EBMT

European Group for Blood and Marrow Transplantation

HLA MANUAL

A Guide to the completion of the EBMT Form

HISTOCOMPATIBILITY

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I How to fill in the HLA form

To start this section, general data are required regarding the Laboratory, which may be different from the Haematology Unit if HLA typing could not be done at the same hospital.

Laboratory
Hospital .....
Unit .....
Telephone ..... Fax .....

It also contains parameters on methods used to perform the HLA typing (several methods should be performed) and on donor (date of birth).

Technique Used:
.....

Donor HLA Phenotype:
Date of birth ..... - ..... - .....
dd *mm* *yyyy*

In the HISTOCOMPATIBILITY allogeneic transplants Form we request HLA typing results for the following loci : A, B, C, DRβ1, DQβ1 and DPβ1.

A revised listing of recognised HLA specificities is issued by the WHO nomenclature and is available in http://www.anthonynolan.org.uk/HIG/nomenc.html

HLA Type	**A**		**B**		**C**	
Serology						
*Molecular biology typing						
HLA Type	**DRB1**		**DQB1**		**DPB1**	
Serology						
*Molecular biology typing						

*Instructions for coding (e.g.: DQB1 0201-0202-0203)
- If result is 02 only and high resolution not done, type is "02 X"
- If result is 02 only and high resolution not discriminant, type is "02 ?"
- If result is 0201-0202-0203, type is "02 *"
- If result is 0201, type is "02 01"

Results obtained by serological methods should be written in the line “Serology”, the line “Molecular biology typing” should be filled in when methods used are molecular like SSP, SSOP …
To fill in this table, the above mentioned nomenclature should be used.

It is very important to identify the right locus and not to truncate the string. If there are any problems understanding the laboratory results please ask your physician. If there is no expert in your department, please attach a copy of the laboratory results to your MED-B form and send it to the EBMT / national registry.

II What is HLA

The **H**uman **L**eukocyte **A**ntigens system contributes to the Immune Response. It's a set of molecules (glycoproteins) expressed at the surface of almost all cells that are responsible for lymphocyte recognition and "antigen presentation". The HLA molecules control the immune response through recognition of "self" and "non-self". They belong to a group of molecules known as the Imunoglobulin Supergene Family, which includes immunoglobulins, T-cell receptors, CD4, CD8, and others. The main function of the HLA molecules is presenting the antigen (protein chain of antigen) to the T Lymphocytes and initiating the specific immune response.

HLA molecules are coded by two groups of genes, HLA class I and HLA class II, and the functions of both groups are really quite distinct.

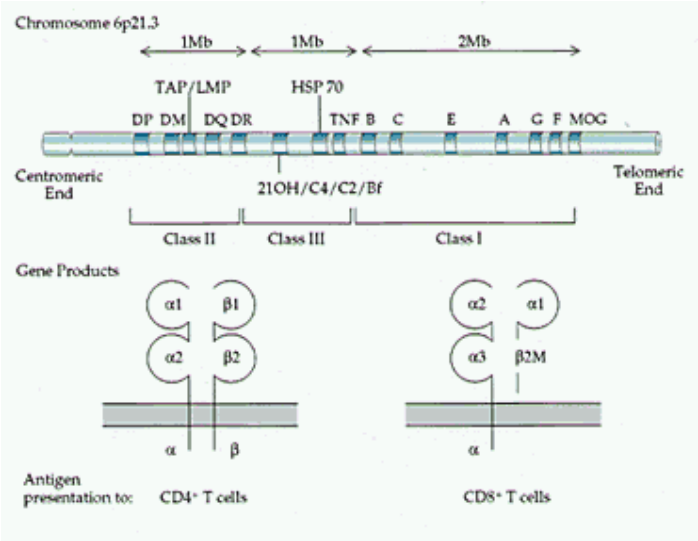
HLA class I proteins are coded by the genes **HLA-A**, **HLA-B**, and **HLA-Cw**.
Class I molecules are found on virtually every cell in the human bodyand they present antigen to cytotoxic T-cells (CTLs) (the $CD8^+$ T Cell). Class I molecules present "endogenous" antigen. An endogenous antigen might be fragments of viral proteins or tumour proteins. Presentation of such antigens would indicate internal cellular alterations that if not contained could spread throughout the body. Hence, destruction of these cells by CTLs is advantageous to the body as a whole.

