High Throughput HLA Class I & II Allelic Typing Using Automated SBT and SSOP Methods

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Introduction
Human leukocyte antigens (HLA), as central components in immune function, are a major barrier for solid organ or bone marrow transplantation. HLA typing is performed to identify identical or compatible HLA alleles for transplantation donors. The HLA Lab of the PJ Region of the American Red Cross provides allelic level HLA typing to support bone marrow transplantation for hospitals and intermediate resolution HLA typing for unrelated bone marrow donors as a contractor for the National Marrow Donor Program (NMDP). We process more than 2000 samples/week identifying HLA class I and II polymorphisms using molecular methods that include sequence specific primer (SSP), sequence specific oligonucleotide probe (SSOP) hybridization, and sequence-based typing (SBT). Our HLA typing turn-around time (TAT) is 3 to 9 days. For the National Marrow Donor Program alone, our lab receives between 720 to 1000 donor samples/week as buccal swabs for HLA-A, B & DRB1 typing. Greater than 85% of the typing results are sent to NMDP within 14 days. We chose to automate these high throughput projects using several liquid handling robotic systems. Our first goal was automation of the labor-intensive steps for our most demanding project, i.e., NMDP’s unrelated donor typing, in order to decrease assay setup time while maintaining high quality standards.

Materials & Methods

Automated HLA Kit Typing Assays - Development and Validation

One Lambda LABType® SSO Kits – reverse PCR/SSOP HLA class I and II typing using Lumimmul®-APC/Microbead Array Technology. LABScan™ 100 flow analyzer, and LABType™ software for HLA allele assignments.

Atria Genetics AlleleSEQ® HLA SBT Kits – high resolution HLA class I and II typing using ABI Prism® DNA sequencing system and Atria-AutoSEQ™ software for allele assignments.

1 Automated SSOP - Amp Assay Setup

JANUS WinPREP® Test & Deck view – Universal Amp

2 Automated SSOP – Pre-Hybridization

WinPREP® Test & Deck View – Denaturation & Neutralization Setup

3 Allele Assignments - Automated SSBP Results

Example of automated SSBP typing results for a 96 well sample plate. LABScan™ trimming scan data were analyzed using the LABTYPE SSO DRB1 Locus template.

7 Allele Assignments - Automated SBT Analysis

Electropherogram & Allele Assignment for an HLA-A Locus Sequence. Sample data for an automated SBT assay analyzed using an ABI PRISM® 3730XL DNA Analyzer and Atria-AutoSEQ™ software is shown below. Manual and automated processing gave the same results.

8 Conclusions

Productive HLA DNA typing strategies require initial low to medium resolution screening to identify potential HLA-matched donors. (SBT) is the most specific allelic typing method and allows identification & assignment of all alleles. PCR/SSOP is notable, cost-effective, with decreased labor and assay time. We chose to automate the most tedious and time-consuming steps for both SBT and SBT assays using multiple JANUS workstations. Ongoing assay development using these robotic liquid handling platforms features the following:

• Workstation protocols have to date automated multiple HLA typing assay steps including primary amp setup for SBT and SSBP methods, complex SBT cycle sequencing reactions and pre-hybridization steps for SSBP.
• Flexible JANUS WinPREP software allows easy creation and implementation of new automation protocols.
• Automated protocols are more robust and faster than manual setup.
• Validation results are comparable or better than manual data. Importantly, no contamination was found.
• Automation resulted in reduced hands-on assay setup time, decreased user errors, and increased throughput improving laboratory productivity.