Strategies for Resolving and Reporting Uncommon Null Alleles in Deceased Donors for Organ Allocation

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BACKGROUND

Comprehensive and accurate human leukocyte antigen (HLA) typing within a short turnaround time is a crucial initial step for allocating deceased donor (DD) organs for transplantation. Erroneous HLA typing of DDs can have catastrophic consequences, resulting in recipient death, failed transplantations, and organ wastage due to inappropriate donor matches. The real-time polymerase chain reaction method (RT-PCR) is widely used as the primary method for HLA typing of DDs due to its simplified workflow. We concurrently utilize two RT-PCR trays (LinkSeq from One Lambda and QTYPE from CareDx) for all DD workups to enhance the accuracy of our workflow. Any discrepancies encountered between the two test results are resolved using SSP (Olerup from CareDX).

METHODS AND TESTING

In this case, we encountered a DD with inconsistent DPB1 typing results from two different RT-PCR trays. The QTYPE tray yielded ambiguous DPB1 typing, showing DPB1*04:01, *25:01, or DPB1*04:01, *545:01, or DPB1*04:01, *61:01N. On the other hand, the LinkSeq tray assigned a rare DPB1*04:01, *61:01N typing, but the three wells specific to DPB1*61:01N were clearly negative. Upon closer examination of the well specificities, it was discovered that there was a “?” suffix for DPB1*61:01N, indicating uncertainty regarding allele amplification or reactivity at the primer location by the analysis software. To verify this null allele, a DPB1 SSP tray was utilized, confirming the typing as DPB1*04:01, *61:01N. Null alleles are typically recorded as blank or “No Antigen Detected” in UNET. However, since this null allele was rare, we reported as “Other allele,” and the Organ Procurement Organization (OPO) was informed to ensure a prospective crossmatch for organ allocation. Subsequent retrospective NGS typing confirmed the assignment of DPB1*04:01, *61:01N.

RESULTS

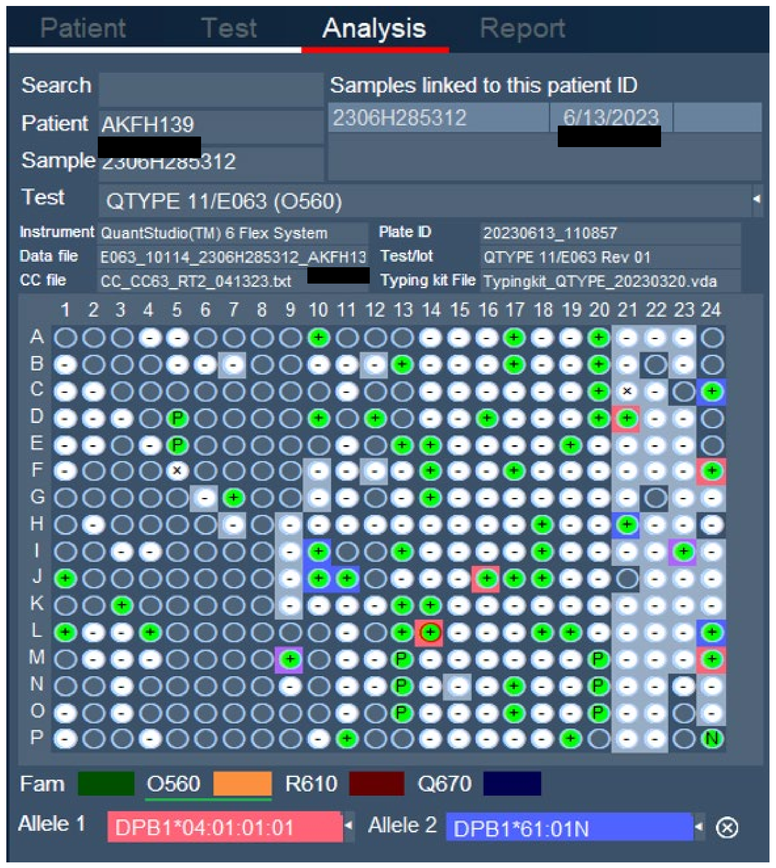


Figure 1. Olerup QType HLA typing results showing that all wells are accounted for allele combination DPB1*04:01, *61:01N.

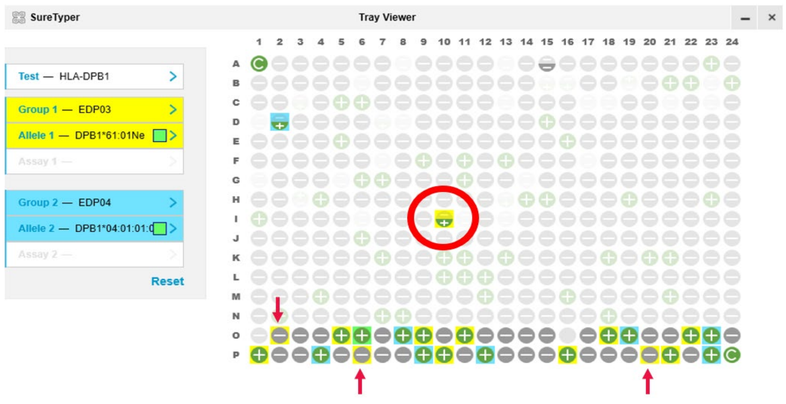


Figure 2. Linkage SureTyper HLA typing results showing that wells O2, P6, and P20 are negative for *DPB1*61:01Ne* (highlighted in yellow) with well I10 (circled in red) being unique for DPB1*61:01Ne.