Class I molecules are made up of 2 chains, a heavy chain (transmembrane polypeptide) coded by the genes **HLA-A**, **HLA-B** and **HLA-Cw,** and a light chain beta-2 microglobulin (a no-transmembrane polypeptide).

HLA class II proteins are coded by the gene **HLA-DR**, **HLA-DQ**, and **HLA-DP**.
Class II molecules, in contrast to Class I molecules, are found only in B-cells, macrophages and other "antigen-presenting cells" (APCs). Class II molecules present antigen to helper T-cells (TH-cells) ($CD4^+$ T cells - The $CD4^+$ T cells that activate B cells are called **Helper T cells**.). Class II molecules present "exogenous" antigens. Exogenous antigens, in contrast, might be fragments of bacterial cells or viruses that are engulfed and processed by e.g. a macrophage and then presented to helper T-cells. The TH-cells, in turn, could activate B-cells to produce antibody that would lead to the destruction of the pathogen.

Class II molecules consist of two transmembrane polypeptides, Alpha Chain and Beta Chain. The Beta chain is much more polymorphic compared to the Alpha chain For this reason HLA typing is currently done on Beta chain (**HLA-DRB1**, **HLA-DRB3**, **HLA-DRB4, HLA-DRB5**, **HLA-DQB1**, and **HLA-DPB1)**.

