DNA-TECHNOLOGY

GENETICS

HLA-B27

KIT FOR DETECTING HLA-B27 ALLELES BY REAL-TIME PCR. CE/IVD

HLA-B27 is the major human histocompatibility complex (HLA) class I, locus B. The HLA gene complex (Human Leukocyte Antigens) is located on the short arm of chromosome 6 and includes several dozen genes, providing the immune response function.

Protein products of the HLA class I genes (antigens) play a crucial role in maintaining the genetic constancy of tissues and ensuring human resistance to infections, mainly viral.

In some cases, due to several reasons (for example, a genetic predisposition, a decrease in the ability of the immune system to reverse regulation of immune responses), the normal immune response to some microorganisms become pathological or autoimmune. The autoimmune response is directed against the body's normal tissues, causing damage and the development of autoimmune inflammation.

It was found that the most significant role in the development of autoimmune processes (diseases) belongs to the HLA genes. However, the mechanisms for realizing the genetic predisposition associated with these genes have not been fully established. "Molecular mimicry" is one of the leading hypotheses explaining the relationship of the HLA genes with the development of the autoimmune process, according to which the proteins of infectious pathogens may have regions similar to the proteins of specific human tissues. Under certain conditions, this similarity can lead to the development of an autoimmune reaction.

HLA-B27 (a genetic variation of the HLA gene at the B locus) is one of the most striking examples demonstrating the relationship of HLA genes with the development of diseases. For example, it has been established that this molecule is a "marker" of several inflammatory arthritis, combined into the group of seronegative spondyloarthritis (SSpA).

Diseases included in the SSpA group have common pathogenetic mechanisms and several similar clinical and radiological signs:

- tests negative for rheumatoid factor (RF),
- damage to the sacroiliac joints and spine,
- asymmetric arthritis with predominant involvement of the joints of the lower extremities,
- family predisposition to the development of diseases,
- «overlap syndrome» (Fig. 1).

A key feature of SSpA is damage to the musculoskeletal system and other organs and systems: eyes, skin, mucous membranes, heart, aorta, and kidneys. The variety of extra-articular manifestations reflects the clinical polymorphism of SSpA. It characterizes them as diseases with systemic inflammation, in which the pathology of the joints and the spine can appear at different times in various combinations with damage to other organs.

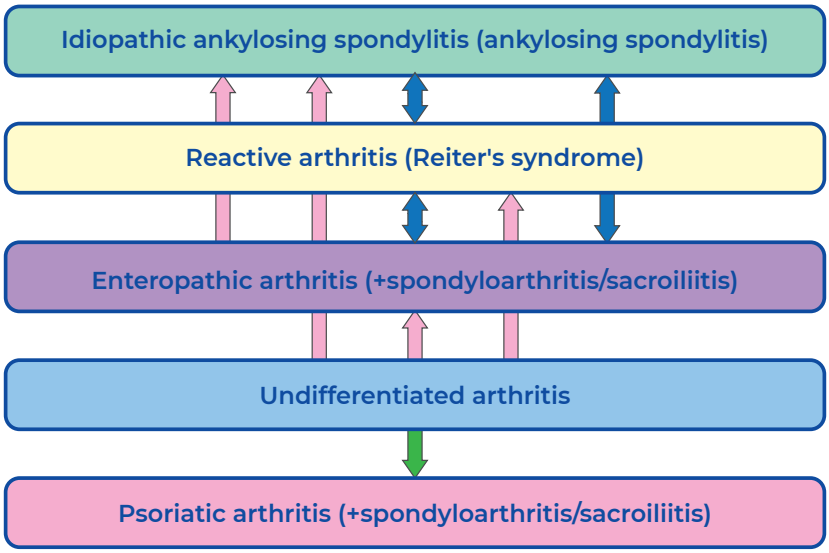


Fig 1. The strength of association of HLA-B27 with diseases (%) from the SSpA group and the relationship between various spondyloarthropathies (pink and green arrows indicate unidirectional relationships between diseases, blue — bidirectional)

The SSpA group includes the following diseases:

- idiopathic ankylosing spondylitis (ankylosing spondylitis),
- psoriatic arthritis,
- reactive arthritis (Reiter's syndrome) following extra-articular infections most commonly caused by Klebsiella, Salmonella, Yersinia, Shigella, Chlamydia trachomatis,
- enteropathic arthritis (with Crohn's disease, Whipple's disease, ulcerative colitis),
- acute anterior uveitis,
- juvenile spondylitis,
- undifferentiated arthritis.

The association of ankylosing spondylitis with HLA-B27 is an example of one of the most robust genetic links of the HLA gene complex with rheumatic disorders. This genetic link was discovered in the early 1970s when it was found that more than 95% of patients with ankylosing spondylitis had HLA-B27, while the frequency of this gene in the general population was below 10%.

