UMCG laboratoriumgeneeskunde

skml
Stichting Kwaliteitsbewaking
Medische Laboratoriumdiagnostiek

SKML Congres
Kwaliteit in Harmonisatie
Nijmegen, 14 juni 2011

HLA-B*27 diagnostiek: is sequentie analyse the way to go?

14 juni 2011

Bouke Hepkema
Transplantatie-Immunologie
Laboratoriumgeneeskunde
UMCG

skml
Stichting Kwaliteitsbewaking
Medische Laboratoriumdiagnostiek

SKML Congres
Kwaliteit in Harmonisatie
Nijmegen, 14 juni 2011

Kwaliteit in Harmonisatie

of

Harmonisatie in Kwaliteit

Laboratorium voor Transplantatie-Immunologie
Laboratoriumgeneeskunde
UMCG

UMCG laboratoriumgeneeskunde

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ISO 9001
DNV
CERTIFIED ORGANISATION

EUROPEAN FEDERATION FOR IMMUNOGENETICS

JACIE
joint accreditation committee
isct ebmt

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ACCREDITATION CATEGORIES

Renal transplantation:

Recipient typing	Yes ☐	No ☐
Antibody screening	Yes ☐	No ☐
Antibody identification	Yes ☐	No ☐
Donor typing	Yes ☐	No ☐
Cross-matching	Yes ☐	No ☐

Non renal transplantation:

Recipient typing	Yes ☐	No ☐
Antibody screening	Yes ☐	No ☐
Antibody identification	Yes ☐	No ☐
Donor typing	Yes ☐	No ☐
Cross-matching	Yes ☐	No ☐

Haematopoietic stem cell transplantation (HSCT):

Donor registry typing	Yes ☐	No ☐
Related donor typing	Yes ☐	No ☐
Unrelated donor typing	Yes ☐	No ☐
Cord Blood typing	Yes ☐	No ☐
Cross-matching	Yes ☐	No ☐

Disease association studies	Yes ☐	No ☐
Transfusion	Yes ☐	No ☐

EUROPEAN FEDERATION FOR IMMUNOGENETICS

ACCREDITATION TECHNIQUES:

Class I typing by:

CDC	Yes ☐	No ☐
Flow cytometry (HLA-B27,etc.)	Yes ☐	No ☐
DNA: 2 digits	Yes ☐	No ☐
DNA: 4 digits	Yes ☐	No ☐

Class II typing by:

CDC	Yes ☐	No ☐
DNA: 2-digits	Yes ☐	No ☐
DNA: 4 digits	Yes ☐	No ☐

Antibody testing

Screening:

CDC	Yes ☐	No ☐
Flow cytometry	Yes ☐	No ☐
ELISA	Yes ☐	No ☐
Bead array	Yes ☐	No ☐

Identification:

CDC	Yes ☐	No ☐
Flow cytometry	Yes ☐	No ☐
ELISA	Yes ☐	No ☐
Bead array	Yes ☐	No ☐

Cross-matching:

CDC	Yes ☐	No ☐
Flow cytometry	Yes ☐	No ☐
ELISA	Yes ☐	No ☐
Bead array	Yes ☐	No ☐

A - GENERAL POLICIES
B - PERSONNEL QUALIFICATIONS
C - QUALITY ASSURANCE
C4.000 Quality Assurance.

C4.100 **External Proficiency Testing(EPT) and Competency Evaluation.**

C4.110 The laboratory must participate in EPT programme(s) to cover all the accredited laboratory applications (HLA typing, antibody screening and identification, crossmatching, etc.). EPT results must be obtained for all techniques individually or in combination as routinely used to produce a final result. The procedure for testing EPT samples including the allocation to techniques must be documented prior to the annual commencement of the EPT cycle.

C4.120 For proficiency testing, the laboratory must be in compliance with published regulations formulated by the EFI EPT Committee and approved by the EFI Board.

K - DISEASE ASSOCIATION

K3.100 **Typing for a single allele-group by molecular techniques (e.g. HLA-B*27).**

K3.110 Where typing for a single allele-group is performed a positive control DNA known to encode the allele-group of interest must be included in each test.

K3.120 Where typing for a single allele-group is performed a negative control DNA known not to encode an allele belonging to the allele-group of interest must be included in each test.

L – NUCLEIC ACID ANALYSIS

L1.0000 General laboratory design, equipment and reagents.

L1.2000 **Contamination control (″wipe-test″).**

L3.2550 Databases of HLA sequences used for allele assignment must be accurate and updated at least each year.

Standards for PROVIDERS of External Proficiency Testing (EPT) schemes – Version 5.0.