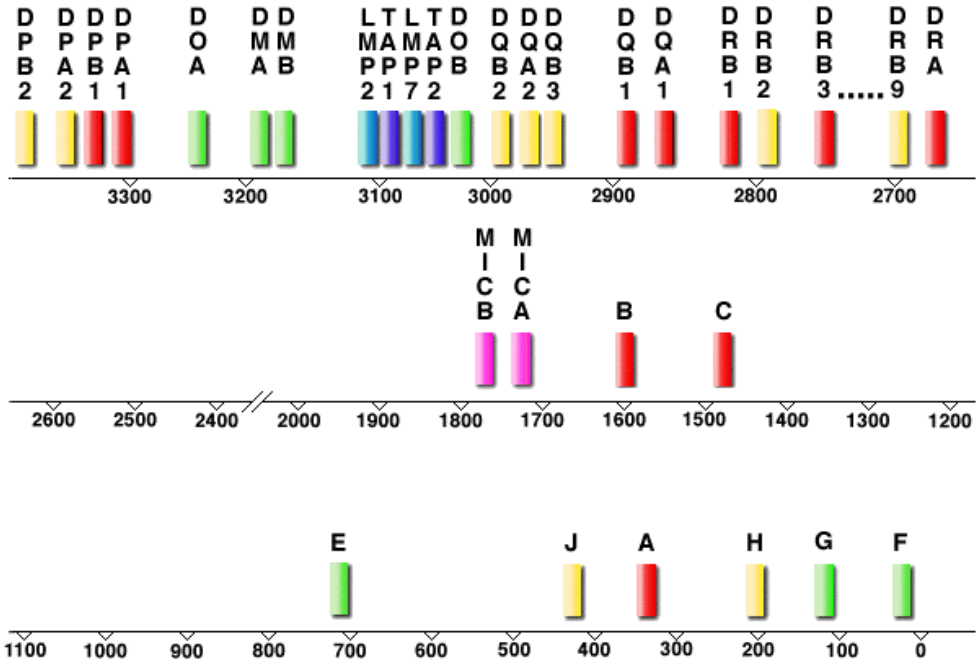
CLASS I vs CLASS II MOLECULES



III Genomic Structure and Polymorphism

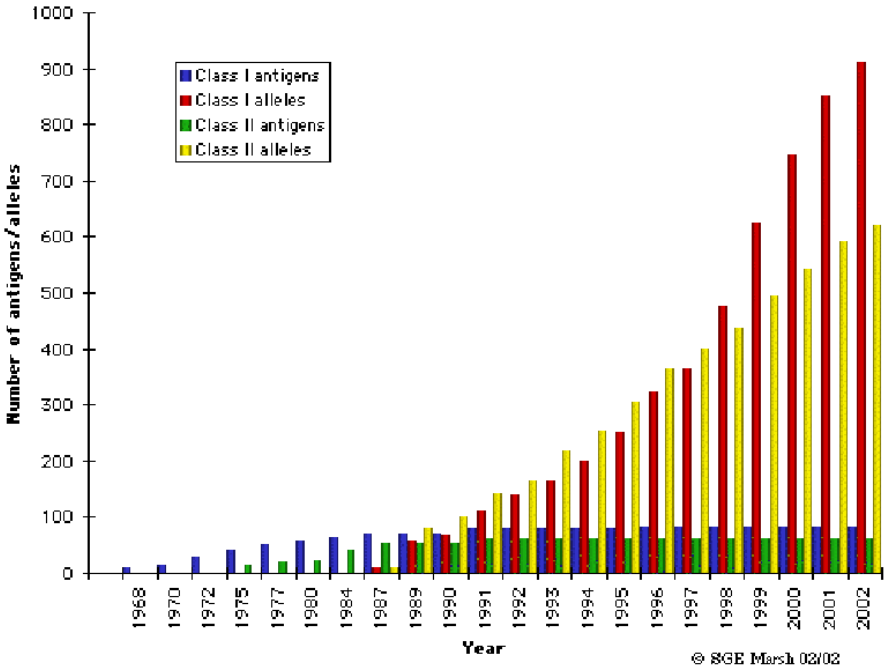
The HLA is located on chromosome 6p21.31 and covers a region of about 3.6 Mbp depending on the haplotype. The classical HLA antigens encoded in each region are HLA-A, -B, and -Cw in the class I region, and HLA-DR, -DQ and -DP in the class II region. All class I genes are between 3 and 6 kb, whereas class II genes are 4-11 kb long.

MAP of the HLA REGION



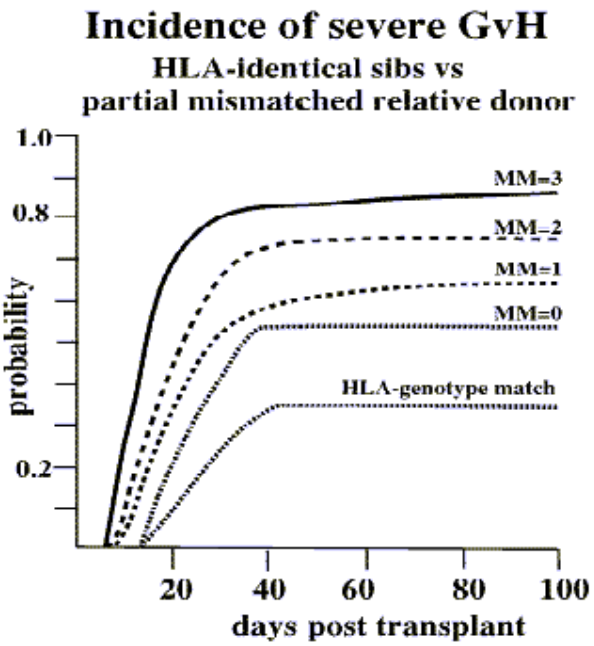
One of the main characteristics of the HLA is its extreme polymorphism. Among the expressed loci, the HLA has the greatest degree of polymorphism in the human genome. The numbers of known alleles increase constantly, as shown in the following graph.

Numbers of HLA antigens and alleles 1968 - 2002



IV Importance of the HLA system in the transplantation

For Haemopoietic stem cell (HSC) transplants, the degree of HLA matching is critical in determining the probability of Graft-versus-Host disease (GvHD). This is the major form of rejection in clinical HSC transplants. In an attempt to minimise these alloresponses, the HLA class I and class II types of the donor and recipient are matched as closely as possible. However, because of extensive polymorphism, an HLA identical donor is only rarely available. Most transplant recipients therefore receive immunosuppressive drugs to prevent or stop detrimental alloresponses, but this non-specific approach also compromises beneficial immune responses to infection. Most centres will only transplant across 1 HLA class I (HLA-A or B locus) difference. The following graph shows the probability of serious GvHD (so-called grade III or IV) versus time for different degrees of mismatch.



HLA matching at the highest level of molecular resolution appears to be most beneficial to a successful therapeutic outcome. Thus, the incidence and severity of graft-versus-host disease (GvHD, a syndrome caused by the transplantation of immunocompetent donor haematopoietic stem cells into an immunocompromised recipient) or failure of the graft, can be reduced significantly with HLA genotypic matched transplants.

