Everything but the kitchen sink: Resolving an unusual DRB1*04:04 – DRB4*01:03N – DQB1*03:02 haplotype in a deceased donor

Challenging assumptions by using all tools in the HLA toolbox; the value of historical and evolving methods coexisting with each other.

Common strong haplotype associations can be a useful tool in the Histocompatibility Laboratory to resolve ambiguities; however, less common haplotypes are being reported more frequently with advances in high-resolution typing technologies and must be considered. This case demonstrates the value of utilizing multiple testing platforms, and that caution and correlation should be used when interpreting test results.

ABSTRACT:

A deceased donor of unknown ethnicity was being assessed for solid organ transplant. Initial medium-resolution typing by rSSO identified class II alleles DRB1*04,07; **DRB4*01,01N**; and DQB1*03(8),03(9).

Follow-up high-resolution testing was performed by NGS and showed DRB1*04:04,07:01; **DRB4*01:03N,01:03N**; DQB1*03:02,03:03. Close review of genomic site 9985 at the end of intron 2 found only A nucleotides, verifying that an expressed DRB4*01 allele was not present. Additional testing was performed by real-time PCR and SSP, all showing only DRB4*01:03N alleles in concordance with the NGS results. Timeline of testing methods is shown in Figure 1 and the Class II HLA typing results by all methods is displayed in Table 1.

A surrogate flow crossmatch was performed against a stored serum with only DR53 antibody (MFI: 21079) and was negative (Figure 2), further confirming the absence of a DR53 antigen. Retrospective review of the original rSSO results found that bead #042 was flagged by the software as a close reaction and if this bead was adjusted to negative, DRB4*01 was no longer present leaving only the DRB4*01N remaining.

Assuming one haplotype was the well-documented DRB1*07:01-DRB4*01:03N-DQB1*03:03, we sought to differentiate between homozygosity and hemizygosity of the DRB4*01:03N allele. Performance of a research-use-only NGS application by CareDx confirmed there were two copies of the DRB4*01:03N allele (Figure 3), thus neither haplotype contained a deletion of the DRB4 gene. It was therefore determined that the donor's two Class II haplotypes were the well-documented DRB1*07:01 – DRB4*01:03N – DQB1*03:03 and the uncommon DRB1*04:04 – DRB4*01:03N – DQB1*03:02. Though rare, a handful of DRB1*04:XX – DRB4*01:03N – DQB1*03:02 haplotypes have been documented, including DRB1*04:01, DRB1*04:02, and as in our case, DRB1*04:04.

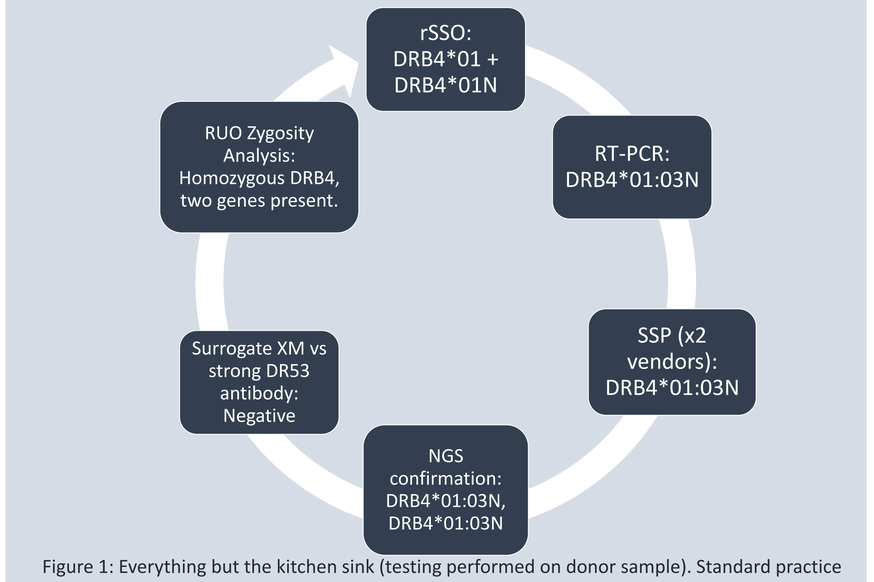

Figure 1: Everything but the kitchen sink (testing performed on donor sample). Standard practice rSSO was followed by RT-PCR due to ongoing validation process; SSP to resolve ambiguity between methods; standard confirmation NGS was rushed; surrogate XM and zygosity analysis and retrospective review of initial rSSO results/interpretation.

