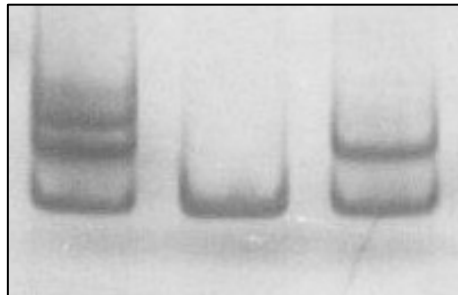




CYP 2C9 A1075C – CYP2C9*3

Detection system of the A1075C mutation in the cytochrome P450 2C9 gene.



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CYP2C9*3

 ATGen SISTEMAS MOLÉCULARES	Code: IDK-012	Ver: 1
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Kit Utility

The kit analyzes the presence of a polymorphism in the position 1075 (A1075C) in the gene that encodes for the cytochrome P450 CYP2C9 (allele cyp2C9*3).

Principle of the Assay

The analysis for the detection of the A1075C mutation, requires a PCR amplification of the segment of the cyp2C9 gene (allele cyp2C9*3) that contains the mutation. The presence or absence of a base substitution is later detected by RFLP.

There are three possible test results:

Homozygote AA: when the A1075C mutation cannot be detected in neither of the gene alleles.

Heterozygote AC: when the A1075C mutation is detected in only one of the gene alleles.

Homozygote CC: when the A1075C mutation is detected in both alleles.

Introduction

Cytochrome P450 (CYP) 2C9 is a monooxygenase, which produces the hydroxylation of 16% the most commonly used drugs. It metabolizes different xenobiotics including S-warfarin, tolbutamide and phenytoin. The alteration of the CYP2C9 enzymatic activity might cause drug adjustment difficulties or toxicity problems.

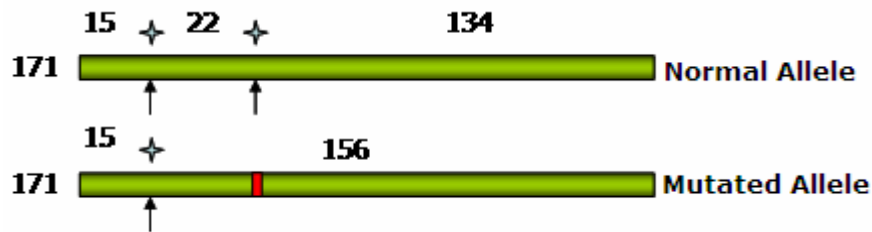
Several polymorphisms consisting in a single nucleotide substitution have been identified in the CYP2C9 gene. These polymorphisms determine metabolic phenotypes, which explain the individual and ethnical differences. Apart from the native protein CYP2C9*1, there exist at least five other allelic variants that produce alloenzymes with a reduced or deficient metabolic activity. Among Caucasian populations only the CYP2C9*2 and CYP2C9*3 variants are significant. Their allelic frequencies are 0.08-0.14 and 0.04-0.016, respectively.

It has been described that those enzymes encoded by the CYP 2C9*2 (substitution C430T/ R144C) and CYP 2C9*3 (substitution A1075C/ I359L) allelic variants, have very low effectiveness in the in-vitro hydroxylation of warfarin.



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Experimental Strategy:



Kit presentation:

Color that identifies the kit: dark grey

The kit includes:

- 1 CYP2C9*3 Reaction Mix tube (light grey).
- 1 CYP2C9*3 Restriction Enzyme tube.
- 1 CYP2C9*3 DNA Positive Control, containing heterozygous control DNA (once defrosted it is recommended to keep it at 4 °C).
- 1 CYP2C9*3 Taq DNA polymerase tube.
- 1 CYP2C9*3 Molecular Weight tube. It the amplification product band and all the 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 after the expiration date indicated on the package.



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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 50-100 ng / µl concentration, apt for PCR amplification.

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

Protocol

Pre-amplification zone:

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 CYP2C9*3 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 CYP2C9*3.



CYP2C9*3

Program: 35 cycles at 94 °C/0:30', 58 °C/0:30', 72 °C/0:30'; an initial denaturing step: 5 minutes at 94 °C and a final extension step: 5 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 171 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 overnight at 37°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 of CYP2C9*3 molecular weight marker in a 10% acrylamide gel.
3. Migrate the bromophenol blue (of the loading buffer): 8 cm in acrylamide.
4. Use silver nitrate for staining the acrylamide.

Interpretation of the results

Assay	Homocygote 1075 AA	Heterocygote 1075 AC	Homocygote 1075 CC
CYP 2C9	134 bp	156 + 134 bp	156 bp

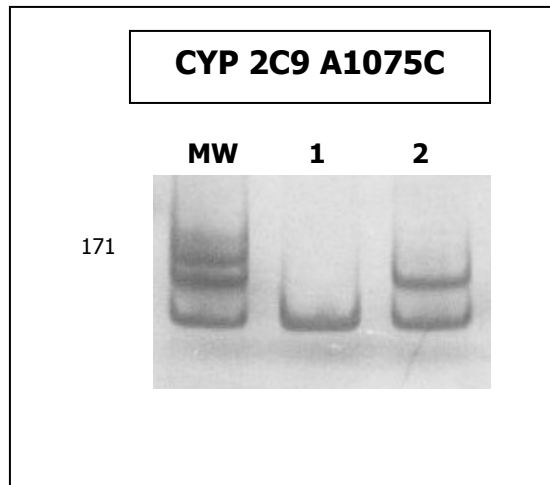
Note:

After the digestion, the amplification product band must not be present. If this 171 bp band appears, it means that a partial digestion has occurred.



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Example:



10 % acrylamide gel stained with silver nitrate showing the following results:
1: AA homozygote individual.
2: AC heterozygote individual. The control DNA must show this result.
MW: CYP2C9*3 molecular weight marker with the amplification band and the possible digestion bands.

Bibliography

1. Lancet 1999; 353: 717-719
2. J.A.M.A. 2002; 287: 1690-1698