http://www.aruplab.com/guides/clt/tests/clt_290a.jsp#1929266

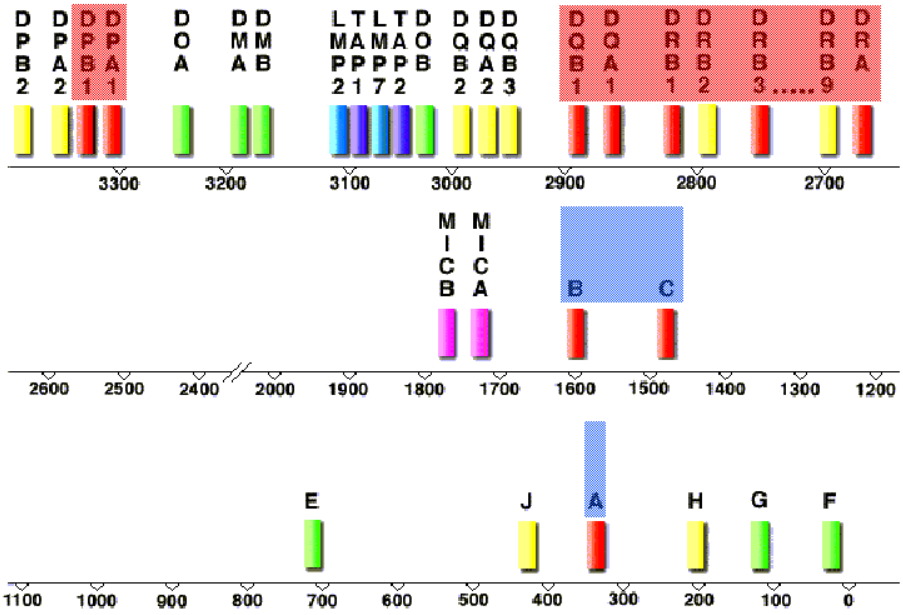
V The search for a donor

When a patient begins to look for a bone marrow donor, the first step is to look at his or her brothers and sisters. If no HLA-identical sibling donor is found or if the patient has no siblings, a decision is made based on the patient's disease, medical condition, and HLA typing to either look at extended family members (aunts, uncles, cousins, etc.) or proceed directly to a search for an unrelated donor. An unrelated donor search is performed through national and international bone marrow transplant registries which have HLA typings on thousands, and in some cases, millions of potential donors.

The actual HLA testing is performed on a blood sample from the patient and potential donors. There are different ways that an HLA typing test can be done. These methods differ in their ability to detect differences between a patient and donor. HLA typing can be performed by « serologic typing » or by « DNA molecular typing » techniques. Serologic typing is often the first step in HLA typing, which identifies the major transplantation antigens that make up a patient's HLA (at least A, B & DR, but now matches are defined to a level of 10/10 on loci A, B, Cw, DR & DQ). This test can be done rather quickly with results available within two to three days. Although serologic testing is sufficient in some cases to identify a compatible sibling donor, more sensitive techniques using molecular typing of DNA are needed for identification of compatible "unrelated" donors (a first quick search is done using serological or molecular low resolution methods, and a second more specific search is carried out using high resolution methods on locus A,B, Cw, DR & DQ and facultative DP). The molecular typing

is very sensitive, more accurate than serology, and can detect even small differences between patients and prospective donors.

HLA genes typed in HSCT



Blue : Class I & Red : Class II

VI What are the methods for HLA Typing?

Currently, donor/patient matching relies mainly on serological matching for HLA-A and -B loci, and DNA-based typing techniques for HLA-Cw, -DR, -DQ and DP loci.

A Serological Methods

Serologic-based HLA typing uses antigen-specific sera to determine a person's HLA type. Sera are human-derived preparations that react to specific HLA antigens expressed on white blood cells. A serologic-based test determines a person's HLA tissue type by noting which types of sera react to the person's white blood cells and which sera do not react. After an incubation step that permits time for the antibody to bind to any corresponding antigens in the cells, a complement is added to facilitate cell lysis. The reactions are then viewed under the microscope and graded according to the amount of cell lysis that has occurred. The type is assigned after reviewing the reaction patterns for the various sera.

Serologic-based typing is a less specific test than molecular-based tissue typing.

B Molecular Methods

Molecular-based tissue typing uses standardized synthetic probes and primers to determine a person's HLA tissue type. These probes and primers do not react to the antigens expressed on a person's white blood cells, but to the DNA that specifies which antigens are present. Production of an amplicon indicates in the DNA sequence encoding that a specific allele is present in the patient's sample. In some cases, the primer pair can only eliminate some of the possible allelic types. The resulting product is then subjected to restriction enzyme digestion and fragment length analysis to determine the allelic assignment. In other cases, the only sure way to determine what allele(s) is/are present is to take the amplified product and perform DNA sequence analysis. Molecular-based methods do not require live white blood cells; any source of cells can serve as a sample for molecular-based tissue typing. Because

of the standardized probes and primers, molecular typing is more accurate than serologic typing. It also provides more typing information at a higher level of detail because it is able to identify alleles as well as antigens. Alleles determine which antigens are present on the cells.

