

DEFINITIONS OF HISTOCOMPATIBILITY TYPING TERMS

The definitions below are intended as general concepts. There will be exceptions to these general definitions. These definitions do not imply any specific requirements for HLA typing but are only meant to define useful terms.

Definitions of typing resolution. HLA laboratories should have a written agreement with each entity requesting HLA typing regarding the specifications for the resolution of typing. The following terms might be used in the agreement. These definitions do not imply any specific requirements for typing; that decision is made by the HLA typing laboratory in compliance with testing standards and requirements of the test requesting entity.

Allelic resolution: The DNA-based typing result is consistent with a single allele as defined in a given version of the WHO HLA Nomenclature Report as described on the reference web site, <http://hla.alleles.org>. An allele is defined as a unique nucleotide sequence for a gene as defined by the use of all of the digits in a current allele name. Examples include A*01:01:01:01; A*02:07 (based on the IMGT/HLA Database version 3.1.0, July 2010).

High resolution: A high resolution typing result is defined as a set of alleles that specify and encode the same protein sequence for the peptide binding region of an HLA molecule and that excludes alleles that are not expressed as cell-surface proteins. Examples are typings with the following characteristics: (1) includes only alleles within a P group designation eg., A*02:01P, DPB1*04:02P or (2) includes alleles within a G group designation (eg., A*02:01:01G, DRB1*12:01:01G) with the exception that it does not include non-expressed alleles with the same nucleotide sequence (e.g., A*02:01:01G without A*02:43N and A*02:83N). A list of P and G groups can be found at <http://hla.alleles.org>. The appendix shows another example.

Confirmed allele high resolution: A “confirmed” allele is one that has been identified in two or more unrelated individuals and is designated as “confirmed” in the IMGT/HLA database (http://www.ebi.ac.uk/imgt/hla/all_confirm.html).

A confirmed allele high resolution typing result is defined as a set of alleles that

1. specify and encode the same protein sequence for the peptide binding region of an HLA molecule, and
2. exclude “confirmed” alleles that are not expressed as cell-surface proteins and
3. include alternative genotypes with two unconfirmed alleles that specify different protein sequences, when applicable.

An example of confirmed allele high resolution typing is: A*02:01:01G in which the non-expressed alleles, A*02:43N and A*02:83N, are not excluded because both are unconfirmed. The appendix shows another example.

Common and well-documented (CWD) allele high resolution: This resolution is based on a list of alleles designated as CWD and distinguished from 'rare alleles'. An initial report defining CWD alleles has been published (Cano P, et.al., Common and well-documented HLA alleles: report of the ad-hoc committee of the American Society for Histocompatibility and Immunogenetics. Hum Immunol 2007; 68(5):392-417); however, it is suggested that laboratories rely on the most current definition of CWD alleles.

Common alleles appear with gene frequencies greater than 0.001 in any reference population. Accurate frequency estimations can be made for alleles observed three or more times; thus, population studies with a sample size of 1,500 unrelated subjects ($2n = 3,000$ chromosomes) can detect with accuracy only allele frequencies of 0.001 or greater.

Well-documented alleles are a second category which includes those alleles that have been observed in at least three unrelated individuals. In certain cases, the gene frequency of well-documented alleles may not be estimated accurately. This is the case for alleles found only in isolated populations such that their frequencies are likely to be less than 0.001 in any outbred population.

Rare alleles have extremely low frequencies and are not likely to be found again in a significant number of unrelated subjects.

Intermediate resolution: Intermediate resolution is defined as a DNA-based typing result that includes a subset of alleles sharing the digits in the first field of their allele name and that excludes some alleles sharing those digits. Examples include: A*02:01 or A*02:02 or A*02:07 or A*02:20 but not other A*02 alleles. There may be cases in which the subset of alleles includes one or more alleles with a name beginning with different digits but these alleles should be the exception i.e., the majority of the alleles should share the same first digits eg., A*01:01 or A*01:02 or A*01:14 or A*36:04.

Low resolution: The DNA-based typing result is at the level of the digits comprising the first field in the DNA-based nomenclature. Examples include: A*01; A*02. The exception is B*15 in which low resolution is defined as a subset of alleles that might be considered as sharing a broad serologic type, either B15 or B70.

Replacement of the term "confirmatory typing". It was felt that the activities encompassed within the term "confirmatory typing" have become unclear. It is recommended that the following two terms be used in the place of the words "confirmatory typing."

Verification typing: HLA typing performed on an independent sample (or, for a cord blood unit, from an attached segment or from the unit itself) with the purpose of verifying concordance of that typing assignment with the initial HLA typing assignment. Concordance does not require identical levels of resolution for the two sets of typing but requires the two assignments to be consistent with one another.