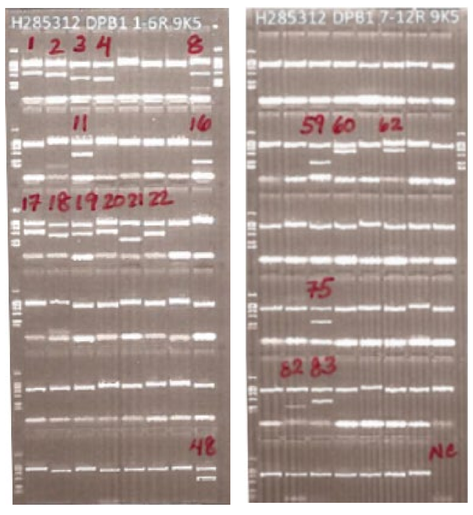
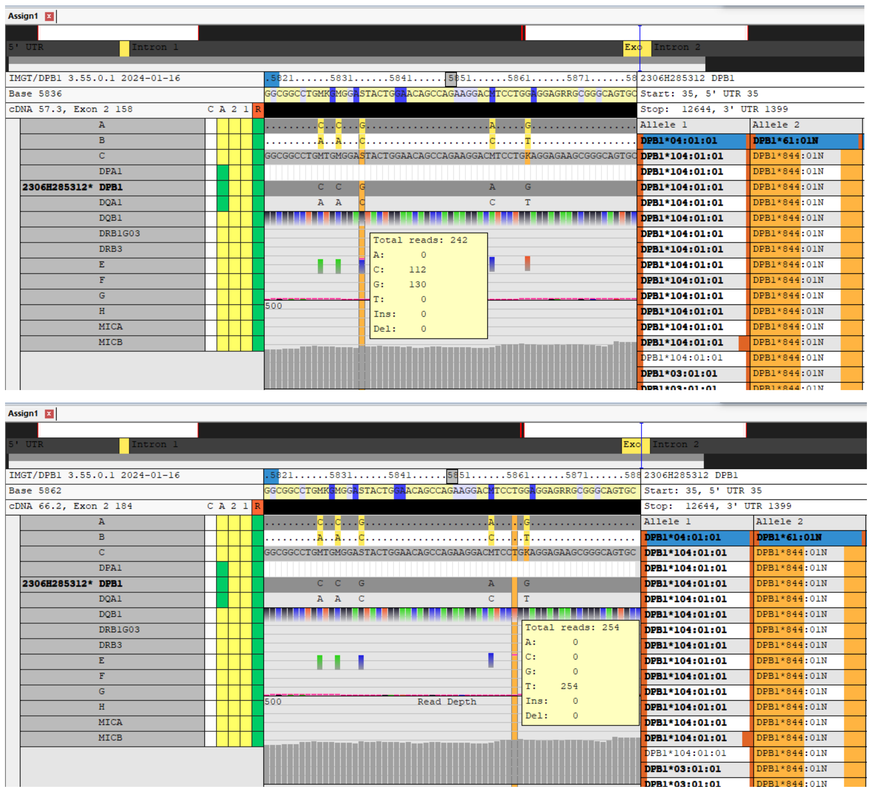


Figure 3. Gel image of SSP HLA typing results for *DPB1*04:01* (wells 3, 4, 8, 11, 22, 60, 82, 83) and *DPB1*61:01N* (wells 1, 2, 16, 17, 18, 19, 20, 21, 48, 75) marked in red.