It has been established that other diseases from the SSA group have different, but lower, than for ankylosing spondylitis, association with HLA-B27 (Table 1).

Table 1. Spondyloarthritis associated with HLA-B27

Disorder	Prevalence of HLA-B27, %
Ankylosing spondylitis	90–95
Psoriatic arthritis	50–60
Reactive arthritis	60–90
Enteropathic arthritis	50–60
Juvenile spondylitis	80–90
Undifferentiated arthritis	50–70

The problems of diagnosis and treatment of seronegative spondyloarthritis are especially relevant because diseases belonging to the SSpA group affect young people of working age. Moreover, they are the cause of long-term disability of patients and often end with their disability.

Significant clinical symptoms of spondyloarthropathies are inflammatory back pain, asymmetric peripheral oligoarthritis mainly of the lower extremities, enthesitis, and/or tendosynovitis with severe pain that impedes walking, as well as extra-articular manifestations involving other organs, such as anterior uveitis, psoriasis, and chronic bowel disease.

An HLA-B27 test in combination with other laboratory and X-ray studies significantly increases the possibility of making a correct diagnosis and prognosis of diseases belonging to the group of seronegative spondyloarthritis. However, the final diagnosis can be made by a rheumatologist based on the entire set of clinical, X-ray, laboratory, and anamnestic data.

Indications for prescribing an HLA-B27 test:

- the presence of clinical symptoms of spondyloarthropathies: inflammatory back pain, asymmetric peripheral oligoarthritis mainly of the lower extremities, enthesitis and/or tendosynovitis;
- as an additional laboratory marker in order to predict the severity of the disease.

DNA-Technology has developed a kit for the detection of HLA-B27 alleles by real-time PCR.

Technical characteristics and kit content:

Number of tests	48 tests
Reagents format	Paraffin sealed PCR-mix: 48 tubes or six 8-tube strips (20 µL per tube)
Taq-polymerase solution	One tube (500 µL)
Mineral oil	One tube (1,0 ml)
Positive control	One tube (75 µL)
Sample	Peripheral blood
Shelf life	12 months
Storage temperature	from 2°C to 8°C

Recommended kits for DNA extraction:
- «PREP-RAPID Genetics»;
- «PREP-GS Genetics»

To perform the analysis, the following consumables and equipment are required:
- rack and Vortex rotor for striped plastic.

Advantages of using the kit for detection of HLA-B27 alleles by real-time PCR:
- manufacturability (standard PCR methods with real-time results detection);
- high speed (determine the genotype within 24 hours);
- automatic results delivery (for «DT» devices);
- low cost of analysis;
- high sensitivity;
- CE/IVD certified.

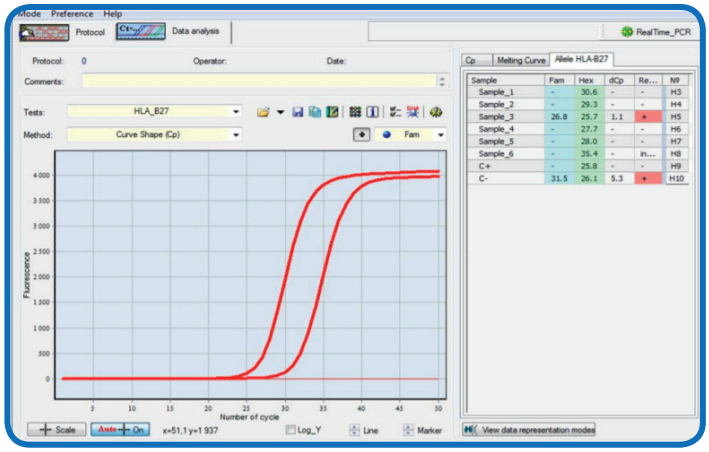
Devices required for analysis

The kit is intended for use in laboratories equipped with real-time PCR instruments ("DT" series devices manufactured by DNA-Technology R&P): "DTlite", "DTprime" (Fig. 2).

«DT» series devices are equipped with specially developed English-language software that supports automatic data processing and gives output in the convenient interpretation format. In addition, the unique technical characteristics of the instruments make it possible to reduce the amplification time to 1 hour 20 minutes, and the total analysis time — to 2 hours 30 minutes. This significantly saves analysis time and provides high laboratory throughput.

DTprime

DTlite



Moreover, the software allows displaying the results in a convenient and descriptive format for clinicians to analyze the data.

HLA*B27 gene detection

Number:
Tube:
Patient:
Sex:
Age:
Physician:
Comment:

Logotype

Information about laboratory

Sample ID: 1

Name of research	Result
allele HLA-B27	Not detected

Study was carried out by:

Date:
Signature:

Attention! The information contained in the brochure may not correspond to the current version of the specification for the product.

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