Long-Read Next-Generation DNA Sequencing for Deceased Donor HLA Genotyping Enables Resolution of HLA Serotypes

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Introduction

- Adoption of HLA serotypes aims to refine HLA nomenclature for describing serologic reactivity of HLA antigens beyond WHO serologic antigen nomenclature.
- This increased granularity has the potential to improve prediction of donor-recipient serologic compatibility for organ transplantation but requires additional information about the DNA sequences of donor HLA genes.
- Our laboratory has addressed this information gap by implementing long-read next-generation DNA sequencing (LR-NGS) for high-resolution HLA genotyping of deceased donors

Aim

- We evaluated the impact of LR-NGS on HLA typing and serotype assignment in 88 consecutive deceased donors

Materials and Methods

- HLA typing for 88 consecutive deceased donors was performed in parallel using both LR-NGS and standard-of-care real-time PCR (RT-PCR) (N = 88)
- LR-NGS was performed using nanopore technology on NanoTyper software (Werfen)
- RT-PCR was performed using LinkSeq (Thermo-Fisher)
- Molecular typing results obtained by nanopore were compared to RT-PCR results for all 88 donors
- Serotypes assigned for all CIWD alleles in allele string by manual assignment according to IMGT/HLA assignment using HATS "FULL" designation

HLA Serotype Assignment: RT-PCR vs. LR-NGS

LR-NGS provided additional allele-level typing information necessary for HLA serotype assignment **in all 88 donors** and was **100% concordant** with RT-PCR for serologic-split antigen-level typing.

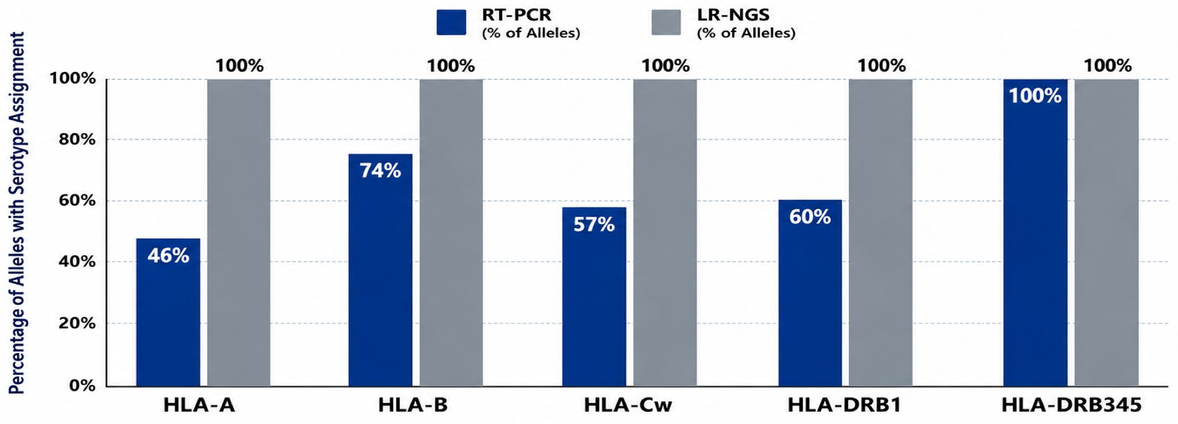


Figure 1. Serotype Assignment with RT-PCR vs LR-NGS

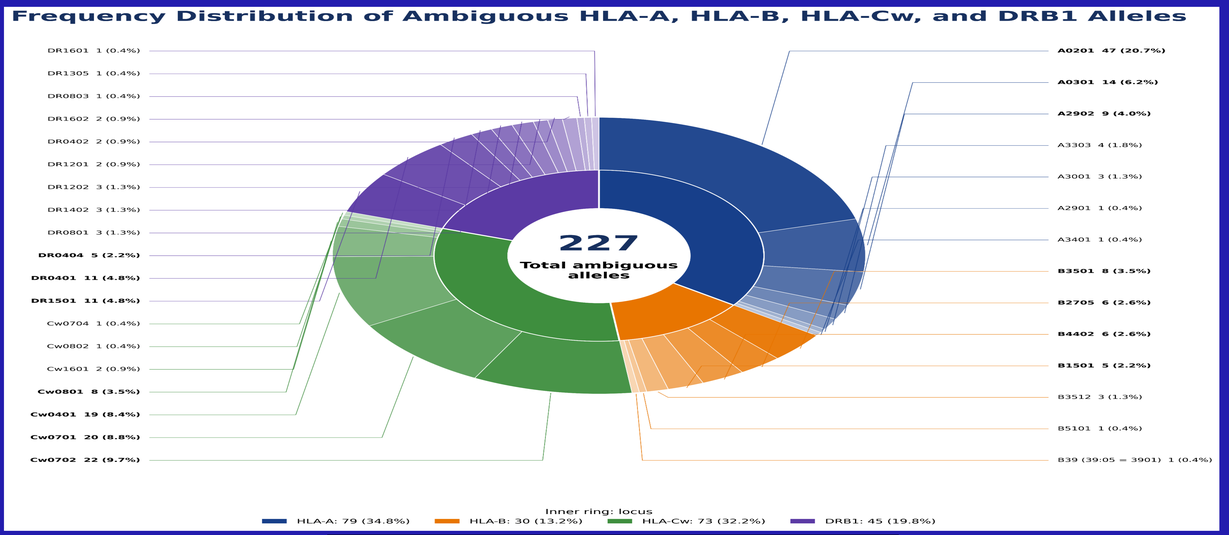


Figure 2. Distribution of Ambiguous Alleles

Results

- LR-NGS provided additional allele-level typing information necessary for HLA serotype assignment in all (88/88) donors
- LR-NGS was 100% concordant with RT-PCR for serologic-split antigen-level typing
- RT-PCR was only capable of identifying serotypes present in 81/176 (46%) HLA-A, 130/176 (74%) HLA-B, 101/176 (57%) HLA-Cw, 106/176 (60%) HLA-DRB1, and 176/176 (100%) HLA-DRB345 alleles tested
- LR-NGS additionally provided important information about the allele-level typing of DQA1 and DQB1 that distinguished antigens with potentially different serologic reactivity (i.e. DQB1*05:01 and DQB1*05:02).

Conclusions

- LR-NGS provides essential genotyping information necessary for assignment of HLA serotypes for deceased donor HLA typing
- Our data demonstrates the improved precision of high-resolution HLA genotyping by LR-NGS for HLA typing for deceased organ donors, and supports integration of LR-NGS into donor typing workflows
- Notably, several instances of HLA typing assignment defaulted to HLA serologic split-antigen-level typing due to the absence of defined serotypes for specific alleles, though other alleles in that group had distinct serotypes (i.e. A*02:06 reported as A2), which likely accounts for low performance of RT-PCR at HLA-A locus
- This highlights potential limitations in HLA serotype nomenclature, which may not yet be completely inclusive of serologically-reactive polymorphisms.

References

[Marsh SGE, Osoegawa K, Bodmer WF, et al. Nomenclature for Factors of the HLA System, 2026. *HLA*. 2026;107(3):e70595. doi:10.1111/tan.70595