Different Level:

The level of resolution of molecular methods is due to the specificity of the probe used for the typing. Some probes can distinguish an antigen, by low level resolution methods, whereas other probes make it possible to distinguish alleles. The latter requires a high level of resolution

The oligotyping procedure (using SSP [Sequence Specific Primer] or SSOP [Sequence Specific Oligonucleotide Probe]) provides low to medium resolution.

Ambiguity

Certain probes used in the molecular methods can detect the presence of several alleles of the same gene, and do not determine with precision which specific allele is present. In this case we speak of ambiguity or medium level resolution.

SSOP methods (Sequence Specific Oligotide Probes):

This technique that is widely used in the laboratory is the sequence specific oligonucleotide probe (SSOP) assay in which the PCR product is tested against a series of known probe sequences. From the pattern of probe annealing the allelic type can be deduced. Class II typing (DRB1 and DQB1) is performed using this technique and it is used extensively to augment the results of serological typing for class I antigens.

SSP methods (Sequence Specific Primer):

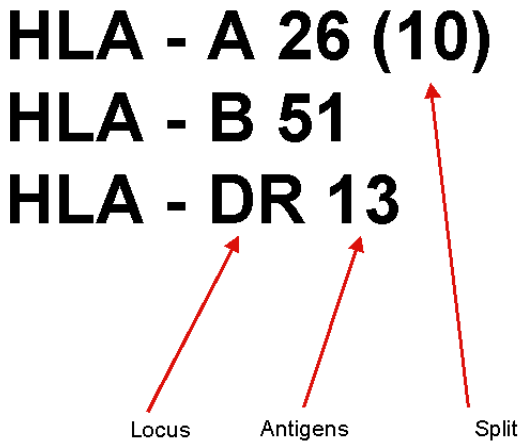
The basis of modern molecular typing techniques is the polymerase chain reaction (PCR), a technique that allows the amplification of specific fragments of deoxyribonucleic acid (DNA) to produce literally millions of fragment copies which can then be further analysed by an increasing array of techniques. Selection of the primer sequences that drive the PCR can make the reaction specific for a given allele (which translates to a tissue antigen) in a sequence specific primer (SSP) assay.

VII Nomenclature

A For Serological Methods

The official nomenclature used for serologically defined antigens is the WHO nomenclature.
The letter (A or B etc) refers to the genetic locus and the number to the particular antigen coded by that locus. Numbers in parentheses denote "broad" parent antigens from which the "split" specificities have been derived.