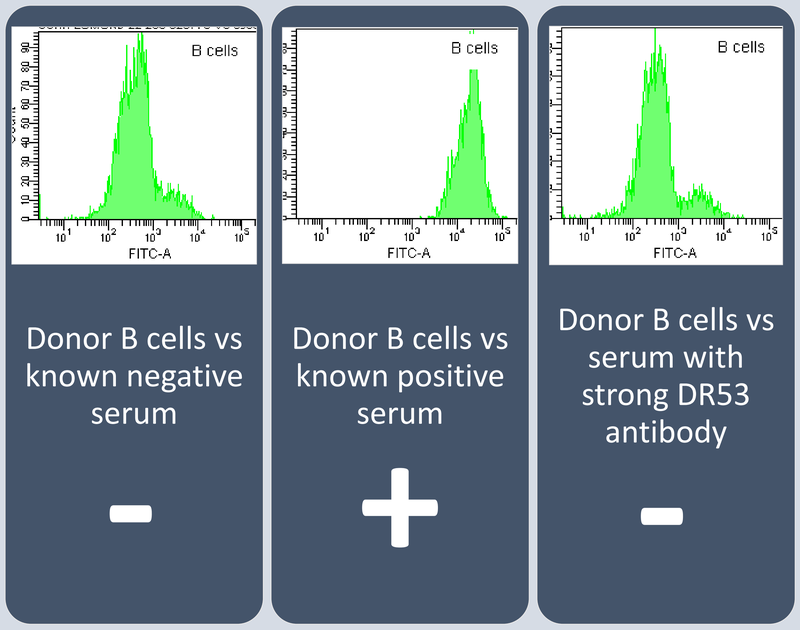

Figure 2: Flow crossmatch results for donor B cells showing absence of DR53 antigen.

METHOD	DRB1	DRB3/4/5	DQA1	DQB1
rSSO *(Thermo Fisher Scientific)*	07	4*01N	02	03(9)
	04	***4*01***	***03***	***03(8)***
NGS *(Werfen)*	07:01	4*01:03N	02:01	03:03
	04:04	*4*01:03N*	***03:01***	***03:02***
SSP *(CareDx)*		***4*01:03N***		
SSP *(Thermo Fisher Scientific)*		***4*01:03N***		
RT-PCR *(Thermo Fisher Scientific)*	07	4*01:03N	02	03(9)
	04	*4*01:03N*	***03***	***03(8)***
NGS RUO *(CareDx)*		***DRB4 homozygous***		

Table 1: Class II HLA typing results obtained by variety of methods, with haplotype of interest highlighted.

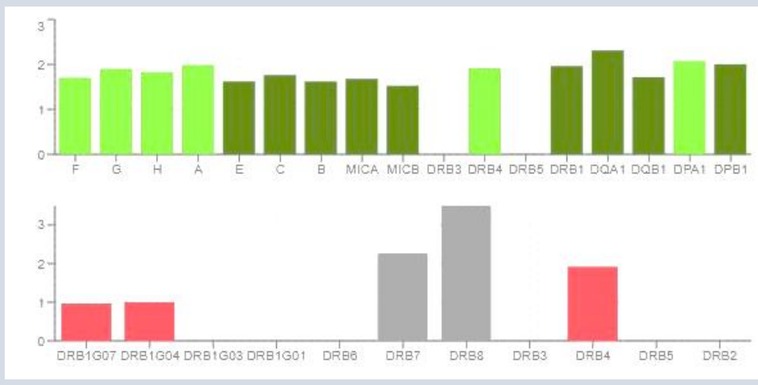

Figure 3: Research-use only application NGS showing heterozygosity vs homozygosity for donor HLA alleles, including hemizygous vs homozygous analysis of DRB alleles.

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