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Nanopore Typing Validation for Clinic Use in the HLA Laboratory

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ABSTRACT

Aim: To assess the performance of the OMIXON® (now Werfen) NanoTYPE™, a third-generation sequencing method, for HLA typing, compared with rSSO (reverse sequence-specific oligonucleotide sequencing) and NGS (next-generation sequencing) in a clinical laboratory setting.

Methods: Seventy-three patients previously typed by rSSO (One Lambda®) and NGS (CareDx®) were sequenced using the NanoTYPE™ HLA typing assay. Samples were selected to represent a mix of routine samples received in the lab and included a variety of patient diagnostic types, races, and ages (Table 1). DNA was extracted from both peripheral blood and buccal cell samples. Typing results were compared for accuracy between all three assays. Assay precision was measured using three samples tested by three different technologists on three separate occasions.

Results: When comparing previous typing results with the NanoTYPE™ HLA typing assay, an agreement rate of 99.6% was achieved. Disagreement was due to a known limitation of the assay with dropouts in the DQ locus. Precision testing resulted in a 100% agreement between and within technologists' assays.

Conclusions: OMIXON® (now Werfen) NanoTYPE™ HLA typing assay allows for amplification of all 11 HLA loci through a long-read sequencing technique that occurs in a considerably shorter amount of time than the current NGS and rSSO assays used in the laboratory, while still maintaining, or even exceeding, the precision and accuracy needed in a clinical setting. The availability of at least two-field typing results and faster turnaround time is advantageous for a clinical laboratory with regard to making decisions about transplantation

BACKGROUND

OMIXON® (Werfen) NanoTYPE™ is an HLA typing assay leveraging third-generation sequencing technology developed by Oxford Nanopore Technologies (ONT). This innovative assay involves amplifying all 11 HLA loci through long-range multiplex PCR process. After barcoding, labelled PCR products are subsequently loaded into flow cells for sequencing. These flow cells contain integrated nanopores and advanced electronics. As DNA fragments pass through the nanopores, each DNA base disrupts the surrounding electrical field in a distinctive manner, serving as a unique signature for individual bases. The electrical signals are then deciphered using a base-caller, which translates them into a DNA sequence formatted as FASTQ output. Later, this FASTQ file is imported into NanoTYPER™, the dedicated genotyping software, for comprehensive analysis and result generation.
The NanoTYPE™ workflow allows for a streamlined process that decreases the amount of time for accurate, high-resolution typing results.

METHODS

- LABType™ SSO typing using One Lambda® commercial kits
- Next Generation Sequencing (NGS) using CareDx® commercial kits
- OMIXON® (now Werfen) NanoTYPE™

RESULTS

All seventy-three specimens (836 HLA loci total) which were subjected to the new typing method (OMIXON® NanoTYPE™ HLA typing assay) exhibited strong concordance with previous test results (from both rSSO and NGS), achieving a 99.6% agreement rate. Discordance was limited to three HLA DQ loci, and these discrepancies can be attributed to a known limitation of the NanoTYPE™ typing method, specifically an HLA-DQB1 dropout. NanoTYPE™ correctly identified every null allele, the single novel allele, and all homozygous allele calls previously detected by rSSO and NGS.

To evaluate precision, three specimens (one buccal swab and two peripheral blood samples) were tested by three technologists using the NanoTYPE™ assay. Each technologist performed three independent sequencing runs on each of two MinION instruments (MN43885 and MN43899). In every run, the three specimens were each prepared and sequenced in triplicate to assess intra-assay variability. Comparisons across the three runs for each technologist assessed inter-assay variability, comparisons among technologists assessed inter-technologist variability, and parallel testing on both instruments assessed inter-instrument performance. Across all comparisons-within run, between runs, between instruments, and between technologists-allele calls demonstrated 100% concordance, confirming excellent repeatability and reproducibility of the typing method.

Table 1. Distribution of sample types used for validation study. N=number of individuals.

Patient type	N
Kidney Patient	31
Kidney Living Donor	1
Heart Patient	10
Hematopoietic Stem Cell patient	12
Hematopoietic stem cell donor	5
Disease study	1
Prior Proficiency samples	13
Adult patients	67
Children	6
White	37
Black	29
Asian	4
Hispanic	2
Pacific Islander	1

CONCLUSIONS

This study demonstrated the capability of the OMIXON® NanoTYPE™ HLA typing assay to yield precise and consistent HLA typing outcomes. Its accuracy, precision, and rapid turnaround times make it a suitable tool for analyzing patient and donor HLA typing results, aiding in quicker transplantation decision-making, and it is ready for implementation in clinical practice.