Extended typing: HLA typing performed to add additional information to an existing HLA assignment. This additional HLA typing may: (1) extend the typing to include assignments at additional HLA loci (e.g., to type HLA-C for an HLA-A,-B,-DRB1 typed volunteer donor) and/or (2) include additional typing to increase the resolution at any previously typed HLA locus (e.g., to type an HLA-B serologically typed individual to identify the alleles potentially carried at the HLA-B locus at intermediate, at high or at allelic resolution).

Format for the report of HLA assignments. HLA typing assignments must be clearly understood by the end user. HLA laboratories should have a written agreement with each entity requesting HLA typing regarding the specifications for the typing. This must include the loci to be tested, the level of resolution of the typing, criteria for selection of a donor for allogeneic transplant, and the format in which typing results will be reported. The impact of any uncertainty in HLA assignments of either the potential donor or patient on matching should be addressed in the report.

Unresolved alternative assignments: It is strongly recommended that the report to the end user include all uncertainty in the typing assignment. This means that genotypes and/or alleles that have not been excluded should be listed in the final report. If it is not possible to provide a list of unresolved alternatives on the report, the laboratory should indicate that alternative assignments exist and might provide a rationale for the HLA assignment that is selected to be included in the report.

Database used in interpretation of typing results: The version of the IMGT/HLA Database used to interpret the HLA results should be included in the report to the end user.

Reporting a string of alleles: Slashes should be used to separate a string of alternative alleles e.g., A*02:01/02:02/02:07/02:20 to mean A*02:01 or A*02:02 or A*02:07 or A*02:20. When the laboratory wishes to shorten the length of the string, it is recommended that the laboratory use the following format: A*02:01/02/07/20; DRB1*01:01:01/02. The more complex assignment to represent A*02:01:01 or A*02:01:02 or A*02:05 would be A*02:01:01/02/A*02:05. Other notations may be utilized as long as the typing assignment is clearly understood by the end user.

Matching: If the laboratory is providing HLA assignments for a potential donor and patient for allogeneic transplantation, the laboratory might include a description of the matching status of the pair. Matching denotes interpretation of the comparison of HLA types of patient (P) and a putative donor (D). The outcome of this interpretation is either a match (they share the type) or a mismatch (they do not).

Directionality of match: The laboratory may wish to refer to directionality (or vector) of the mismatch in cases where the patient or a potential donor is homozygous at a locus

or has a non-expressed allele. If the patient is homozygous and one of the HLA assignments is identical to an assignment of the heterozygous donor (e.g., patient A*01:01:01:01, potential donor A*01:01:01:01, A*23:01), the mismatch may be referred to as a mismatch in the host versus graft vector direction. If the potential donor is homozygous and one of the HLA assignments is identical to an assignment of the heterozygous patient, the mismatch may be referred to as a mismatch in the graft versus host vector direction.

Matching within a family:

HLA haplotypes identical by descent: This phrase may be used when (1) parental HLA assignments are available, (2) all four haplotypes are unequivocally defined in the family, (3) the HLA assignments of the parents are clearly distinguishable from one another, and (4) the assignments include HLA-A, -B, -C, DRB1, DQB1 and DPB1. Other loci (DRA, DQA1, DPA1, DRB3/4/5) may be included. The patient and potential donor who share both haplotypes may be described as HLA identical by descent.

HLA identical for all loci tested: This phrase may be used to refer to matching of related donors who appear to share the HLA loci tested with the patient based on segregation within the family. This phrase would refer to matching in which not all HLA loci (that is, HLA-A, -B, -C, DRB1, DQB1 and DPB1) are tested (e.g. not DPB1) so the possibility of recombination is not excluded.

Families where segregation to confirm identity by descent is not possible: For example, if there are two common haplotypes present within a family, and it has not been possible to exclude the possibility that a phenotypically identical sibling donor is actually only haploidentical, the phrases used to describe matching should be those used for an unrelated donor (see below).

Matching of patient to unrelated donor or matching within a family where identity by descent can not be ascertained:

Matched for {insert} at {insert} resolution: A phrase like this might be used to refer to the number of loci tested (i.e., two assignments at three loci yielding 6 assignments to include HLA-A,-B,-DRB1 or four loci yielding 8 assignments with the addition of HLA-C or 10 assignments with the addition of HLA-DQB1 or 12 assignments with DPB1), the potential identity of the assignments (e.g., 8/8 or 7/8 or 9/10), and the level of resolution used to determine the potential identity (e.g., confirmed allele high resolution or intermediate resolution). The number of loci to be included in this grading of match should be agreed locally with the service users.

For example, an umbilical cord blood unit may be described as “matched for 6/6 at low resolution for HLA-A and HLA-B and at allelic level resolution for HLA-DRB1” with the

patient. For example, an adult volunteer donor may be described as “matched for 9/10 at high resolution” with the patient.

