EUROArray HLA-B57:01 Direct

- Detection of all currently known HLA-B*57:01 subtypes
- High result security due to various integrated controls
- Direct use of EDTA blood – no separate DNA isolation required

Technical data

Substrate: Single-stranded DNA probes, length: 20 to 50 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 5210-0505-V, -1005-V, -2005-V, -0803-V

Clinical significance

The EUROArray HLA-B57:01 Direct is designed for the molecular genetic detection of the HLA-B*57:01 allele associated with a hypersensitivity to the HIV chemotherapeutic agent abacavir. All HIV-infected patients, regardless of their ethnicity, should be tested for the presence of HLA-B*57:01 before starting treatment with drugs containing abacavir sulphate (e.g. ZIAGEN tablets and suspension, KIVEXA tablets and TRIZIVIR tablets).

Around 8% of people carry the HLA-B*57:01 allele (deviation range: Japanese 0.1%, South Africans 19.6%). It can be assumed that 8 to 61% of HIV patients who carry this allele and are treated with abacavir will develop a hypersensitivity to the drug within six weeks. Reactions have been proven to occur in 48 to 61% of Caucasian, 8 to 16% of black South African and 20 to 22% of Hispanic carriers undergoing treatment, compared to 0 to 4% of individuals who do not carry the HLA-B*57:01 allele.

When treated with abacavir, HIV patients with a hypersensitivity generally develop fever, exanthema and pruritus, sometimes also gastrointestinal and respiratory problems, joint pain and increased liver/kidney parameters with a progressive course up to death, especially with re-exposure.

In HLA-B*57:01-negative individuals the negative predictive value for (skin-test-confirmed) hypersensitivity reactions (HSR) to abacavir is virtually 100%. However, individual cases of severe allergic reactions can occur despite HLA-B*57:01 negativity, so patients must still be monitored accordingly.

Diagnostic application

The EUROArray HLA-B57:01 Direct allows fast and simple HLA-B*57:01 detection in one reaction by including all currently known HLA-B*57:01 alleles. In the direct method full blood samples can be used directly without the need of separate DNA isolation.
**Test principle**

The test system is used for molecular genetic detection of HLA-B*57:01 alleles. 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 pretreating the blood with the extraction solutions provided in the test kit according to a specific protocol. In the first reaction step, one section of the HLA-B gene and a β-globin gene fragment as positive control are amplified by PCR from the extract or, alternatively, from a purified genomic patient DNA sample. During their formation, all PCR products are labelled with a fluorescence dye. In the second reaction step, the PCR products are analysed using the microarray, which contains immobilised probes 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). If the patient DNA sample contains an HLA-B*57:01 allele, a fluorescence signal is generated at the HLA-B*57:01-specific spots. All spot signals are evaluated automatically using the EUROArrayScan software.

**Test performance**

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.

**Sensitivity and specificity**

Sensitivity and specificity of the test system were determined with samples precharacterised using a molecular genetic method.

<table>
<thead>
<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>76 EDTA blood samples(^1) from blood donors, Germany, (16 precharacterised as HLA-B<em>57:01 positive, 59 precharacterised as HLA-B</em>57:01 negative)</td>
<td>molecular genetic</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>85 DNA samples(^2) from the &quot;International Histocompatibility Working Group&quot; (IHWG): &quot;Sequence Polymorphism Reference Panel (SP Reference Panel)&quot; and &quot;HLA (Anthropology) Reference Panel&quot;, <a href="http://www.ihwg.org">www.ihwg.org</a></td>
<td>molecular genetic</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

\(^1\)The investigations were performed both with EDTA blood using the direct method and with DNA samples isolated from EDTA blood using the "QIAamp® DSP DNA Blood Mini Kit" (QIAGEN). This investigation was not performed for the direct method.

**Robustness**

111 DNA samples and 135 EDTA blood samples from blood donors were investigated twice. The determinations were successful in 100% (DNA samples) and 97% (EDTA blood samples).

**Literature references**