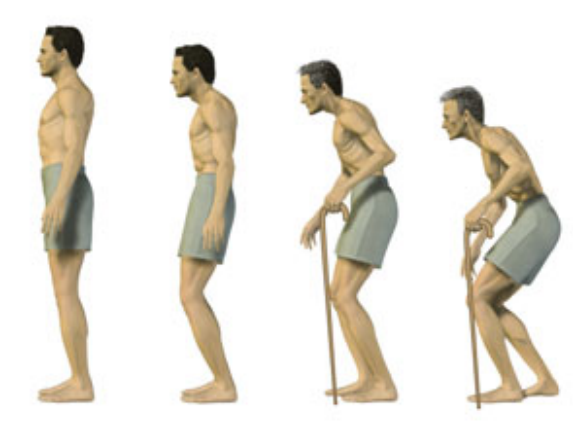
8 DNA-based EPT

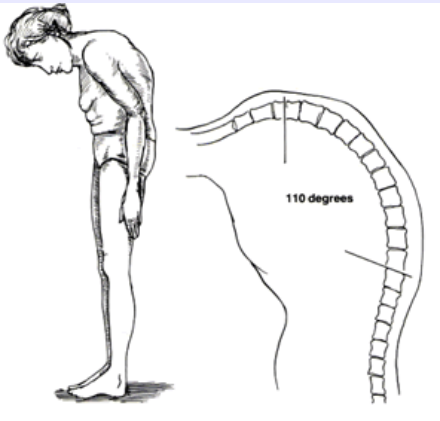
8.1 For all DNA based EPT (low or high resolution) the HLA typing accepted by the EPT Provider is designated as the correct result.

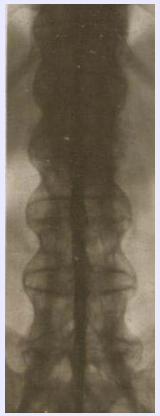
Laboratorium voor Transplantatie-Immunologie
Laboratoriumgeneeskunde, UMCG

- B*27 SSP (Sequence Specific Priming):
  alleen B*27
- HLA-B SSO (Sequence Specific Oligonucleotides)
  complete B-locus typering
- HLA-B SBT (Sequence Based Typing)

Morbus Bechterew of Ankylosing spondylitis







SKML WMBD 2007.1 sample 4

Resultaten:

- B*27 2x GR
  6x neg
  20x pos
  consensus **Positief**

UMCG:

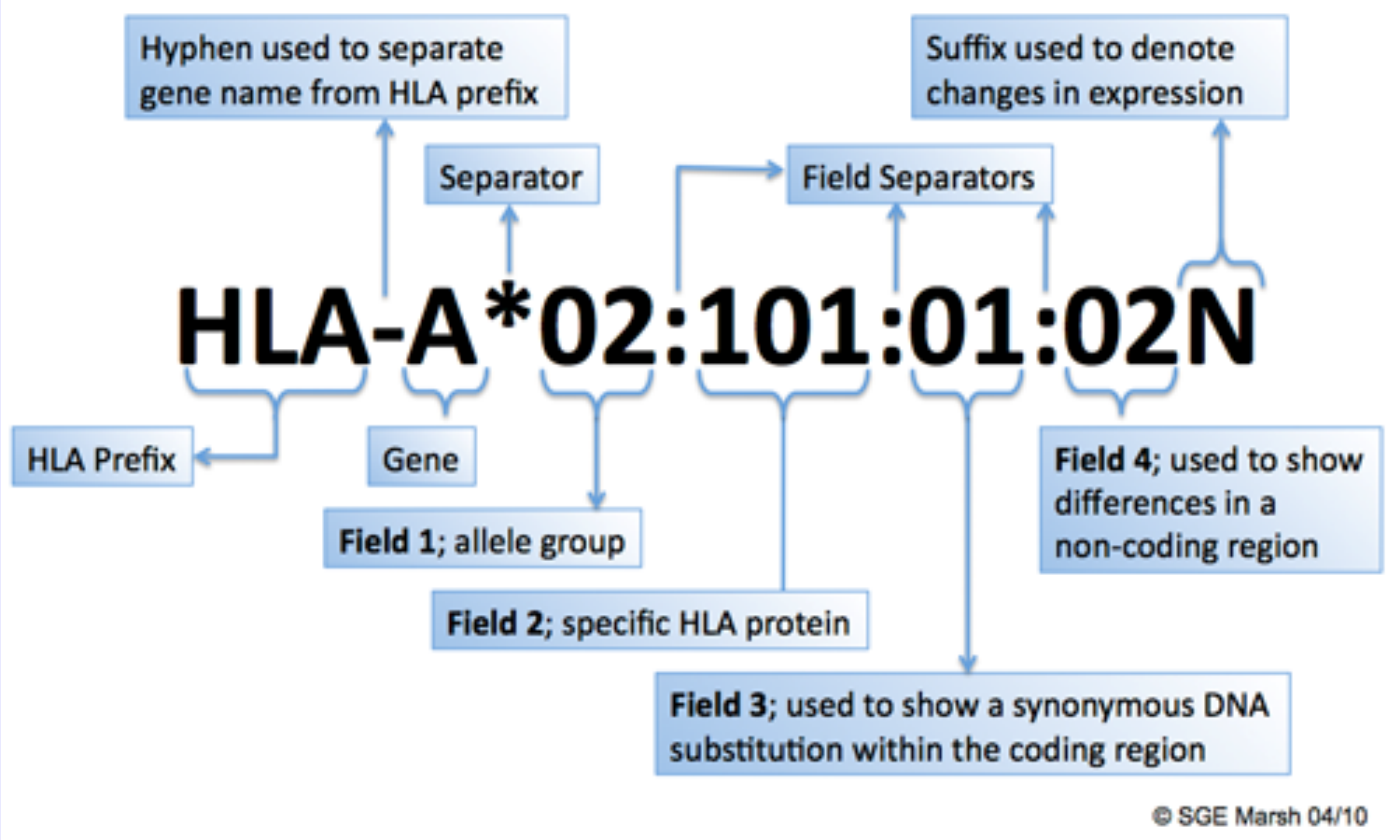
- HLA-B*18, *40 (SSO)
- HLA-B*18:02, *40:01 (SBT)

