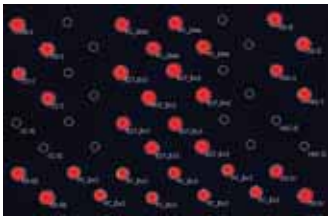
EUROArray HLA-B27 Direct



- **Detection of all currently known HLA-B*27 subtypes and indication of the presence of the non-disease-associated alleles HLA-B*27:06 and HLA-B*27:09**
- **High result safety 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 5110-0505-V, -1005-V, -2005-V, -0803-V**

Clinical significance

The EUROArray HLA-B27 Direct is designed for the molecular genetic detection of disease-associated HLA-B*27 alleles. In total, 130 different subtypes (B*27:01–B*27:105) have been described for HLA-B27, which differ only in some bases and can all be detected together using the EUROArray HLA-B27 Direct.

The membrane-bound HLA-B27 protein is associated with the occurrence of several autoimmune diseases such as Bechterew's disease (spondylitis ankylosans, ankylosing spondylitis, AS), urethro-oculo-articular syndrome (Reiter's disease, combination of symptoms from urethritis, conjunctivitis/uveitis, arthritis), reactive arthritis (para-/postinfectious arthritis), acute uveitis anterior or acute iridocyclitis, periarthritis (periarthropathia) humeroscapularis, arthritis psoriatica (psoriasis arthritis) and juvenile idiopathic arthritis. Enteropathies (chronic inflammatory bowel diseases, IBD) are also associated with HLA-B*27.

There is a clear relationship between Bechterew's disease and the presence of HLA-B27. Around 3 to 6% of HLA-B*27 carriers develop ankylosing spondylitis. Around 90% of Bechterew's disease patients are carriers of this tissue antigen, in particular subtypes B*27:02, B*27:04 and B*27:05. The subtypes B*27:06 and B*27:09 on the other hand are not associated with Bechterew's disease. Therefore, subtype differentiation is necessary for confirmation of diagnosis in particular populations. This is provided by the EUROArray HLA-B27 at a very high level of quality.

Molecular genetic detection of HLA-B27 competes with the lymphocytotoxicity tests frequently used in the past. The antibodies can cause cross reactions (with e.g. HLA-B27), which may produce false-negative results in immunophenotyping in low HLA-B*27 expression. Due to the use of allele-specific primers the molecular genetic detection of HLA-B*27 is more specific and sensitive than serological methods.

Diagnostic application

The EUROArray HLA-B27 Direct allows fast and simple HLA-B*27 detection in a single reaction. In the direct method, full blood samples can be used directly without the need of separate DNA isolation.

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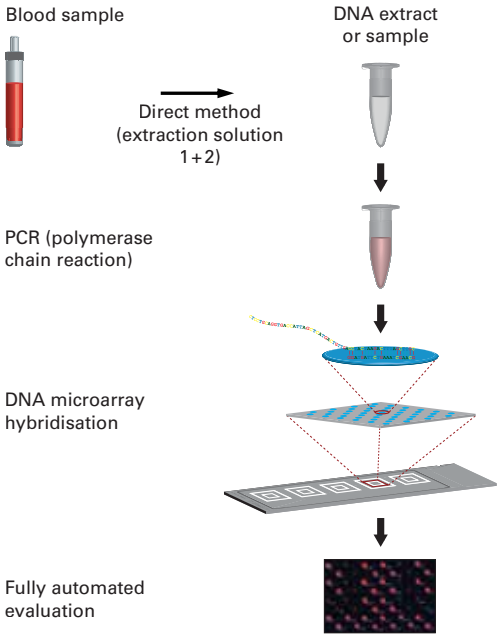
Test principle

The EUROArray is an in vitro test for molecular genetic determination of disease-associated HLA-B*27 alleles in human genomic DNA. 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, two sections 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. The HLA-B gene sections are only amplified if the sample contains an HLA-B*27 allele. 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 binding (hybridisation) of the fluorescing PCR product to the corresponding microarray spot is detected using the EUROIMMUN Microarray Scanner. All spot signals are evaluated automatically using the EUROArrayScan software. A fluorescence signal on the HLA-B*27-specific spots indicates the presence of an HLA-B*27 allele in the patient DNA.



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 EUROArrayScan system (Microarray Scanner incl. EUROArrayScan software). This provides fully automated evaluation of EUROArray analyses and detailed documentation of results.

Sensitivity and specificity

The microarray test system detects all HLA-B*27 subtypes and indicates the possible presence of the non-disease associated subtypes HLA-B*27:06 and HLA-B*27:09.

Reference samples	Reference method	Sensitivity with resp. to reference method	Specificity with resp. to reference method
100 EDTA blood samples[1] from blood donors, Germany (13 precharacterised as HLA-B*27 positive, 37 precharacterised as HLA-B*27 negative)	molecular genetic	100 %	100 %
55 DNA samples[2] from the "International Histocompatibility Working Group" (IHWG): "Sequence Polymorphism Reference Panel (SP Reference Panel)" and "HLA (Anthropology) Reference Panel" (6 precharacterised as HLA-B*27 positive, 49 precharacterised as HLA-B*27 negative)	molecular genetic	100 %	100 %

[1]The investigations were performed both with EDTA blood using the direct method and with DNA samples isolated from EDTA blood using the "DNA whole blood kit S CE IVD". [2]This investigation was not performed for the direct method.

Robustness

For 100 analysed EDTA blood samples the determinations were successful in all cases (100%) using the direct method. For DNA samples the determinations were also successful in all cases (100 %) (n = 150).

Literature references

1. Bowness P. HLA B27 in health and disease: a double-edged sword? Rheumatology (Oxford). 2002 Aug; 41(8):857-68.
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3. EUROIMUNN AG. Köhler S, Komorowski L, Stöcker W. Diagnosis of inflammatory bowel diseases, more particularly ulcerative colitis. Deutsches Patent Nr. DE102004018418 (2005).
4. Khan MA, Mathieu A, Sorrentino R, Akkoc N. The pathogenetic role of HLA-B27 and its subtypes. Autoimmun Rev. 2007 Jan; 6(3):183-9.

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