

# HLA-DRB1-Shared Epitope-Kit

## A standardised test for the rheumatoid arthritis

### Background

Rheumatoid Arthritis (RA) is a chronic, progressive inflammatory disease which occurs in approx. 1% of the population. It can vary from a mild, self-limiting disease to one that is treatment-resistant and highly aggressive. Although RA mainly causes erosive joint damage, systemic extraarticular manifestations are also common, particularly rheumatoid vasculitis which is associated with increased mortality in RA patients. Thus markers that can help predict these manifestations and also a patient's response to treatment will contribute significantly to the clinical management of patients with RA. Its been known for some time that there is a strong genetic association in RA with HLA-DRB1 and, more specifically, with a conserved amino acid sequence in the 3rd hypervariable region.

This sequence is known as the shared epitope (SE) and is expressed by several DRB1 alleles.

Interestingly, recent studies now suggest that the actual role of the SE, and the SE genotype in particular, is in the development of erosive disease and rheumatoid vasculitis (both severe manifestations of RA) (1,2). It also appears to play a significant part in determining the response to early treatment (3) and, in association with smoking, the risk of developing seropositive RA (4). Moreover, there is a clear SE gene dosage effect in all the above with two alleles conferring greater risk.

Thus the SE and the SE genotype is an ideal marker for screening and monitoring patients with RA.

### Indications

- prognosis of rheumatoid arthritis
- response to drug therapy
- reindication of therapy

Treatment group	Shared Epitope		
	2 copies n = 98	1 copy n = 207	0 copies n = 129
Methotrexat	48	41	38
Etanercept, 10 mg	27	35	28
Etanercept, 25 mg	<b>76</b>	41	48

Proportion of patients who respond to treatment after 12 months, according to treatment group and number of HLA-DRB1 SE copies inherited. Values are in percent.

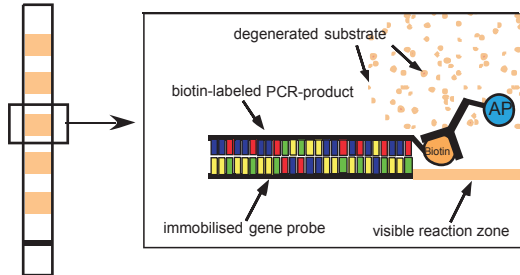
The results of the study from Lindsey et al. show that RA-patients who had inherited 2 copies of the shared epitope significantly more respond to the treatment with etanercept than with methotrexat (76% versus 48%).

### Advantages of using the AID SE-kit :

- sensitive and highly specific molecular assay based on PCR and reverse hybridisation technology
- accurate and reliable detection of the SE by screening all HLA-DRB1-alleles and differentiation in QKRAA, QRRAA, RRRRAA
- clear differentiation between heterozygous and homozygous individuals
- fast and reproducible
- contains all sensitivity and specificity controls for the PCR and hybridisation procedures
- easily automated
- certified and accredited according to CE

## Test principal

The GenID HLA-DRB1 "Shared Epitope"- Kit provides an accurate, reliable and fast method for detecting amino acid motives QKRAA, QRRAA and RRRAA that characterize the SE of all known HLA-DRB1 alleles.

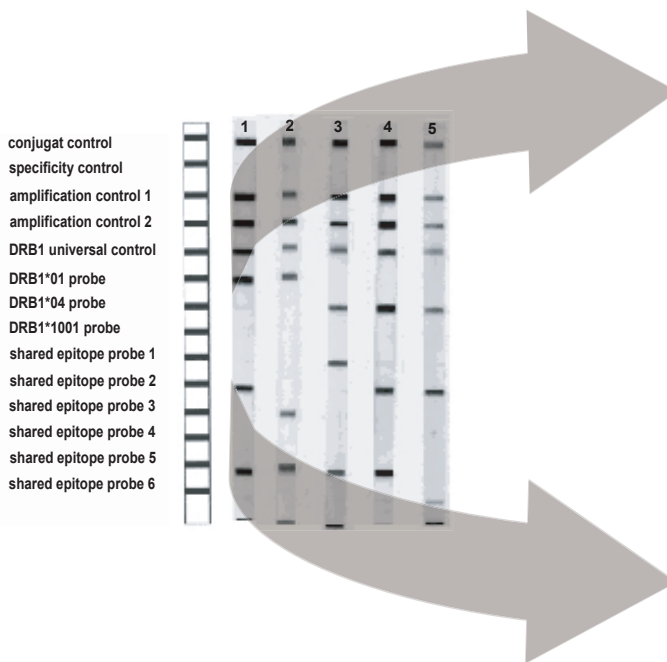


The test is carried out in two steps:

1. Amplification of biotinylated HLA-DRB1-alleles from genomic DNA by standard PCR
2. Hybridisation of the PCR-products to strips precoated with oligonucleotide probes specific for the various DRB1 alleles.

A highly specific washing procedure ensures that only hybrids with 100 % complementary sequences survive. Detection is subsequently achieved by the sequential addition of streptavidin-coupled alkaline phosphatase and the substrate BCIP/NBT to give a coloured product.

## Interpretation



- interpretation by template and scheme instancing the study of Grözl et al.

	Probe 1	Probe 2	Probe 3	Probe 4	Probe 5
	DR1	DR1	DR4	DR4	DR4
conjugat control	x	x	x	x	x
specificity control	-	-	-	-	-
amplification control 1	x	x	x	x	x
amplification control 2	x	x	x	x	x
DRB1 universal control	x	x	x	x	x
DRB1*01 probe	x	x			
DRB1*04 probe			x	x	x
DRB1*1001 probe					
shared epitope probe 1			x		
shared epitope probe 2	x			x	x
shared epitope probe 3		x			
shared epitope probe 4	x				
shared epitope probe 5	x	x	x	x	
shared epitope probe 6					x
position 70-74 AA subtypes DRB1	QKRAA	DERAA	QKRAA	QRRAA	QRRAE
	*0101	*0103	*04011	*0404	*0403
	*01021		*04012	*04051	*0406
	*0104		*0409	*04052	*0407
	*0105		*0413 ect.	*0408 ect.	*0411 ect.

- detailed interpretation with the AID-Scanning System



## Technical Data

**Specimen** EDTA- or Citrate-anti-coagulated whole blood

**Time required** 2.5 hrs. for DNA-Isolation and PCR  
2.5 hrs. for the reverse hybridisation step

**Sample quantity** 150-300ng DNA per PCR

**Controls** Amplification-, specificity- and conjugat controls

## Literature

- 1) Grözl, D., Heide K.-G., Bauerfeind S. HLA-DRB1 in Rheumatik Diseases: Rapid direct typing of the Shared Epitope based on a combined SSP- and SSOP PCR. *Präsentiert auf dem 20. ERW Workshop 2003 in Oxford, England*
- 2) Lindsey et al. The Influence of Genetic Variation in the HLA-DRB1 and LTA-TNF Regions on the Response to Treatment of Early Rheumatoid Arthritis With Methotrexate or Etanercept. *Arthritis & Rheumatism Vol. 50 (9), 2750-2756, 2004*