HISTO SPOT SSO Kits

Technical Report

Background on allele frequencies and levels of resolution

Introduction

When reporting HLA typing results achieved with different methods it is important to understand the possibilities and limitations of the techniques and the interpretation algorithms that were applied. The aim of this report is to give some fundamental information on HLA nomenclature, allele frequencies and allele filters, and to explain the different levels of resolution for the HISTO SPOT kits on this basis.

HLA Nomenclature and levels of resolution

The WHO nomenclature (Figure 1) starts with the name of the gene locus followed by 4 fields indicating different levels of variation in the DNA sequence and the resulting protein. The fields are separated by a colon. The first field defines the allele group that usually corresponds to the serologically defined specificity of the HLA protein. Referring to the older nomenclature resolution on this level is called 2 digit (2D) resolution. The second field indicates differences in the DNA sequence that lead to a difference in the amino acid sequence of the resulting protein. The second field needs to be resolved to achieve 4 digit (4D) resolution. The third field is used to indicate synonymous DNA substitutions in the coding region and the fourth field refers to differences in the non-coding regions. Since differences in the third and fourth field do not have any influence on the resulting protein, it is usually not required to resolve them.
At the end of the name for an HLA allele there may be a suffix indicating changes in the expression of the protein (N=not expressed Null allele, L= Low expression, Q= expression questionable). Null alleles are clinically relevant and usually have to be excluded or confirmed.

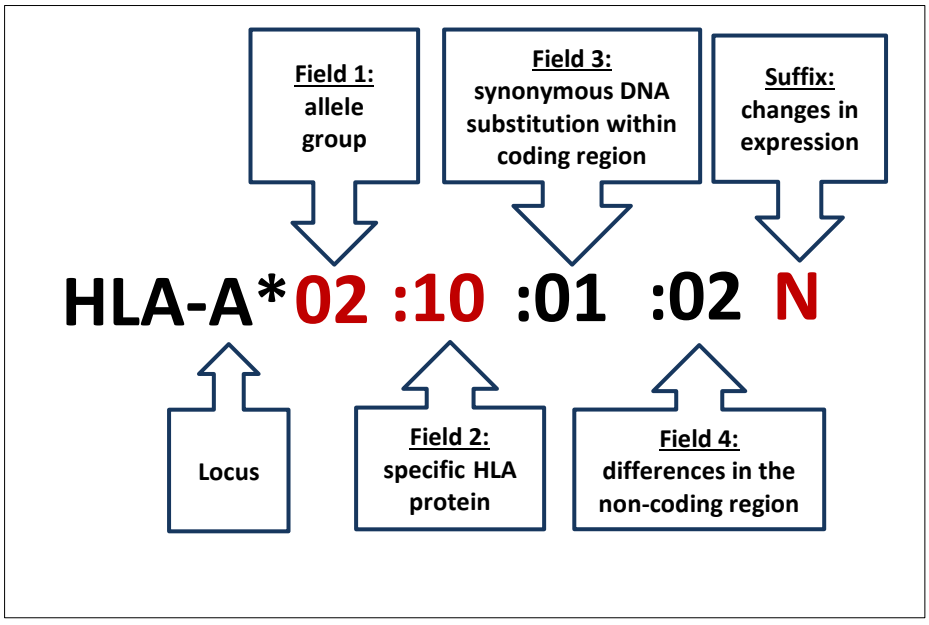

Figure 1: WHO nomenclature for HLA alleles

The definition of high resolution in the EFI standards (V6.1) requires the identification of HLA alleles that encode the same protein sequence within the antigen binding site (P-groups). Specifically, the following requirements have to be met:

- First and second field of the WHO nomenclature resolved.
- All ambiguities resulting from polymorphisms in exon 2 and 3 for class I and from exon 2 for class II resolved.
- All ambiguities resolved that encompass a null allele wherever the polymorphism is located, unless the presence of an expressed antigen on the cell can be demonstrated (e.g. by serology).

Resolution requirements

The required resolution for HLA typing depends on the clinical application and is defined by the local transplant protocol. The EFI standards provide some minimal requirements that have to be fulfilled. Generally, for solid organ transplantation a 2 digit resolution differentiating the serological splits is required. Most of the low resolution SSP kits do not achieve this. For bone marrow registries a low to medium resolution is the minimum requirement for screening. Most registries request a high resolution (4 digit) typing because this increases the probability that a donor is chosen. For the donor and the recipient of a haematopoietic stem cell transplantation a true high resolution typing is required that can only be achieved by sequencing.

Allele frequencies and filters

With the release 3.18.0 from October 2014 there are more than 12.000 alleles listed in the IMGT database and it is hardly possible to discriminate all of them with an SSO technique. However, most of these alleles have only been seen once or twice in the whole wide world. For this reason the common and well-documented (CWD) alleles catalogue has been published by a group of international histocompatibility and immunogenetics researchers working to identify that subset of HLA alleles for which the frequencies are well known or which have been identified multiple times through the use of sequence-based typing methods (Mack et al. 2013). They have defined the following categories for frequent and rare alleles:

- **Common (C): 415 alleles**
  allele frequency > 0,001 in reference populations of at least 1500 individuals
- **Well-documented (WD): 707 alleles**
  detected by SBT 5 times in unrelated individuals or detected by SBT 3 times and observed in a specific haplotype in unrelated individuals
- **Rare: 11.120 alleles in IMGT Release 3.18.0**
  all other alleles

From the frequencies it can be deduced that more than 99,9% of all HLA types will have two common alleles (C;C), i.e. around 3% of all known alleles make up the vast majority of all types that are really present (Figure 2). This also implies that 4 digit SSO results given with the use of an allele filter that only shows the C alleles will be confirmed by SBT in 99,9% of the cases.

