Comparison of SSOP versus NGS for HLA-A, B, C, DRB1, DRB3/B4/B5, DQA1, DQB1, DPA1, DPB1 typing

Toward single pass high resolution HLA typing in support of solid organ and hematopoietic cell transplant programs

Anajane G. Smith[1], Shalini Pereira[2], Andrés Jaramillo[3], Scott T. Stoll[3], Faisal M. Khan[4], Noureddine Berka[4], Ahmed A. Mostafa[4], Marcelo J. Pando[5], Crystal Y. Usenko[5], Maria P. Bettinotti[6], Chul-Woo Pyo[1], Wyatt C. Nelson[1], Amanda Willis[7], Medhat Askar[7], Daniel E. Geraghty[1].

[1]Fred Hutchinson Cancer Research Center, Seattle, Washington, [2]University of Washington, Seattle, Washington, [3]Mayo Clinic, Phoenix, Arizona, [4]Calgary Laboratory Services, Calgary, Alberta, [5]Scott & White Medical Center, Temple, Texas, [6]Johns Hopkins School of Medicine, Baltimore, Maryland, [7]Baylor University Medical Center, Dallas, Texas.

Introduction

- Many HLA typing laboratories employ a 2-tier process of intermediate resolution followed by high resolution when clinically required. SSP and qPCR provide the rapid turnaround necessary in deceased donor workup for solid organ transplantation, SSOP is employed for routine typing of higher volumes and Sanger sequencing (SBT) has been the "gold standard“ for high resolution. However, both SSOP and SBT often yield ambiguous results, adding costs and extending turnaround time (TAT). Next Generation Sequencing (NGS) now provides HLA allele level genotyping in a 2 day TAT, with all bench work accomplished on day 1.
- Many published studies compare NGS to Sanger SBT in the context of hematopoietic cell transplantation. We compare NGS with SSOP in the context of solid organ transplantation. Donor specific anti-HLA antibodies (DSA) are known to impact organ survival and it has been shown that DSA may involve HLA allele mismatches not discriminated by SSOP typing; suggesting the need for routine high resolution HLA typing of solid organ transplant candidates and, whenever possible, their donors.
- Using commercially available reagents for NGS and SSOP, we compared accuracy, TAT, ease of use, technologist time and level of resolution for HLA typing of 289 specimens from five laboratories supporting solid organ transplant programs.

289 Samples from 5 laboratories

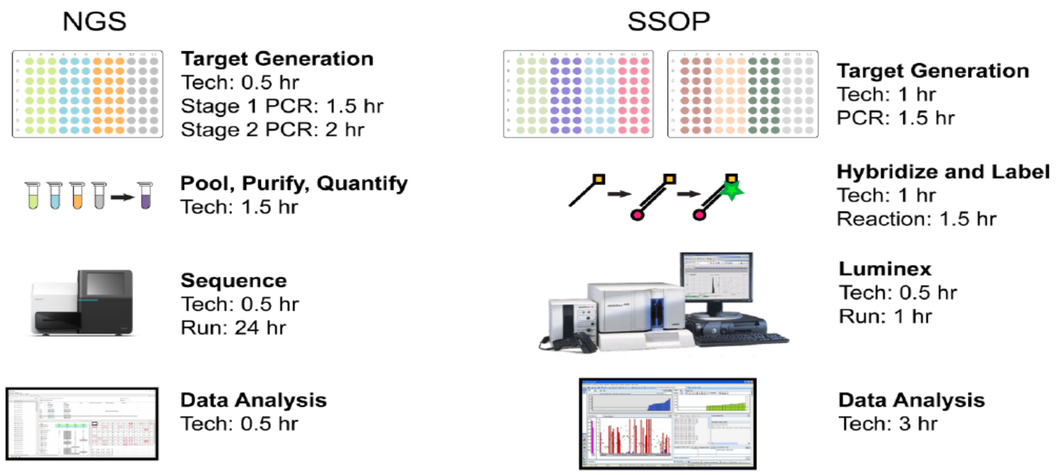
Baylor University Medical Center: 120 samples.
Mayo Clinic: 50.
Baylor Scott and White Medical Center: 50.
Calgary Laboratory Services: 50.
Johns Hopkins School of Medicine: 19.

