

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.

Triple Threat: Three Typing Assays Resulted Three Different HLA-DPB1 Typings Gail Crowther¹, Gregory Lesko¹, Kristin Gay¹, Marlee Folckomer¹, Maria P. Bettinotti¹, Alison J. Gareau¹ ¹Johns Hopkins Immunogenetics Laboratory, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

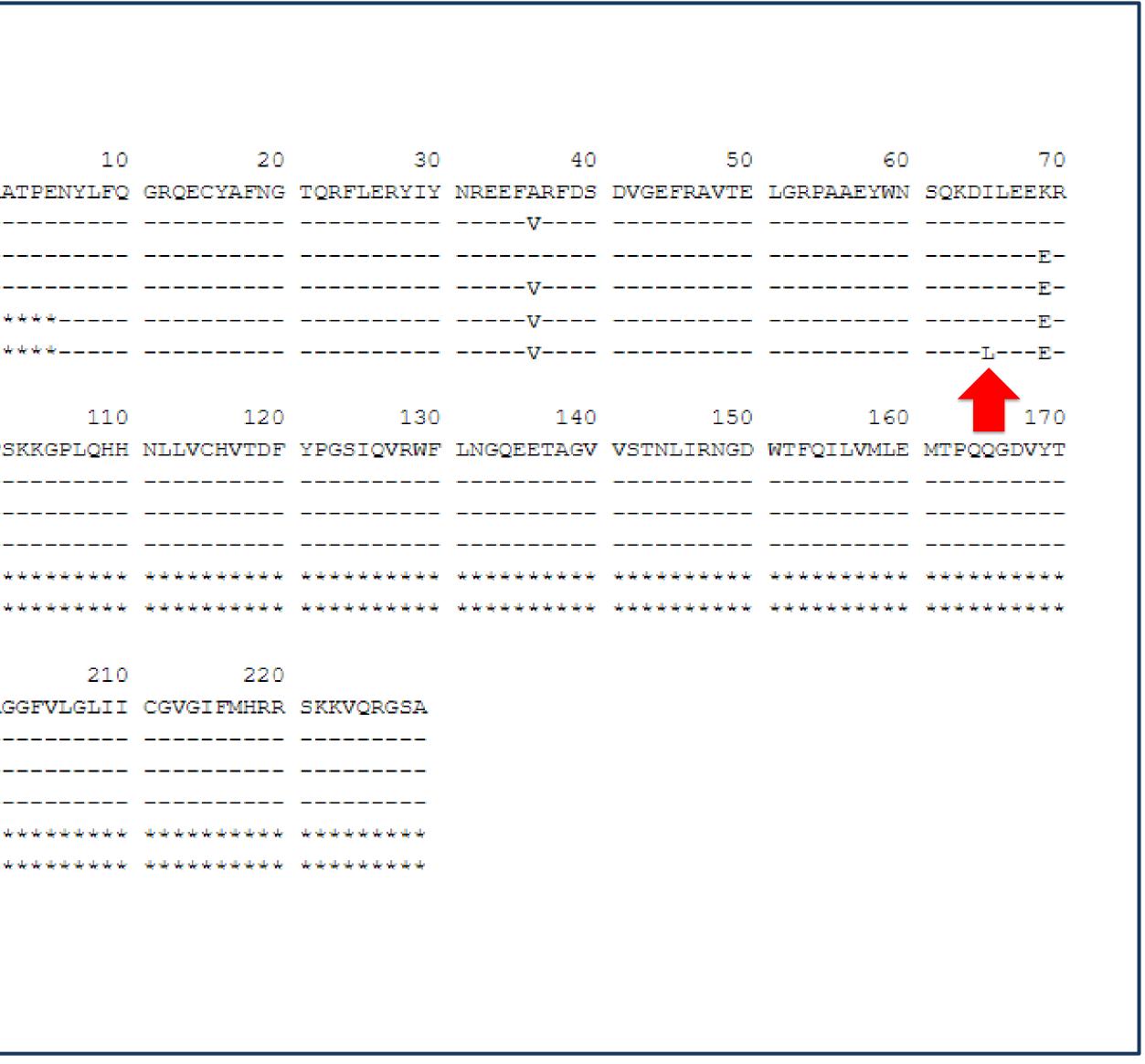
			Results	
SureTyper Hide Rare Alleles	Test Result Details Hide Negative Assays Show P Groups TEST RESULT FOR HLA-DPB1 Epitype — multiple rare		AA Pos. DPB1*04:01:01:01 DPB1*23:01:01:01 DPB1*33:01:01:01 DPB1*71:01:01 DPB1*71:01:02	-21 -11 -1 MMVLQVSAA PRTVALTALL MVLLTSVVQG RA
(Show)	More than one genotype can explain the reaction pattern. Possibilities to consider: Epitype — EDP04 EDP576	CE RARE	DPB1*576:01 AA Pos. DPB1*04:01:01:01 DPB1*23:01:01:01 DPB1*33:01:01:01	******** *****************************
(Show)	OR Epitype — EDP02 EDP04	CE RARE	DPB1*71:01:01 DPB1*71:01:02 DPB1*576:01	
(Show)	OR Epitype — EDP04 EDP71	CE RARE	AA Pos. DFB1*04:01:01:01 DFB1*23:01:01:01 DFB1*33:01:01:01 DFB1*71:01:01 DFB1*71:01:02 DFB1*576:01	180 190 200 CQVEHTSLDS PVTVEWKAQS DSARSKTLTG A0
Figure 1 Linksēq™ SureTy	per RT-PCR results		Figure 3 Protein alignm	nent sequences

Pairs Force Type/SubType Match Sero	Close Bead F
	▲ #072 #076
Group 1: frequent on both alleles	Possible Alle
DPB1*23:01:01:01 DPB1*33:01:01:01(G1)	DPA1*01:XX
Group 2: frequent on one allele	XX1:=:01:03/
DPB1*04:01:01:01 DPB1*71:01:01(G2)	
DPB1*04:01:01:01 DPB1*71:01:02(G2)	DPB1*04:XX
DPB1*23:01:01:01 DPB1*33:01:01:02(G2)	DPB1*23:01
DPB1*23:01:01:01 DPB1*33:01:01:03(G2)	DDP1#22.01
DPB1*23:01:01:01 DPB1*33:01:01:04(G2)	DPB1*33:01
DPB1*23:01:01:01 DPB1*33:01:01:05(G2)	DPB1*71:01
DPB1*23:01:01:02 DPB1*33:01:01:01(G2)	DPB1*71:01
DPB1*23:01:02 DPB1*33:01:01:01(G2)	
DPB1*23:01:03 DPB1*33:01:01:01(G2)	DPB1*71:01
DPB1*33:01:01:01 DPB1*1078:01(G2)	DPB1*71:01
Group 3: no frequent alleles	ThDD1#71.01

Figure 2 LabType™ rSSO and HLA Fusion™ Software results

i Rxn	
6 #095 #097	
llele Code	
X1 DPA1*01:XX1	
3/01:03Q/01:14/01:16/01:1	.7/01:18/01:19/01:20/01:21Q/01:22/01:23/01:24/01:25/01:26/01:27/01:28/01:29N
X2 DPB1*71:01	
1 DPB1*33:01	
1 DPB1*1078:01	
1 DPB1*192:01	
1 DPB1*213:01	
1 DPB1*334:01	
1 DPB1*523:01	
1 10012205.01	

Figure 4 CareDx[®] A 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.



High Resolution Molecu	lar Typing	
DPB1*04:01:01	DPB1*71:01:01	
Motifs: rs9277534:AA		
oSeq™Tx17 Results		