Correctie WMBD: **Negatief**

Nomenclature (april 2010)

www.ebi.ac.uk/imgt/hla

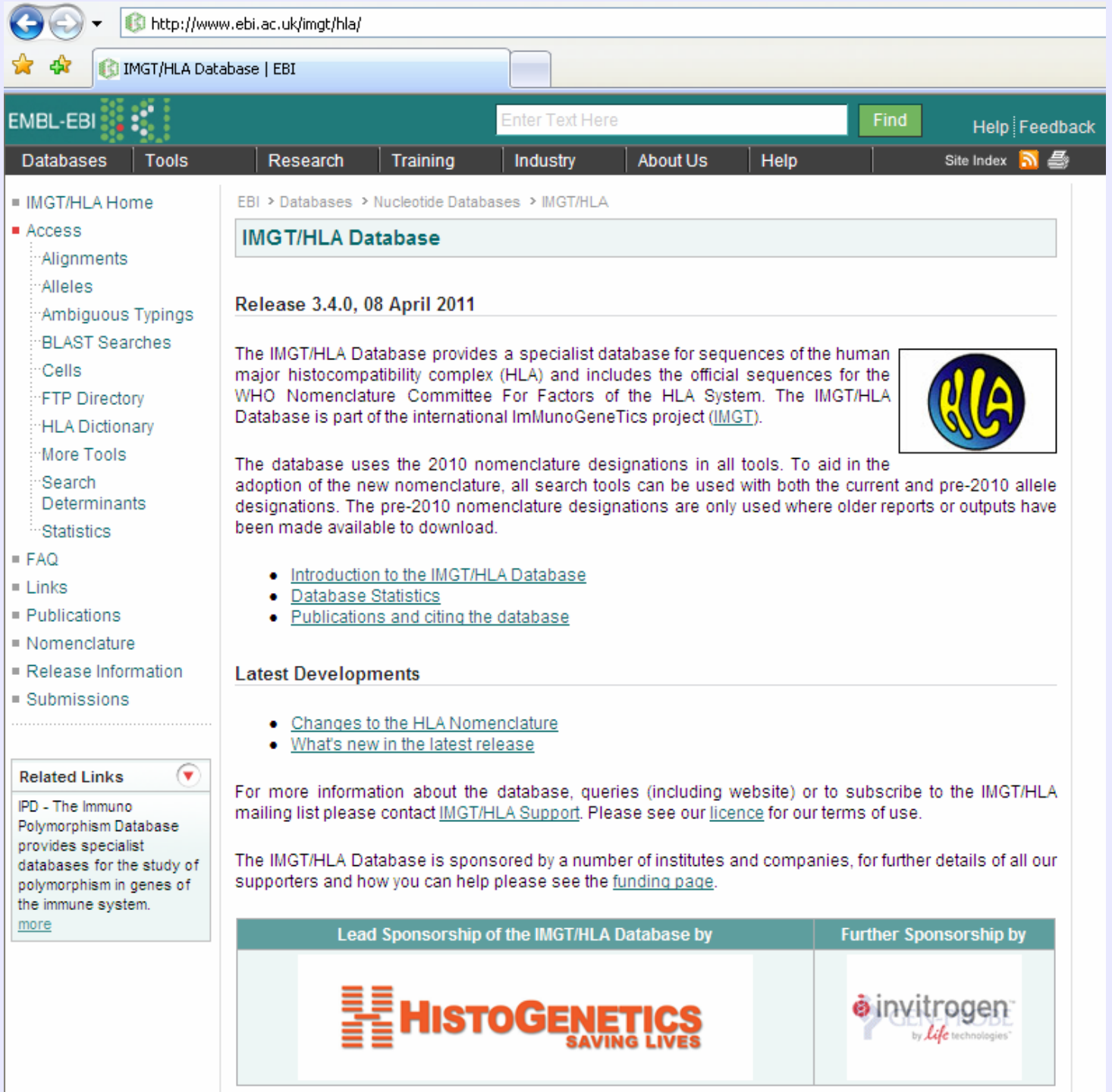
- Serology: Antigens e.g. HLA-A1
- DNA:



Nomenclature

Moleculaire diagnostiek

~~B27~~ → B*27



http://www.ebi.ac.uk/imgt/hla/

IMGT/HLA Database | EBI

EMBL-EBI

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Related Links

IPD - The Immuno Polymorphism Database provides specialist databases for the study of polymorphism in genes of the immune system.
more

EBI > Databases > Nucleotide Databases > IMGT/HLA

IMGT/HLA Database

Release 3.4.0, 08 April 2011

The IMGT/HLA Database provides a specialist database for sequences of the human major histocompatibility complex (HLA) and includes the official sequences for the WHO Nomenclature Committee For Factors of the HLA System. The IMGT/HLA Database is part of the international ImMunoGeneTics project (IMGT).