Coded, blinded DNA samples were submitted to the Fred Hutchinson Cancer Research Center (FHCRC) for NGS typing.

Comparison of time and effort for NGS versus SSOP

Workflow of NGS versus SSOP for typing of 24 samples at all 11 HLA loci. All bench-work is on day 1 and results are reported on day 2.

NGS typing at FHCRC:
- ScisGo HLA v6 reagents and GeMS-UI v80 software (Scisco Genetics) with analysis on the MiSeq platform (Illumina).
  Exons 1-7: HLA-A, B, C.
  Exons 1-4: DRB1, DRB3/DRB4/DRB5, DQA1, DQB1, DPA1, DPB1
  Exons 2-4 of DPA1
- Initial PCR uses 4 primer sets so that 24 samples are amplified in 1 X 96-well plate, with no plate transfers.



SSOP typing at each submitting laboratory:
- LABType and Fusion software (One Lambda) with analysis on a flow cell instrument (Luminex).
- Intermediate resolution (IR) SSOP at 3 labs:
  Exon 2: DRB1/DRB3/DRB4/DRB5.
  Exons 2, 3: A/B/C/DQA1/DQB1/DPA1/DPB1.
- High resolution (HR) SSOP at 1 lab:
  Exons 2-5 of HLA-A, B and exons 2-7 of HLA-C
  Exon 2 of DRB1.
- Initial PCR used 7-8 primer sets so that 24 samples are amplified in 2 X 96-well plates, followed by 2 steps with full plate transfers of samples.

Results: accuracy

At the level of resolution provided by SSOP, NGS and SSOP were fully concordant for HLA-A, B, C. Class II results were concordant except for 11 SSOP results in 8 specimens due to false negative (n=8) or operator changes in probe reactions (n=3): 3 discordant alleles at each of DQB1 and DRB1; 2 each at DPB1 and DRB5 and 1 of DQA1.

Results: level of resolution

Typing technology	Sample Numbers	Percent resolution to a single allele				Mean % allele resolution
		HLA-A	HLA-B	HLA-C	DRB1	
IR SSOP	239	0.4%	2%	0%	2%	**1.1%**
HR SSOP	50	20%	25%	5%	19%	**17%**
NGS	289	99.3%	99.5%	100%	100%	**99.7%**

- **IR SSOP** achieved less than 2% of specific HLA alleles across the HLA-A, B, C, DRB1 loci.
- **HR SSOP** gave 17% of specific allele assignments across those 4 loci.
- **NGS** provided unambiguous alleles assignments for HLA-C, DRB1, DRB3, DRB5, DQA1 and DPA1. NGS identified 1 diploid ambiguity at HLA-A; 2 each at HLA-B and DQB1; and 13 diploid ambiguities at DPB1. All NGS ambiguities are also found within SSOP typing.

Results: 21 novel sequences

- DPA1: 6 new sequences.
- HLA-C, DRB1 and DRB3: 3 new at each.
- DQA1 and DRB5: 2 new at each.
- DRB4 and DPB1: 1 new at each.
- 20 novel sequences were due to SNPs, of which 13 generated an amino acid substitution.
- A novel DRB1*15 allele exhibited a large, multi-exon deletion downstream of exon 1.

Discussion

- The high concordance of NGS and SSOP (>99%) attests to the **accuracy** of both HLA typing systems.
- The amplicon-based NGS protocol provides a **2 day turnaround time** for result reporting comparable to SSOP
- NGS had **fewer manipulations, fewer consumables, and less hand-on tech time**.
- NGS genotyping results in a vast improvement in the **level of resolution**.
- The exquisite sensitivity and specificity of NGS provides critical information, particularly for patients with **allele specific antibodies**.

Conclusions

Availability of 2 day turnaround high resolution HLA genotyping is a strong impetus for laboratories to consider implementing single-pass NGS for routine HLA typing in support of both HCT and solid organ transplantation.