Comparison between Real-Time PCR and Next Generation Sequence for Deceased Donor Typing

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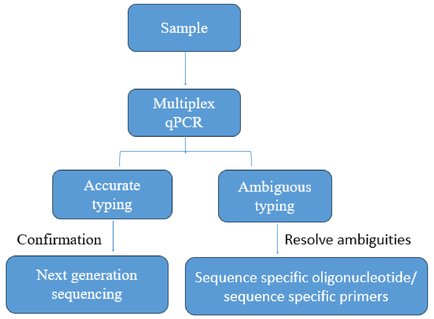
INTRODUCTION

- NGS, a powerful tool that delivers high resolution typing result at more than 2 Field levels which enables massive parallel sequencing of many DNA strands at the same time generating millions to billions of 50-500 bps reads per run.
- Short read next generation sequencing (NGS) offers superior resolution of HLA typing compared to Real-time PCR (RT-PCR), yet RT-PCR is the most feasible and preferred method in many transplant centers for deceased donor typing due to its rapid turnaround time. However, RT-PCR may be unable to resolve ambiguities and may also incorrectly assign alleles, thereby negatively impacting virtual crossmatch. Here, we sought to compare RT-PCR and NGS technologies for deceased donor typing and impact on virtual crossmatch.

Comparison of RT-PCR and NGS Platforms

Factor	RT-PCR	NGS
Target scope	Specific, targeted sequences	Genome-wide or broad panel coverage
Sensitivity	High for known targets	High for detecting unknown or rare variants
Turnaround time	Fast, ideal for rapid diagnostics	Longer, more detailed results
Cost	Lower for small panels	Higher for comprehensive sequencing

METHODS



- Our cohort comprised of 32 deceased donors who were typed by Thermo-Fisher/One Lambda LinkSēq™ at all 11 loci. The most likely common alleles given by SureTyper™ software were converted to serological equivalents for virtual crossmatch assessment. Each donor was retrospectively typed at high resolution by NGS (CareDx-AlloSeq ™ and Immucor- MIAFORA ™). RT-PCR and NGS HLA typings were compared at the two-field, serological equivalent, and P group levels to determine potential impact on virtual crossmatch.

RESULTS AND CONCLUSIONS

- At the two-field level, the total concordance was 91% between RT-PCR and NGS, with B, C, DRB1 and DQA1 loci having 100% concordance (Table 1). A lower concordance was observed in DRB345, DQB1, and DPB1 at 82%, 84%, and 70% respectively, likely due to technical limitations of RT-PCR, including limited reference sequences in the database and incomplete gene coverage. Furthermore, the accuracy and sensitivity demonstrate that overall RT-PCR is efficient with values of above 90% across all loci, including loci with lower concordance.

Concordance between RT-PCR and NGS

Loci	Sensitivity (%)	Positive predictive value (%)	Accuracy (%)	PCR:NGS Concordance (%)
A	96.9	100	96.9	97
B	100	98.4	98.4	100
C	100	96.9	96.9	100
DRB1	100	96.9	96.9	100
DRB345	92.6	98.0	90.9	82
DQA1	100	98.4	98.4	100
DQB1	98.4	100	98.4	84
DPA1	98.4	96.8	95.3	93
DPB1	96.9	100	96.9	70

Performance of RT-PCR

- 7 instances where RT-PCR and NGS was different, none changed the serological equivalent
- In 2 cases where there was no serological equivalent, these typing were matched at the P group level
- 4 instances of ambiguous typing given by RT-PCR
- 2 of these had unknown serological equivalent and were in a different P group level
- 1 case of ambiguous DQB1*03:01/03:02/06:03 in the same allele string
- 1/1 successful resolution of null DRB4 allele

Conclusion : While RT-PCR is efficient and a better alternative to SSO, it may leave some ambiguities unresolved. In most cases, RT-PCR alone was sufficient to provide an accurate serological equivalent typing for the purposes of virtual crossmatch. While our current NGS platform is not feasible for deceased donor typing, future adoption of long read Nanopore technologies may be able to provide high resolution typing with a shorter turnaround time.