The database uses the 2010 nomenclature designations in all tools. To aid in the adoption of the new nomenclature, all search tools can be used with both the current and pre-2010 allele designations. The pre-2010 nomenclature designations are only used where older reports or outputs have been made available to download.

- Introduction to the IMGT/HLA Database
- Database Statistics
- Publications and citing the database

Latest Developments

- Changes to the HLA Nomenclature
- What's new in the latest release

For more information about the database, queries (including website) or to subscribe to the IMGT/HLA mailing list please contact IMGT/HLA Support. Please see our licence for our terms of use.

The IMGT/HLA Database is sponsored by a number of institutes and companies, for further details of all our supporters and how you can help please see the funding page.

Lead Sponsorship of the IMGT/HLA Database by	Further Sponsorship by
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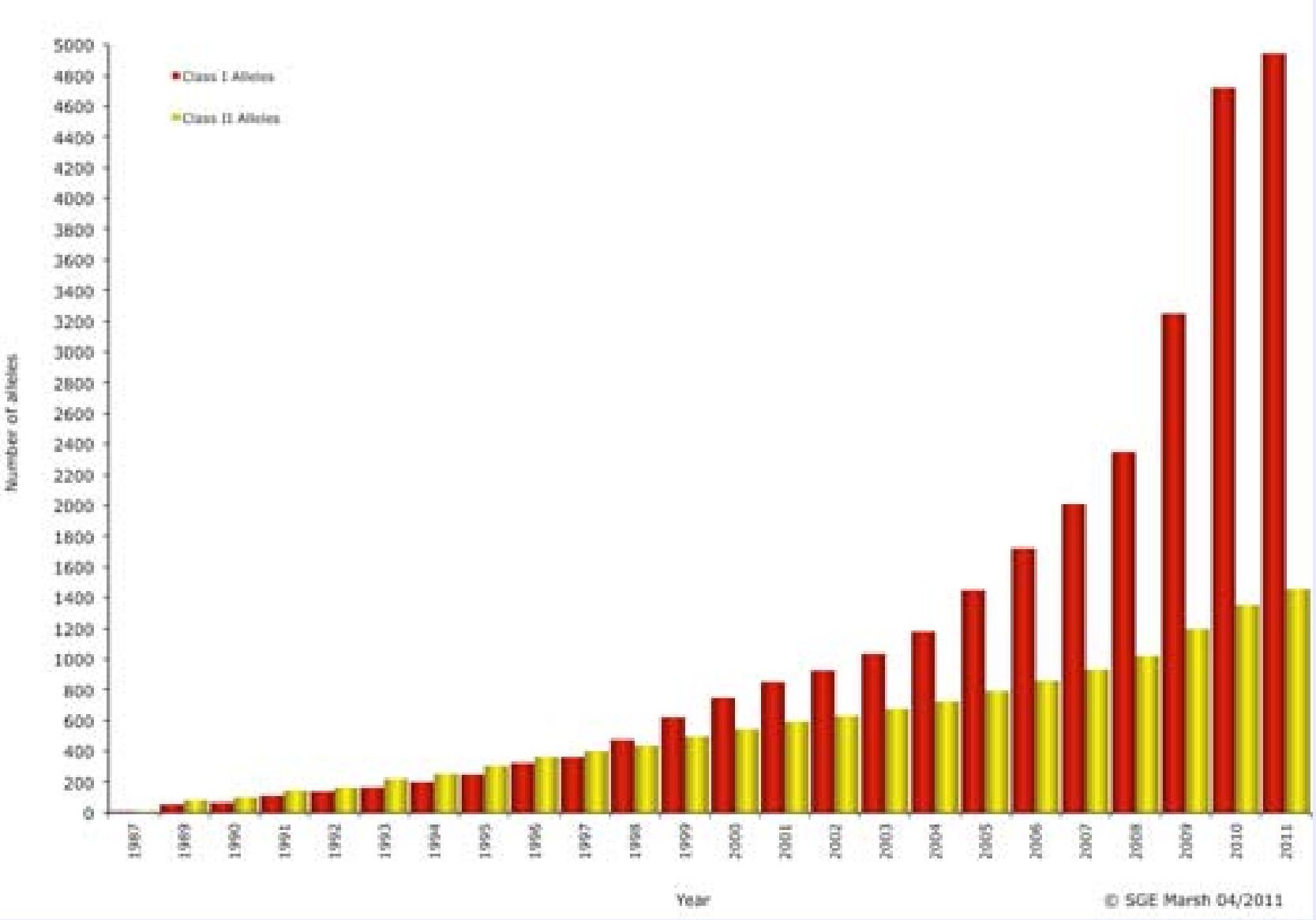
UMCG laboratoriumgeneeskunde



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Olerup O; HLA-B27 typing by a group-specific PCR amplification. Tissue Antigens 43; 253-256, 1994