Reference web site: Information on the current status of this document can be found at <http://hla.alleles.org>.

Approval: These definitions have been reviewed by representatives from the following organizations. The extent to which each organization is able to incorporate the definitions above can be found on the web site of each organization.

AABB

American Society for Histocompatibility and Immunogenetics (ASHI)

College of American Pathologists (CAP)

European Federation for Immunogenetics (EFI)

Foundation for the Accreditation of Cellular Therapy (FACT)

National Marrow Donor Program (NMDP)

World Marrow Donor Association (WMDA)

American Society for Blood and Marrow Transplantation (ASBMT)

Appendix I: Examples of Resolution

Typing required to reach resolution when the following alternative genotypes are present:

A*01:01:01G+A*02:01:01G or A*01:01:13+A*02:01:02 or A*01:14+A*02:101 or A*02:36+A*36:04

The high resolution typing result would be:

A*01:01:01:01/A*01:32/A*01:37/A*01:45 +
 A*02:01:01:01/A*02:01:01:02L/A*02:01:01:03/A*02:01:08/A*02:01:11/A*02:01:14/
 A*02:01:15/A*02:01:21/A*02:09/A*02:66/A*02:75/A*02:89/A*02:97:01/A*02:97:02/A
 *02:132/A*02:134/A*02:140 or A*01:01:13+A*02:01:02

Alternative Genotypes Present	To Achieve a High Resolution Typing Result, The Typing Result Must Be Narrowed Down to the Following Alleles:
A*01:01:01G+A*02:01:01G A*01:01:01G= A*01:01:01:01/ <u>A*01:01:01:02N/</u> <u>A*01:04N/ A*01:22N/</u> A*01:32/ A*01:34N/ A*01:37/ A*01:45 A*02:01:01G= A*02:01:01:01/ A*02:01:01:02L/ A*02:01:01:03/ A*02:01:08/ A*02:01:11/ A*02:01:14/ A*02:01:15/ A*02:01:21/ A*02:09/ <u>A*02:43N/ A*02:66/</u> A*02:75/ <u>A*02:83N/</u> A*02:89/ A*02:97:01/ A*02:97:02/ A*02:132/ A*02:134/ A*02:140	Non-expressed alleles are excluded to give: A*01:01:01:01/ A*01:32/ A*01:37/ A*01:45 A*02:01:01:01/ A*02:01:01:02L/ A*02:01:01:03/ A*02:01:08/ A*02:01:11/ A*02:01:14/ A*02:01:15/ A*02:01:21/ A*02:09/ A*02:66/ A*02:75/ A*02:89/ A*02:97:01/ A*02:97:02/ A*02:132/ A*02:134/ A*02:140
A*01:01:13+A*02:01:02	Same peptide binding region so further resolution is not required A*01:01:13+A*02:01:02
A*01:14+A*02:101	Different peptide binding regions; Testing must exclude this genotype
A*02:36+A*36:04	Different peptide binding regions; Testing must exclude this genotype

The confirmed allele high resolution typing would be:

A*01:01:01:01/A*01:01:01:02N/A*01:22N/A*01:32/A*01:34N/A*01:37/A*01:45 +
A*02:01:01G or A*01:01:13+A*02:01:02 or A*01:14+A*02:101 or A*02:36+A*36:04

Alternative Genotypes	To Achieve Confirmed Allele High Resolution Typing Result, The Typing Result Must Be Narrowed Down to the Following Alleles:
<p>A*01:01:01G+A*02:01:01G A*01:01:01G= A*01:01:01:01/ <u>A*01:01:01:02N/</u> <u>A*01:04N/ A*01:22N/</u> A*01:32/ <u>A*01:34N/</u> A*01:37/ A*01:45 A*02:01:01G= A*02:01:01:01/ A*02:01:01:02L/ A*02:01:01:03/ A*02:01:08/ A*02:01:11/ A*02:01:14/ A*02:01:15/ A*02:01:21/ A*02:09/ <u>A*02:43N/ A*02:66/</u> A*02:75/ <u>A*02:83N/</u> A*02:89/ A*02:97:01/ A*02:97:02/ A*02:132/ A*02:134/ A*02:140</p>	<p><u>Confirmed</u> non-expressed alleles are excluded to give:</p> <p>A*01:01:01:01/ A*01:01:01:02N/ <u>A*01:22N/</u> A*01:32/ <u>A*01:34N/</u> A*01:37/ A*01:45 [Note: A*01:34N has been found in only one individual according to IMGT/HLA but has been sequenced by two labs] A*02:01:01:01/ A*02:01:01:02L/ A*02:01:01:03/ A*02:01:08/ A*02:01:11/ A*02:01:14/ A*02:01:15/ A*02:01:21/ A*02:09/ <u>A*02:43N/</u> A*02:66/ A*02:75/ <u>A*02:83N/</u> A*02:89/ A*02:97:01/ A*02:97:02/ A*02:132/ A*02:134/ A*02:140</p>
<p>A*01:01:13+A*02:01:02</p>	<p>Same peptide binding region so not required to resolve A*01:01:13+A*02:01:02</p>
<p>A*01:14+A*02:101</p>	<p>Both alleles unconfirmed so further typing to exclude this genotype is not required A*01:14+A*02:101</p>
<p>A*02:36+A*36:04</p>	<p>Both alleles unconfirmed so further typing to exclude this genotype not required A*02:36+A*36:04</p>

