Triple Threat: Three Typing Assays Resulted Three Different HLA-DPB1 Typings

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Abstract

A 52 year old African-American female was approved by our laboratory's organ procurement organization (OPO) as a new local deceased donor. The donor was tested by Real-Time PCR (Linksēq™ HLA – ABCDRDQDP+ SABR 384 Kit and SureTyper Software, One Lambda), which resulted in DPB1*04:01/576:01 as the most likely option with multiple rare typings as additional possibilities. The RT-PCR was repeated and resulted the same as the original test. To confirm, rSSO (LabType™ rSSO and HLA Fusion™ Software, One Lambda) was performed using the same DNA sample. rSSO resulted with the likeliest typing (G1) as DPB1*23:01/33:01 with DPB1*04:01/71:01 as a rare option (G2). We concluded that the donor's typing is unlikely to be DPB1*23:01/33:01 because both RT-PCRs did not show reaction patterns to support that result. To determine which typing to enter into UNet (DPB1*71:01 or DPB1*576:01), we performed a protein sequence alignment (Figure 1) using the tool in the IPD-IMGT/HLA database. The alignments show that the only amino acid difference between DPB1*71:01 and DPB1*576:01 is 65L present in DPB1*576:01. To account for this eplet and to eliminate any potential recipients with DPB1 antibody against the 36V, 65L, and 69E eplets, the donor's typing was entered into UNet as DPB1*04:01/576:01. Next Generation Sequencing (CareDx® AlloSeq™Tx17 and Care DxAssign Software, Illumina MiSeq® Library Preparation) was performed to determine the donor's high resolution typing. NGS resulted the donor's typing as DPB1*04:01/71:01, which was the third rare possibility for RT-PCR and the first rare possibility for rSSO. Our OPO was immediately notified and the donor's file in UNet was updated accordingly prior to organ procurement.

Materials and Methods

DNA was isolated from whole blood using EZ1 & 2 DNA Blood kits from Qiagen. HLA intermediate resolution typing was performed using the Linksēq™ HLA – ABCDRDQDP+ SABR 384 Kit and SureTyper Software as well as LabType™ rSSO and HLA Fusion™ Software, both by One Lambda. High resolution typing was performed using the CareDx AlloSeqtX17 and Care DxAssign Software next generation sequencing platform.

Results

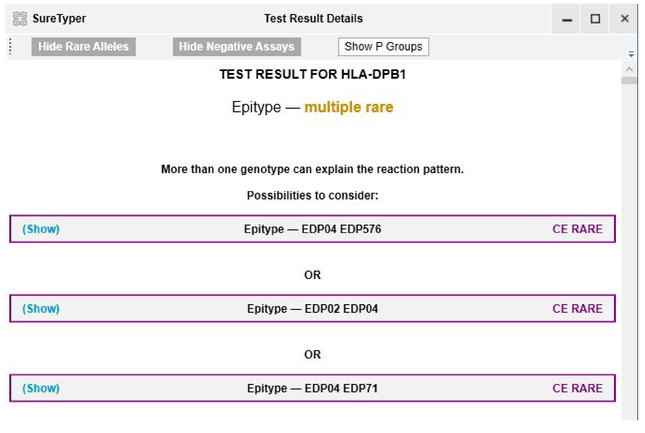

Figure 1
Linksēq™ SureTyper RT-PCR results

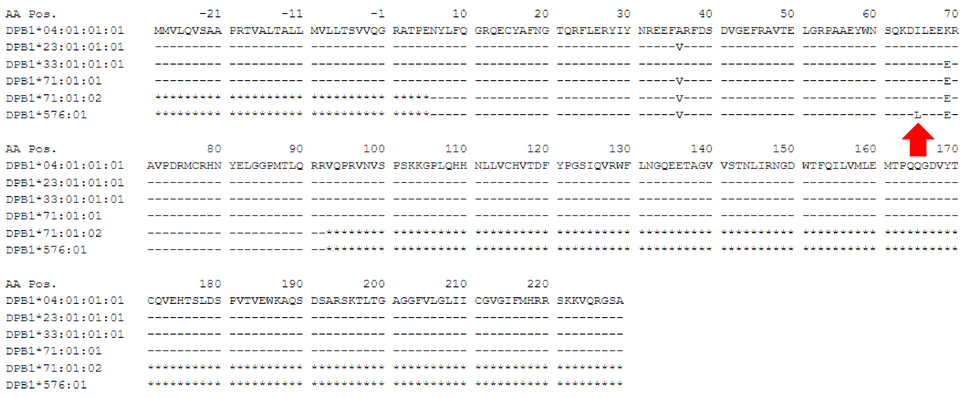

Figure 3
Protein alignment sequences

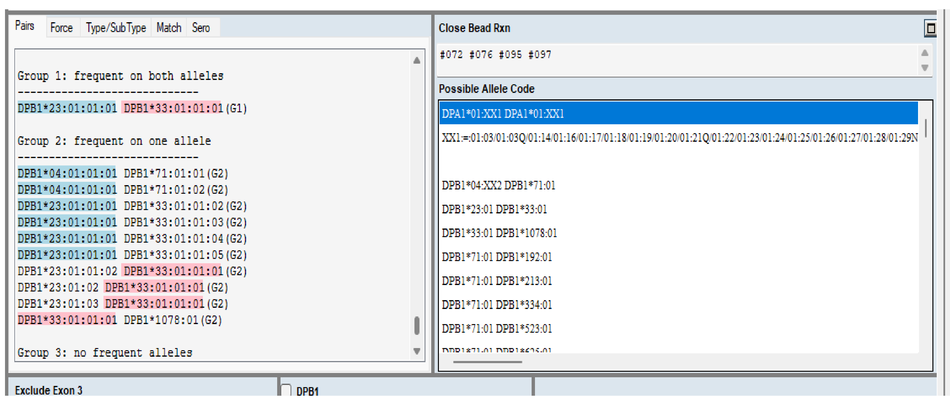

Figure 2
LabType™ rSSO and HLA Fusion™ Software results

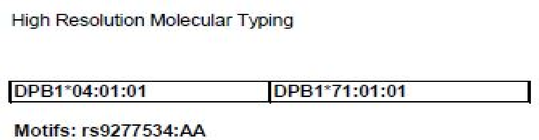

Figure 4
CareDx® AlloSeq™Tx17 Results

Conclusion

This case highlights the importance of using multiple HLA typing methodologies to resolve discrepant results and the need to interrogate sequence alignments when ambiguities cannot be resolved in real-time. The reporting of allele combinations that cover the eplets detected, even if the actual allele combination cannot be defined, precludes the assignment of the donor to an incompatible recipient and gives the laboratory space to perform tests that take a longer time, such as NGS, to obtain an unambiguous high-resolution typing result.