	Exon2	151	161	261	271	281
sample	****	**********	*-----t***	**********	*g----g***	**********
reference	GGGC	TACGTgGACG	ACACGCAGtT	GACACAGATC	TTCAAGACCA	ACACACAGAC
B*27:01	----	----------	------T---	----------	-G----G---	-GG-------
B*27:02	----	----------	------T---	----------	-G----G---	-GG-------
B*27:03	----	----------	------T---	----------	-G----G---	-GG-------
B*27:04:01	----	----------	------T---	----------	-G----G---	-GG-------
B*27:04:02	----	----------	------T---	----------	-G----G---	-GG-------
B*27:04:03	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:02	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:03	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:04	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:05	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:06	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:07	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:08	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:10	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:11	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:12	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:13	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:14	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:15	----	----------	------T---	----------	-G----G---	-GG-------
B*27:05:16	----	----------	------T---	----------	-G----G---	-GG-------
B*27:06	----	----------	------T---	----------	-G----G---	-GG-------
B*27:07	----	----------	------T---	----------	-G----G---	-GG-------
B*27:08	----	----------	------T---	----------	-G----G---	-GG-------
B*27:09	----	----------	------T---	----------	-G----G---	-GG-------
B*27:10	----	----------	------T---	----------	-G----G---	-GG-------
B*27:11	----	----------	------T---	----------	-G----G---	-GG-------
B*27:13	----	----------	------T---	----------	-G----G---	-GG-------
B*27:14	----	----------	------T---	----------	-G----G---	-GG-------
B*27:15	----	----------	------T---	----------	-G----G---	-GG-------
B*27:17	----	----------	------T---	----------	-G----G---	-GG-------
B*27:19	----	----------	------T---	----------	-G----G---	-GG-------
B*27:20	----	----------	------T---	----------	-G----G---	-GG-------

Molecular typing of *HLA-B27* alleles

O. Dominguez[1], E. Coto[1], E. Martinez-Naves[1], S. Y. Choo[2], C. López-Larrea[1]

Immunogenetics 36: 277–282, 1992

	34><Exon3	351	361	371	451	461	471
sample	********	********---	-at*******	**********	**********	**a-----**	**********
reference	GGGTCTCA	CACCCTCCAG	AGGATGTATG	gCTGCGACGt	AACGAGGACC	TGCGCTCCTG	GACCGCGGCG
B*15:129	--------	----------	-AT-------	----------	----------	--A-------	----------
B*18:02	--------	----------	-AT-------	----------	----------	--A-------	----------
B*27:01	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:02	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:03	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:04:01	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:04:02	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:04:03	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:02	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:03	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:04	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:05	--T-----	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:06	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:07	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:08	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:09	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:10	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:11	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:12	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:13	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:14	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:05:15	--------	----------	-AT-------	----------	--T-------	--A-------	------C---
B*27:05:16	--------	------T---	-AT-------	----------	----------	--A-------	------C---
B*27:06	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:08	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:09	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:10	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:12	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:13	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:15	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:16	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:17	--------	----------	-AT-------	----------	----------	--A-------	------C---
B*27:18	--------	----------	-AT-------	----------	----------	--A-------	------C---

Dominguez (exon 3) en Olerup (exon 2) IMGT v 3.4.0

B*15:129	1	
B*18:02	1	
B*27:01	1	2
B*27:02	1	2
B*27:03	1	2
B*27:04:01	1	2
B*27:04:02	1	2
B*27:04:03	1	2
B*27:05:02	1	2
B*27:05:03	1	2
B*27:05:04	1	2
B*27:05:05	1	2
B*27:05:06	1	2
B*27:05:07	1	2
B*27:05:08	1	2
B*27:05:09	1	
B*27:05:10	1	2
B*27:05:11	1	2
B*27:05:12	1	2
B*27:05:13	1	2
B*27:05:14	1	2
B*27:05:15	1	2
B*27:05:16	?	2
B*27:06	1	2
B*27:07		2
B*27:08	1	2
B*27:09	1	2
B*27:10	1	2
B*27:11		2

B*27:12	1	
B*27:13	1	2
B*27:14		2
B*27:15	1	2
B*27:16	1	
B*27:17	1	2
B*27:18	1	
B*27:19		2
B*27:20		2
B*27:21		2
B*27:23	1	
B*27:24		2
B*27:25	1	2
B*27:26	1	?
B*27:27	1	2
B*27:28	1	2
B*27:29	1	
B*27:30		2
B*27:31	1	?
B*27:32		2
B*27:33		2
B*27:34		2
B*27:35	1	2
B*27:36		2
B*27:37	1	2
B*27:38	1	2
B*27:39	1	?
B*27:40	1	2
B*27:41	1	2

B*27:42	1	2
B*27:43		2
B*27:44	1	2
B*27:45	1	2
B*27:46	1	2
B*27:47	1	2
B*27:48	1	2
B*27:49	1	2
B*27:50	1	2
B*27:51	1	2
B*27:52	1	2
B*27:53	1	2
B*27:54	1	2
B*27:55	1	2
B*27:56	1	2
B*27:57	1	2
B*27:58	1	2
B*27:59N	1	?
B*27:60	1	2
B*27:61	1	2
B*27:62	1	2
B*27:63	1	2
B*27:64N	1	2
B*27:65N	1	2
B*27:66N	1	2
B*27:67	1	2
B*27:68	1	2
B*27:69	1	2
B*27:70		2