Typing required to reach resolution when the following alternative genotypes are present:

A*02:01:01G + A*68:02:01G or A*02:34 + A*68:31 or A*02:35:01 + A*68:15

The high resolution typing result would be:

A*02:01:01:01/A*02:01:01:02L/A*02:01:01:03/A*02:01:08/A*02:01:11/A*02:01:14/A*

02:01:15/A*02:01:21/A*02:09/A*02:66/A*02:75/A*02:89/A*02:97:01/A*02:97:02/A*02:132/A*02:134/A*02:140 + A*68:02:01G

The confirmed allele high resolution typing would be:

A*02:01:01G + A*68:02:01G

Alternative Genotypes	To Achieve Confirmed Allele High Resolution Typing Result, The Typing Result Must Be Narrowed Down to the Following Alleles:
<p>A*02:01:01G + A*68:02:01G A*02:01:01G= A*02:01:01:01/ A*02:01:01:02L/ A*02:01:01:03/ A*02:01:08/ A*02:01:11/ A*02:01:14/ A*02:01:15/ A*02:01:21/ A*02:09/ <u>A*02:43N</u>/ A*02:66/ A*02:75/ <u>A*02:83N</u>/ A*02:89/ A*02:97:01/ A*02:97:02/ A*02:132/ A*02:134/ A*02:140</p> <p>A*68:02:01G= A*68:02:01/ A*68:02:01:02/ A*68:01:02:03</p>	<p><u>Confirmed</u> non-expressed alleles are excluded:</p> <p>A*02:01:01G (A*02:01:01:01/ A*02:01:01:02L/ A*02:01:01:03/ A*02:01:08/ A*02:01:11/ A*02:01:14/ A*02:01:15/ A*02:01:21/ A*02:09/ <u>A*02:43N</u>/ A*02:66/ A*02:75/ <u>A*02:83N</u>/ A*02:89/ A*02:97:01/ A*02:97:02/ A*02:132/ A*02:134/ A*02:140)</p> <p>A*68:02:01G</p>
<p>A*02:34 + A*68:31</p>	<p>One allele (A*68:31) is confirmed so further typing to exclude this genotype is required</p>
<p>A*02:35:01 + A*68:15</p>	<p>One allele (A*68:15) is confirmed so further typing to exclude this genotype is required</p>

Appendix II: Further justification and information

HLA typing assigns genotypes or pairs of alleles. The results of DNA based typing are genotypes, that is, combinations of two HLA alleles that might be carried by an individual. For example, the typing result may be consistent with the following 3 genotypes:

B*08:01:01G+B*15:18:01
B*08:21+B*15:93
B*08:35+B*15:10:01

This result, which includes alternative genotypes, is called “ambiguous” because it is not known which of the 3 genotypes the individual actually carries. Depending on the typing method, the list of genotypes can be very long.

If the laboratory decides not to resolve the ambiguity, it has three alternatives for reporting the HLA typing of this individual.

- (1) The list of 3 genotypes can be reported.
- (2) The result might be collapsed into B*08:01/21/35, B*15:10/18/93 or B*08:MDY (01/21/35), B*15:DZBP (10/18/93). Furthermore, if the result included alleles that might be represented by a G or P code, this designation might be used.
- (3) Based on allele and haplotype frequencies, the lab might predict the most likely genotype and indicate this on the report. For example, since B*08:21, B*08:35, and B*15:93 are on the NMDP’s rare allele list, the lab might indicate that B*08:01:01:01+B*15:18:01 is the genotype expected to be present. In this case, the report should note alternative genotypes that were not excluded by testing. If the actual genotype carried by a donor and/or a patient is not known, the transplant center will not know whether or not the pair is an allele match, and this uncertainty should be clearly stated.

A list of ambiguous alleles and ambiguous genotypes can be found on the HLA nomenclature web site (<http://hla.alleles.org>) and the IMGT/HLA website (<http://www.ebi.ac.uk/imgt/hla/>).