LSDPB-055.1	Well O2	DPB1*01: 01:01:01, 01:02:01 / DPB1*26: 01:02:01; DPB1*01: 01:01:02e-01:01:25e, 01:02:02e-01:12e / DPB1*02: 01:05e-01:07e, 01:09e-01:14e, 01:16e-01:18e, 01:21e?, 01:24e?, 01:26e-01:33e, 01:35e-01:36e, 01:38e-01:41e, 01:43e-01:49e, 01:51e?, 02:02e?, 02:04e? / DPB1*03: 01:02e-01:07e / DPB1*04: 01:02e?, 01:05e?, 01:06e?, 01:07e?, 01:08e?, 01:09e?, 01:10e?, 01:11e?, 01:12e?, 01:13e?, 01:14e?, 01:15e?, 01:16e?, 01:17e?, 01:18e?, 01:19e?, 01:20e?, 01:21e?, 01:22e?, 01:23e?, 01:24e?, 01:25e?, 01:26e?, 01:27e?, 01:28e?, 01:29e?, 01:30e?, 01:32e?, 02:02e-02:08e, 02:12e? / DPB1*05: 01:02e-01:06e / DPB1*06: 01:02e-01:03e, 01:05e? / DPB1*08: 01e? / DPB1*09: 01:03e-01:05e / DPB1*10: 01:02e-01:03e / DPB1*11: 01:02e-01:04e / DPB1*14: 01:02e-01:03e, 01:06e-01:09e / DPB1*15: 01:02e? / DPB1*16: 01:02e? / DPB1*17: 01:02e-01:03e / DPB1*18: 01:02e-01:03e / DPB1*20: 01:02e-01:03e / DPB1*23: 01:02e? / DPB1*26: 01:01e?, 01:02:02e-01:03e / DPB1*32: 01e? / DPB1*34: 01:02e? / DPB1*37: 01e? / DPB1*40: 01:02e? / DPB1*41: 01:02e? / DPB1*44: 01e? / DPB1*46: 01:02e? / DPB1*55: 01:02e-01:03e / DPB1*56: 01e? / DPB1*58: 01e? / DPB1*60: 01e? / DPB1*61: 01Ne? / DPB1*62: 01e? / DPB1*65: 01e / DPB1*66: 01e? / DPB1*67: 01e? / DPB1*68: 01e? / DPB1*70: 01e? / DPB1*71: 01:02e? / DPB1*73: 01e? / DPB1*74: 01e? / DPB1*75: 01e? / DPB1*79: 01e? / DPB1*81: 01:02e? / DPB1*82: 01e? / DPB1*83: 01e? / DPB1*84: 01e? / DPB1*85: 01:02e? /
LSDPB-021.1	Well P6	DPB1*03: 01:01:01 / DPB1*06: 01:01:01? / DPB1*14: 01:01:01 / DPB1*20: 01:01:01 / DPB1*28: 01:01:01 / DPB1*29: 01? / DPB1*31: 01:01:01 / DPB1*34: 01:01:01 / DPB1*45: 01 / DPB1*59: 01; DPB1*03: 01:01:02e-01:25e / DPB1*06: 01:01:02e-01:06e / DPB1*14: 01:01:02e-01:14e / DPB1*20: 01:01:02e-01:05e / DPB1*25: 01e / DPB1*28: 01:01:02e / DPB1*31: 01:01:02e / DPB1*34: 01:01:02e-01:02e / DPB1*44: 01e? / DPB1*50: 01e / DPB1*52: 01e / DPB1*56: 01e / *DPB1*57: 01e* / DPB1*61: 01Ne? / DPB1*64: 01Ne? / DPB1*67: 01e / DPB1*70: 01e / DPB1*72: *01:01:01d*, 01:01:02e-01:01:03e / DPB1*73: 01e / DPB1*76: 01e / *DPB1*78: 01d* / DPB1*87: 01e / DPB1*91: 01e / DPB1*92: 01e / DPB1*95: 01e? / DPB1*102: 01e / DPB1*104: *01:01:01d*, 01:01:02e-01:06e / DPB1*108: 01e / DPB1*111: 01e / DPB1*114: 01e / DPB1*119: 01e / DPB1*124: 01e / DPB1*130: 01e / DPB1*139: 01e / DPB1*142: 01e / DPB1*152: 01e? / DPB1*161: 01Ne? / DPB1*164: 01e / DPB1*166: 01e? / DPB1*167:
LSDPB-059.1	Well P20	DPB1*03: 01:01:01 / DPB1*06: 01:01:01? / DPB1*14: 01:01:01 / DPB1*20: 01:01:01 / DPB1*29: 01?; DPB1*03: 01:01:02e-01:25e / DPB1*06: 01:01:02e-01:06e / DPB1*14: 01:01:02e-01:14e / DPB1*20: 01:01:02e-01:05e / DPB1*44: 01e? / DPB1*50: 01e / *DPB1*57: 01e* / DPB1*61: 01Ne? / DPB1*64: 01Ne? / DPB1*69: *01:01:01d?*, 01:01:02e? / DPB1*70: 01e? DPB1*76: 01e / *DPB1*78: 01d* / DPB1*91: 01e / DPB1*92: 01e / DPB1*104: *01:01:01d*, 01:01:02e-01:06e / DPB1*108: 01e / DPB1*111: 01e / DPB1*119: 01e / DPB1*124: 01e / DPB1*130: 01e / DPB1*152: 01e? /

Table 1. Comparison of alleles by assay that are negative for DPB1*61:01Ne denoting “?” (circled in red).



Figures 4a and 4b. Confirmation of DPB1*61:01N typing by AlloSeq Tx NGS HLA typing method by showing that on Exon 2, position 258, codon 57 and Exon 2, position 284, codon 66 are for alleles DPB1*25:01 and DPB1*545:01, respectively.

CONCLUSION

This case demonstrates that employing two concurrent methods when conducting DD typing is the optimal approach to obtain accurate results and instill greater confidence in reporting rare alleles in a timely manner.