B*27:71	1	2
B*27:72	1	2
B*27:73	1	2
B*27:74	1	2
B*27:75	1	
B*27:76	1	2
B*27:77	1	?
B*27:78	1	2
B*27:79	1	2
B*27:80	1	2
B*37:02	1	
B*38:22	1	
B*44:97		2
B*47:04	1	
B*47:05	1	

- Dominguez (exon 3) extra allelen:
  B*15:129
  B*18:02
  B*37:02
  B*38:22
  B*44:97
  B*47:04
  B*47:05

- Olerup (exon 2): alleen B*27
  maar niet alle B*27 allelen

- Beide positief: alleen B*27
  maar niet alle B*27 allelen

UMCG laboratoriumgeneeskunde

Consequences of used primer sets in testing HLA-B*27; results and (non-) learning aspects of a national SKML Quality Survey

H.H.M. Eidhof, B.G. Hepkema, S.P.M. Lems*, J. Danneberg, R. Maatman (klin lab ZGT Hengelo, *UMCG Groningen)*

Introduction

An external quality assurance program for HLA-B*27 by Molecular Biological techniques, twice a year, is operational since 1997 in The Netherlands. Most participants use Sequence Specific Primers (SSP) as described previously by Olerup et all (exon 2) or Dominquez et all (exon 3). Due to the 'all or none respons' type of this technique, co-amplification of a control- gene (eg HGH) is essential. Reported results of the participants have a high degree of consensus but occasionally there are exceptions. We observed this phenomenon two times, in a survey in 2007 and in a survey in 2008. Since 2007, all our survey-samples are typed for HLA-B by sequencing based typing (SBT) to obtain a high resolution. The use of the right primer sets, in combination with a recent HLA allele database (http://www.ebi.ac.uk/imgt/hla/) is important to get the right results.

Method

Blood or purified DNA samples were sent to 34 participants in The Netherlands. Participants used their in-home standard method to analyse the material for HLA-B*27. Results were compared by the distributing laboratory of the Ziekenhuis Groep Twente (Almelo/Hengelo). SBT of the survey was performed, by the UMCG laboratory (Groningen, Holland). The reactivity of the published primer sets were updated with the most recent allele database (v 2.23.0) table 1. Results were presented in a biannual meeting of the CMBD task force.

Results

In the sent out 2007.1, there appeared a great difference between the participants, in the results of the HLA-B*27 typing. Sequencing by the expert laboratory (UMCG), revealed that this patient was HLA-B*1802, 4002.

20 out of 26 laboratories (77%), qualified this material falsely as positive for HLA-B*27.

In the 2008.2 survey, material was used of a patient that was sequenced by the expert laboratory as HLA-B*1802,1501. 14 out of 34 laboratories (41%), reported (false) positive results for HLA-B27. In this sent out there were 13 participants who scored falsely positive in the 2007 sent out and now again failed.

Allele	Domiguez "exon 3"	Olerup "exon 2"
B*1802	+	
B*2701	+	+
B*2702	+	+
B*2703	+	+
B*270401	+	+
B*270402	+	+
B*270502	+	+
B*270503	+	+
B*270504	+	+
B*270505	+	+
B*270506	+	+
B*270507	+	+
B*270508	+	+
B*270509	+	
B*270510	+	+
B*2706	+	+
B*2707		+
B*2708	+	+
B*2709	+	+
B*2710	+	+
B*2711		+
B*2712	+	
B*2713	+	+
B*2714		+
B*2715	+	+
B*2716	+	
B*2717	+	+
B*2718	+	
B*2719		+
B*2720		+
B*2721		+
B*2723	+	
B*2724		+
B*2725	+	+
B*2726	+	
B*2727	+	+
B*2728	+	+
B*2729	+	
B*2730		+
B*2731	+	
B*2732		+
B*2733		+
B*2734		+
B*2735	+	+
B*2736		+
B*2737	+	+
B*2738	+	+

Klinische relevantie; echt B27?

- Andere bevolkingsgroepen
- Sommige allelen géén associatie
- Transgene dieren (+b2M, niet SPF)

Bechterew+	Bechterew -
B*27:02	B*27:06
B*27:04	B*27:09 (?) (sardinië)
B*27:05	
B*27:07 (?)	