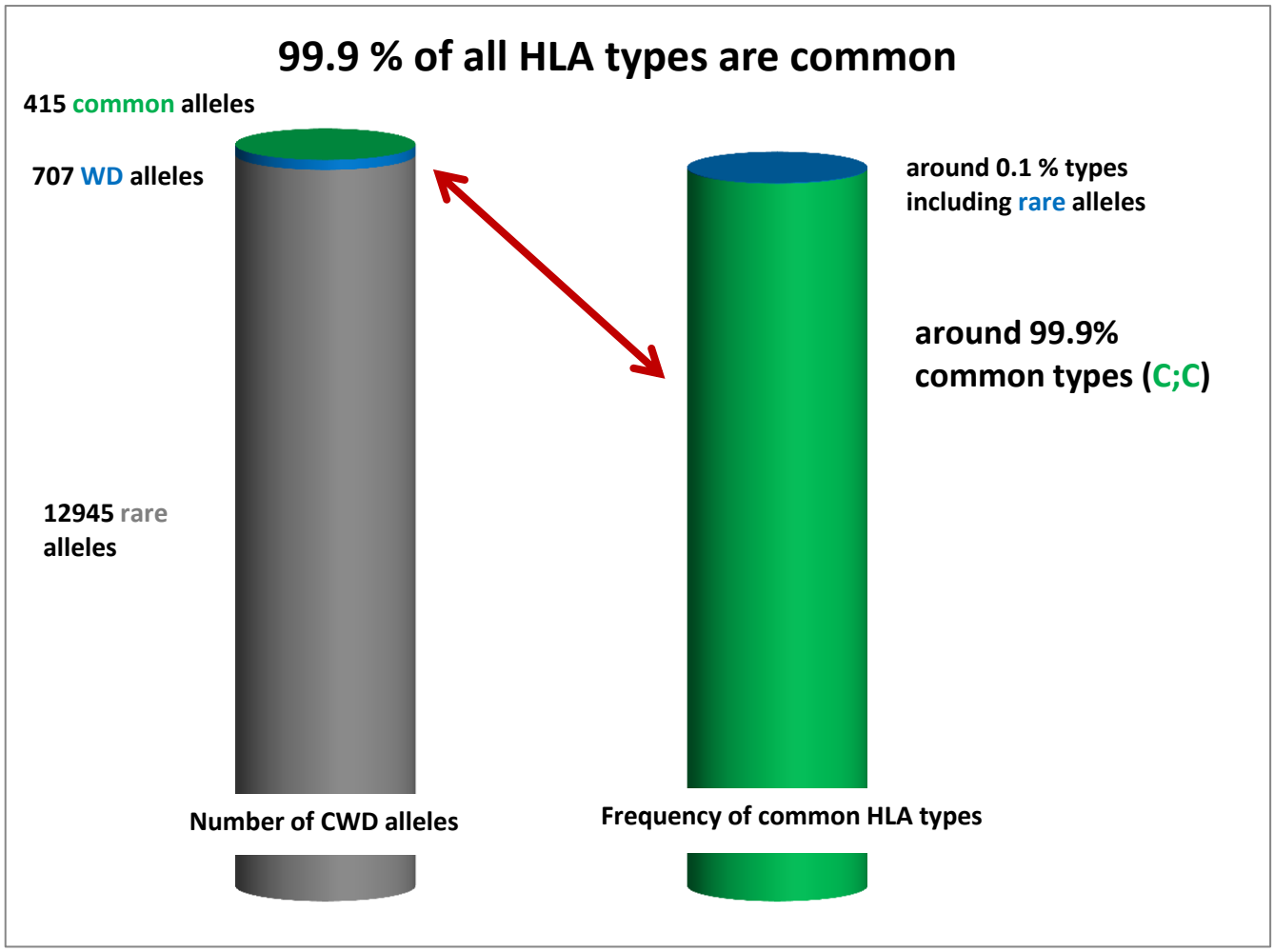

Figure 2: Number of CWD alleles and frequency of common HLA types

Resolution of HISTO SPOT kits

The HISTO SPOT kits are tailored to meet the specific resolution requirements of different labs on the basis of the CWD alleles.

HISTO SPOT 4D Kits

- no ambiguities with common alleles on the low resolution level
- resolve most of the common (C) alleles from the CWD 2.0.0 catalogues on the 4 digit level
- use one well per test for high throughput

HISTO SPOT Xtend Kits

- complementary wells to the HISTO SPOT 4D kits provide a higher resolution including well documented (WD) alleles from the CWD 2.0.0 catalogue
- can be used as a second test to resolve ambiguities or provide higher resolution for selected samples
- unambiguous differentiation of the common alleles from the CWD2.0.0 catalogue
- 99,9 percent of the results will be confirmed by SBT

HISTO SPOT Null CWD high res (available in November 2016)

- determines the presence of CWD class I null alleles based on polymorphisms located in the exons 1, 4 and 7
- CWD null alleles caused by polymorphisms in exon 2 and 3 are resolved by the regular HISTO SPOT 4D kits

HISTO SPOT On-Call Typingkit

- combined test strip for typing of HLA-A, B, C, DRB1, DRB3/4/5, DQB1, DQA1, DPB1 on the 4D level of resolution and a null allele test
- contains ready to use pre-dried primers, PCR buffer and test strips
- simplified on-call workflow in the HISTO MATCH software

Literature

IMGT Database: http://www.ebi.ac.uk/ipd/imgt/hla/

Mack, S.J. et al, Common and well-documented HLA alleles: 2012 update to the CWD catalogue, Tissue Antigens, Volume 81, 2013, 194-203

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