EUROYArray FV/FII+ / MTHFR Direct

- Determination of the most important genetic risk factors for thrombophilia in one test
- Direct use of EDTA blood – no separate DNA isolation required
- Highest reliability of results due to the exclusion of interfering neighbouring mutations

Technical data

Substrate: Single-stranded DNA probes, length: 20 to 45 nucleotides
Test procedure: DNA extraction / PCR (approx. 60 min) / hybridisation (60 min) / fully automated evaluation; total working time approx. 2 min per sample incl. DNA extraction with the direct method (with 40 samples per run)
Reagents: Ready for use
Controls: DNA-negative control and other integrated controls
CE IVD label: Complete process incl. DNA extraction is validated
Test kit format: 5, 10 or 20 slides, each containing 5 test fields, or 8 slides each containing 3 test fields
Order no.: MN 5820-0505-V, -1005-V, -2005-V, -0803-V: EUROArray FV/FII+ / MTHFR Direct
Related products:
- MN 5821-0505-V, -1005-V, -2005-V, -0803-V: EUROArray FV/FII+ Direct
- MN 5822-0505-V, -1005-V, -2005-V, -0803-V: EUROArray FV Leiden Direct
- MN 5823-0505-V, -1005-V, -2005-V, -0803-V: EUROArray FII+ Direct
- MN 5824-0505-V, -1005-V, -2005-V, -0803-V: EUROArray MTHFR Direct

Clinical significance

The EUROArray FV/FII+/MTHFR Direct is designed for the molecular genetic detection of the most important genetic risk factors for thrombophilia.

Thrombophilia is an increased tendency for blood clotting. Deep and superficial venous thrombosis and thromboembolism of the brain, lung and coronary vessels are the most frequent causes of death. The main genetic risk factors for thrombosis are the factor V Leiden mutation (1691G>A), mutation 20210G>A in the factor II (prothrombin) gene and the polymorphisms 677C>T and 1298A>C in the methylene tetrahydrofolate reductase (MTHFR) gene.

The mutated factor V can be only insufficiently inactivated by activated protein C (APC). This so-called APC resistance results in an increased thrombosis tendency. The factor II (prothrombin) 20210G>A mutation is associated with both venous and arterial thrombosis. Due to the increased prothrombin plasma concentration, the heterozygous form alone causes an approximately 3 times higher risk of deep venous thrombosis. Variants 677T and 1298C of the MTHFR gene result in a reduced enzyme activity. This can develop into an increased homocysteine level (hyperhomocysteinaemia), which is a risk factor e.g. for thrombosis.

Diagnostic application

The EUROArray FV/FII+/MTHFR Direct allows fast and simple determination of the point mutations factor V Leiden (1691G>A), factor II (prothrombin) 20210G>A and/or MTHFR 677C>T and 1298A>C in a single reaction. In the direct method full blood samples can be used directly without the need of DNA isolation, which saves time and costs.
The test system is designed for the molecular genetic in vitro determination of point mutations or single-nucleotide polymorphisms (SNP) in the factor V gene (factor V Leiden, 1691G>A, rs6025), factor II (prothrombin) gene (20210G>A, rs1799963) and/or MTHFR gene (677C>T, rs1801133 and 1298A>C, rs1801131). EDTA blood (direct method) or isolated genomic DNA from the patient are used as sample material. In the direct method genomic DNA from blood cells is prepared for polymerase chain reaction (PCR) by diluting the blood with the extraction solution provided in the test kit and incubating it for one minute. In the first reaction step, one section of the factor V, one section of the factor II and/or two sections of the MTHFR gene are amplified by PCR from the extract or, alternatively, from a genomic patient DNA sample. During their formation, the PCR products are labelled with a fluorescent dye. In the second reaction step, the products are analysed using the microarray, which contains allele-specific immobilised probes in the form of small round spots that are complementary to the amplified DNA. The specific binding (hybridisation) of the fluorescence-labelled PCR product to the corresponding oligonucleotide probe is detected using a special microarray scanner (EUROArrayScanner). All spot signals are evaluated automatically using the EUROArrayScan software. For each parameter the genotype is deduced from the proportion of signals generated at the allele-specific probes.

For direct use of EDTA blood, the sample is first incubated with extraction solution 1 for one minute and then extraction solution 2 is added. For PCR an aliquot of the extract or alternatively a purified DNA sample is mixed with the ready-made PCR reagents. The PCRs are incubated in the thermocycler and then, using the TITERPLANE technique, on EUROArray slides containing microarray BIOCHIPS. Scanning and evaluation are performed using the EUROArrayScanner (incl. EUROArrayScan software). This provides fully automated evaluation of EUROArray analyses and detailed documentation of results.

The test system was investigated using sample material precharacterised with a molecular genetic method.

<table>
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<tr>
<th>Reference samples</th>
<th>Reference method</th>
<th>Sensitivity with resp. to reference method</th>
<th>Specificity with resp. to reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>109 (70 DNA and 39 blood samples)</td>
<td>molecular genetic</td>
<td>100%</td>
<td>100%</td>
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For 127 tested DNA samples the determinations were successful in all cases (100%). For 122 analysed EDTA blood samples the determinations were also successful in all cases (100%) using the direct method.