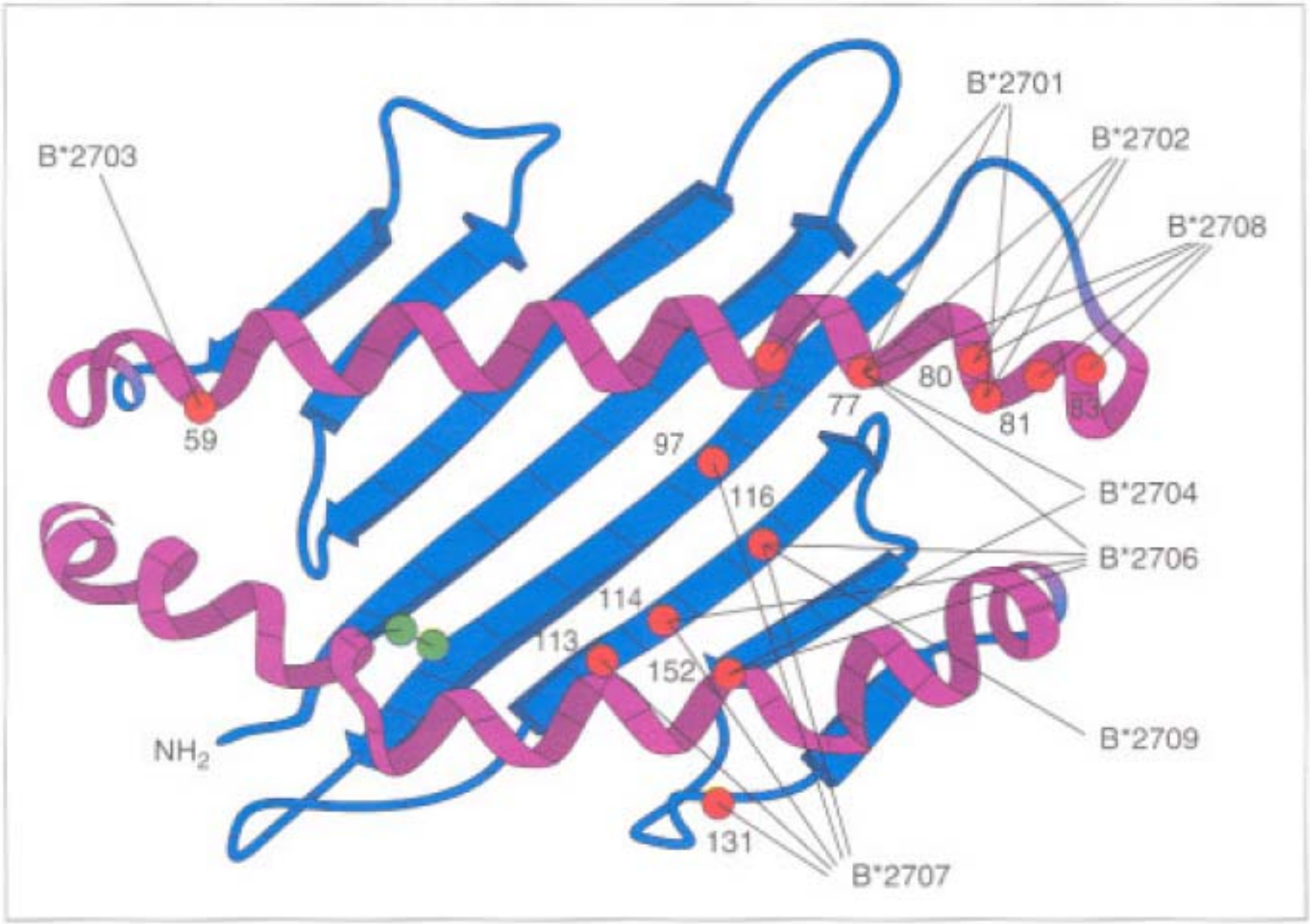
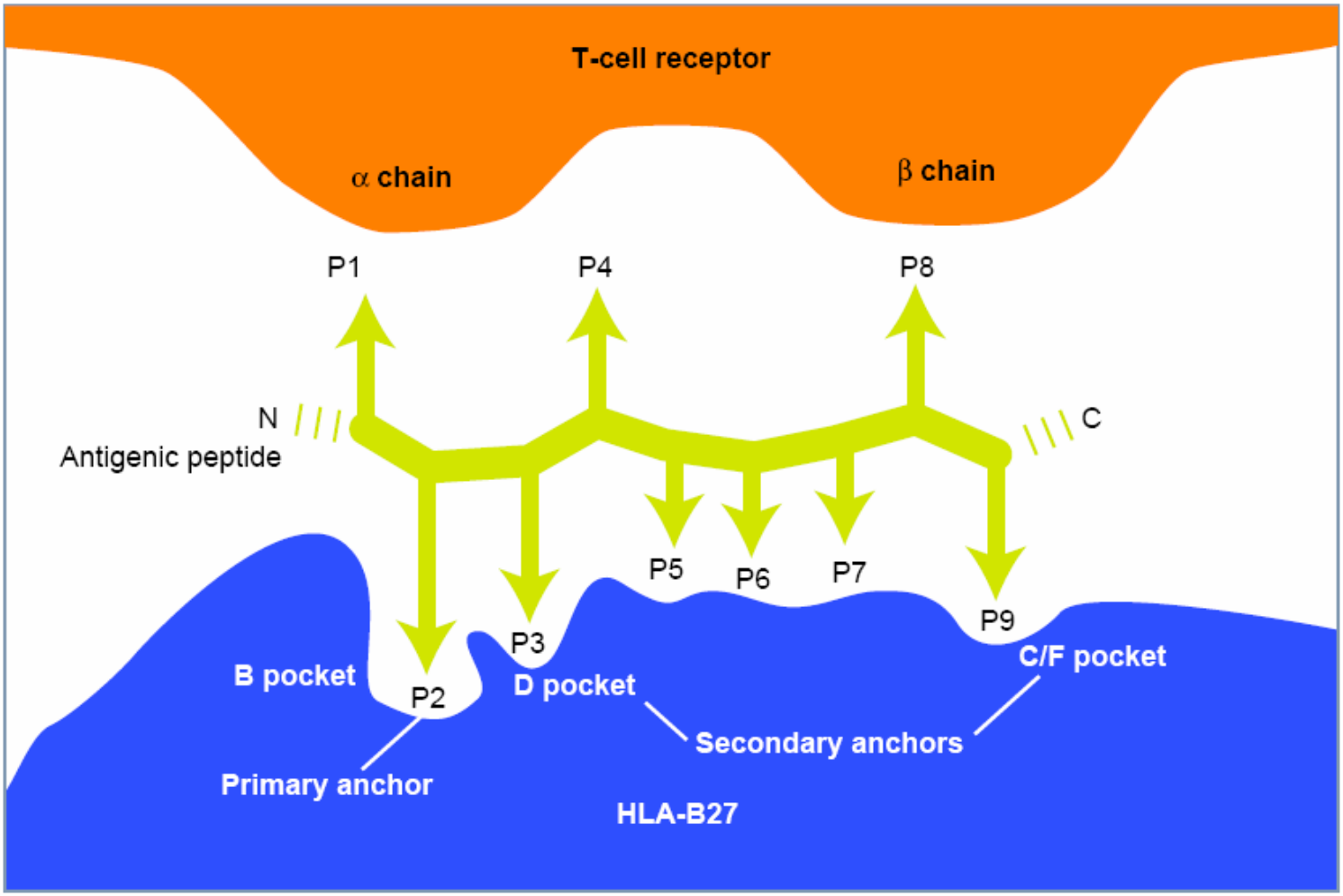