HLA Serological Nomenclature Requirements

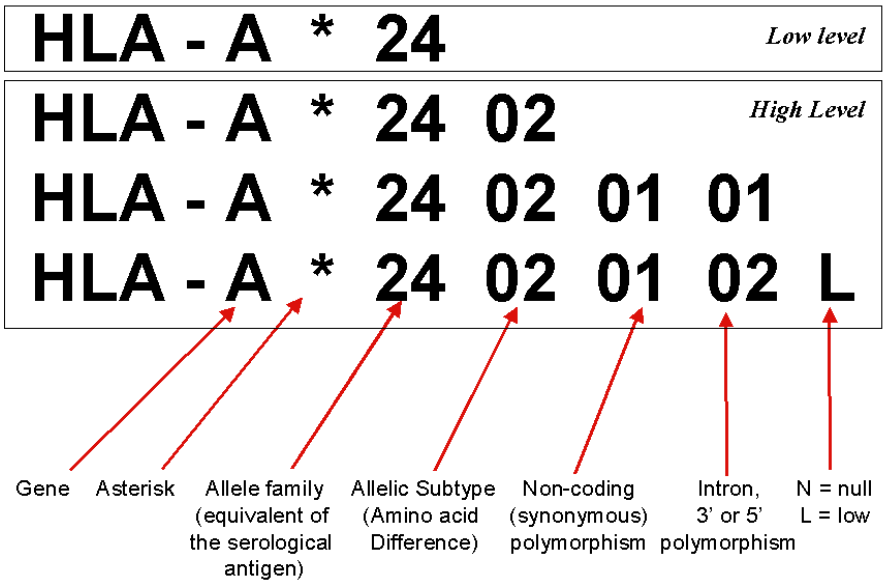


View web site : http://www.anthonynolan.org.uk/HIG/lists/specs.html

B For Molecular Methods

HLA allele assignment for each HLA locus is made using the following nomenclature. Four digits are usually used to identify the allele:
The HLA locus (A, B, C, DR, DR and DP) is separated by the symbol * from two numeric digits, which assigns the serologic equivalent of the antigen. This is followed by another two numeric digits, which assign the specific allele. Occasionally, an additional numeric value is added to identify a different subtype produced by a nucleotide substitution that does not alter the aminoacid sequence and the antigenic expression of the protein product. Thus HLA-A*03012 is an allele from the A locus that encodes the HLA-A3 serologic antigen and is the 01 allelic subtype of A3; this allele was also found to have a silent nucleotide substitution that distinguishes it from A*03011.

HLA Molecular Nomenclature Requirements



Non-coding and Intron digits are very rarely used in our level.
Null and Low indicates the level of expression of the allele and are rarely used too.

Nomenclature	Indicates
HLA	the HLA region and prefix for an HLA gene
HLA-DRB1	a particular HLA locus i.e. DRB1
HLA-DRB1*13	a group of alleles which encode the DR13 antigen
HLA-DRB1*1301	a specific HLA allele
HLA-DRB1*1301N	a null allele
HLA-DRB1*130102	an allele which differs by a synonymous mutation
HLA-DRB1*13010102	an allele which contains a mutation outside the coding region
HLA-DRB1*13010102N	a null allele which contains a mutation outside the coding region
HLA-DRB1*13X	high resolution not done
HLA-DRB1*13 ?	high resolution done but not discriminant

View web site http://www.anthonynolan.org.uk/HIG/lists/class1list.html for Class I
http://www.anthonynolan.org.uk/HIG/lists/class2list.html for Class II

levels of resolution:

Level of Resolution	Allele
Low	**A*02**
Medium	**A*0201/0205/0209/0240**
High	**A*0201**

Ambiguity and medium level of resolution
Ambiguity is due to possible cross reaction of probes used for the typing, and is indicated with two symbols – (score) and / (slash).

- – (score) means all possibilities between the first and the last possibility mentioned
- / (slash) means only the mentioned possibility

See example in the next table.

Ambiguity : Nomenclature

	Indicates the presence
DRB1*0401-04	**DRB1*0401** or **0402** or **0403** or **0404**
DRB1*0401/04	**DRB1*0401** or **DRB1*0404**

B*1501/1502/1505/1515/1521/1545/1556/1570:
The probe can detect the presence of alleles 01, 02, 05, 15, 21, 45, 56 and 70, but cannot detect the presence of alleles 03, 04, 06 etc, therefore for the patient we know that the allele is neither the allele 03 nor 04 etc., nevertheless we do not know if the specific allele is 01 or 02 or 05 or 15 …

When indicating ambiguous results such as B*1501/1502/1505/1515/1521/1545/1556/1570, it is very important not to truncate the string as this may lose important information and may give an incorrect result. Reducing the string above to just B*1501 would be wrong if the individual was actually B*1502

In the results from the laboratory, it is possible to find another nomenclature. This is the simplified alphanumeric code set up by The National Marrow Donor Program® (NMDP).
When the traditional nomenclature is complex, as in the following case :

B*1501/1502/1505/1515/1521/1545/1556/1570

the various possible alleles can be coded by several letters, with the nomenclature used by the NMDP.
This nomenclature is available at the following address : www.nmdpresearch.org

Ambiguity : NMDP code

B*1501/02	**= B*15AB**
B*1501/1502/1505/1515/1521/1545/1556/1570	**= B*15FGR**

VIII References

Web Site References :

- http://www.nmdpresearch.org/index.html
- http://www.anthonynolan.org.uk/research/index.html
- http://www.aruplab.com/guides/clt/tests/clt_a280.htm
- http://www.aruplab.com/media/pdf/testing/tech_bulletins/hlaseq.htm

Other References :

- HLA Nomenclature Report
  Marsh et al. Tissue Antigens (2002) **60** 407-464
- EFI Guidelines for Nomenclature usage in HLA Reports
  Tiercy et al. European Journal of Immunogenetics (2002) **29** 273-274
- HLA Dictionary
  Schreuder et al. Tissue Antigens (2001) **58** 109-140