

HISTO SPOT SSO Kits

Technical Report

Level of resolution achieved with the HISTO SPOT[®] Xtend kits

Introduction

The theoretical analysis of the resolution of an SSO kit is difficult, and not always helpful to understand how many ambiguities will come up in the routine work. For an ambiguity analysis the reaction patterns of all possible allele combinations (or combinations of common and well documented alleles) are calculated and ambiguous combinations are listed. This usually results in quite a long list of ambiguous combinations, but most of these combinations will never be seen in reality because they are so rare. On the other hand, the theoretical ambiguity analysis assumes that all probes react correctly. In reality some probes might have to be ignored and this reduces the actual resolution of the test. Therefore, 96 randomly chosen routine DNA samples were analysed with the HISTO SPOT[®] Xtend kits to determine the level of resolution experimentally. The same test was performed with the HISTO SPOT[®] C 4D test which should reach the same level of resolution with a single well (without an additional Xtend kit).

Material and methods

DNA was isolated from 96 unselected buffy coats from a bloodbank with Qiagen DNA columns. The DNAs were typed with the HISTO SPOT[®] 4D for all four loci and additionally with the Xtend kits for HLA-A, -B and -DRB1. The results were interpreted with the HISTO MATCH software using the different allele filters. The CWD (common and well documented) and the C (common) filter reduce the result to the common and well documented alleles in the CWD 2.0.0 catalogue (Mack et al. 2013). The 4D filter includes a set of more common alleles with a frequency $\geq 0,05$ in populations with a sample size of ≥ 1000 on the www.allelefrequencies.net website. The results were analyzed on the P group level.

The following levels of resolution were defined:

1. CWD: unambiguous with the CWD filter (only two CWD alleles found)
2. C: unambiguous with the C filter (only two C alleles found)
3. 4D unambiguous with the 4D filter (only two 4D alleles found)

Alleles were only considered as “different” when there were differences on the 2 fields level of resolution (exception: Null alleles) and if the differences were in Exon 2 for DRB1 alleles.

Therefore:

A*03:01:01:01 = A*03:01:01:03	(=A*03:01 for two fields)
B*15:01:01:01 \neq B*15:01:01:02N	(Null allele)
DRB1*14:01 = DRB1*14:54	(polymorphism in Exon 3)

Results:

Figure 1 shows that virtually all results are unambiguous with the 4D filter. More than 95% of the results can be resolved with the C filter, too, for all tested loci. The percentage of results solved on the CWD level is considerably lower for the class I loci. For HLA-DRB1 60 % of the results were unambiguous even on the CWD level.

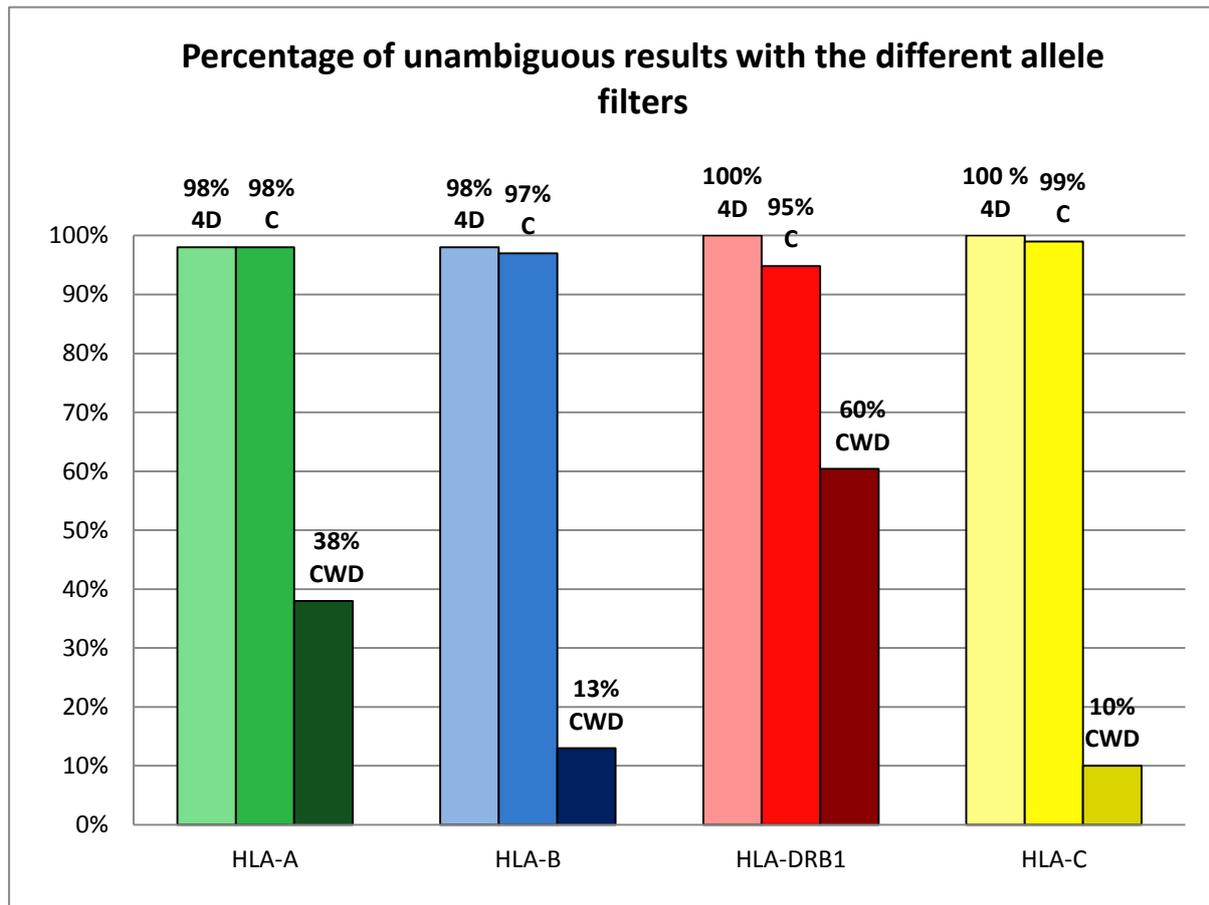


Figure 1: Percentage of results on the different levels of resolution (P groups)

Conclusion:

The HISTO SPOT[®] Xtend kits and the HISTO SPOT[®] C 4D kit can be used for HLA typing on the C allele level for P groups. The HISTO SPOT[®] DRB1 Xtend kit often gives results on the CWD level.

Literature:

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

August 2016

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