Fig. 1. *Top view of HLA-B*2705 indicating the sites (shown in red) at which it differs from the other subtypes of HLA-B27. Disulfide bonds are shown in green.*



Schematic diagram showing binding of antigenic peptides to HLA-B27 and recognition by the T-cell receptor

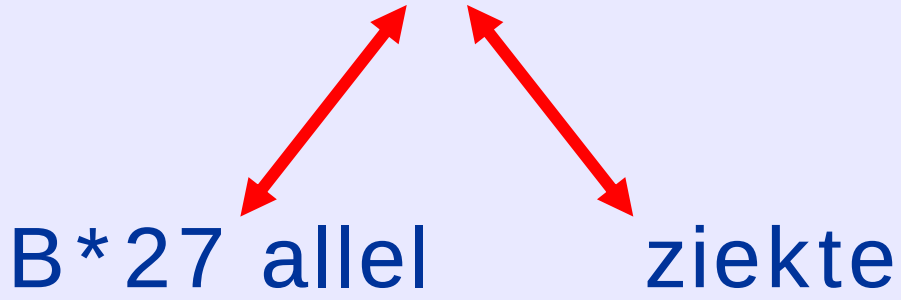
Summary of statistically significant motifs among natural ligands of HLA-B27 subtypes[a]

Subtype	Changes relative to B*2705	Pocket	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	*n*
Decamers													
B*2705			G, H, **R**	**R**	**F**, I, W, Y	–	**G**	I, K	Y	I	**G**, T, Y	K, L, **Y**	39
Nonamers													
B*2705			**G**, **R**	**R**	**F**, **I**, V, **Y**	G, **P**	**G**, I	**I**, V	**I**, Y	G, **N**, **T**	**F**, **K**, **L**, M, R, **Y**		108
B*2709	D116H	F	**G**	**R**	**F**, N	G, **P**		**I**	Y	**N**	**F**, **L**, V		37
B*2704	D77S	F	**G**, R	**R**	**F**, I, V, **Y**	F, G	G, Q	–	L, T	E, T	F, **L**, **V**, Y		35
	V152E	E											
B*2706	D77S	F	**G**, **R**	**R**	H, **Y**	G	**G**	–	T	T	**F**, **L**, **V**		33
	H114D	D/E											
	D116Y	F											
	V152E	E											
B*2703	Y59H	A	**K**, **R**	**R**	**F**, I, **Y**	–	I	**I**	**Y**	–	L, **Y**		24

[a]Residues with $P < 0.05$ (with Yates' correction) at each peptide position (P) are indicated. Those with $P < 0.05$ after Bonferroni's correction are in boldface. Subtype changes relative to B*2705 and their location in the side-chain-binding pockets of the peptide-binding site are indicated. Subtypes for which less than 20 sequences of the same size are available (B*2701, B*2702, B*2707, and B*2710) are not included. The number of peptides in each peptide series (*n*) is indicated.

Conclusie:

Géén duidelijke associatie:



Waarom bestaat B27 (nog)

Evolutionaire voordelen?

Selectie?

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Escape from the Dominant HLA-B27-Restricted Cytotoxic T-Lymphocyte Response in Gag Is Associated with a Dramatic Reduction in Human Immunodeficiency Virus Type 1 Replication▽

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AIDS-protective HLA-B*27/B*57 and chimpanzee MHC class I molecules target analogous conserved areas of HIV-1/SIV$_{cpz}$

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