.
- 10. Homogenize using the pipette.
- 11. Incubate for 2:30 hrs at 37 °C (it is possible to incubate overnight) and then 10 minutes at 65 °C.

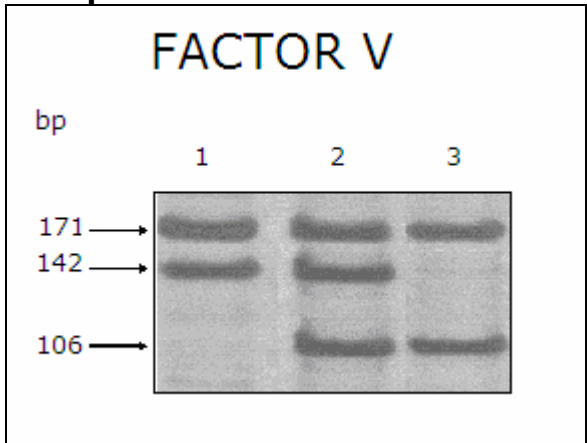
Obtention of test results

1. Prepare samples with the indicated quantity of an adequate loading buffer (e.g. glycerol 30% p/v, xilencianol blue 0.25% p/v, bromophenol blue 0.25% p/v).
2. Load 5 µl of each digested amplification product and 5 µl FV Leiden molecular weight marker in a 6% acrylamide gel or 20 µl of each one in a 2% agarose gel pre-stained with ethidium bromure (0.5 µg/ml).
3. Migrate the bromophenol blue (of the loading buffer): 8 cm in acrylamide or 3.5 cm in agarose.
4. Use silver nitrate for staining the acrylamide or use uv light for viewing the agarose gel.

Interpretation of the results

Electrophoretic pattern	Result
142 + 171 bp	Mutated Homozygote
36 + 106 + 142 + 171 bp	Heterozygote
36 + 106 + 171 bp	Normal Homozygote

Example:



6% Acrylamide gel showing the possible results:

- Lane 1. Homozygote mutated individual.
- Lane 2. Heterozygote mutated individual (The control DNA must show this pattern)
- Lane 3. Normal individual

Band of 171 bp = "control band"
 Band of 142 bp = "mutated band"
 Band of 106 bp = "normal band"

The 313 bp must not be present if the digestion was complete.

Bibliography

OMIM *227400 FACTOR V DEFICIENCY

American College of Medical Genetics Consensus Statement on Factor V Leiden Mutation Testing .Wayne W. Grody et al. (ACMG Factor V Leiden Working Group)