HLA IN TRANSPLANTATION:
PAST, PRESENT AND FUTURE

Donna L. Phelan, MT CHS
Technical Supervisor, HLA Laboratory
Barnes-Jewish Hospital
St. Louis, Missouri
chromosome 6

M N
Q R

M M N N
Q Q R R

M Q R R
N Q R R

M Q R R
N Q R R
DEFINITIONS

PHENOTYPE: VISABLE TRAITS WHICH CAN BE ASCERTAINED IN A SINGLE INDIVIDUAL

GENOTYPE: GENETIC PROFILE OF INHERITED TRAITS

HAPLOTYPE ASSIGNMENT OF GENES TO MATERNAL OR PATERNAL CHROMOSOME

A1, B7, B8
A1, A1, B7, B8
A1 B7
A1 B8
Identification of HLA Antigens / Alleles

- **Serology** - Tissue source: lymphocytes

  - **CDC** - Complement-Dependent Cytotoxicity

- **Molecular** - Tissue source - any nucleated cell

  - **SSP** - Sequence specific primer
  - **SSOP** - Sequence specific probe
  - **SBT** - Sequence based typing
Sequence Specific PCR (SSP)

Nucleated Cell → DNA Extraction → Target Gene

Detection by Agarose Gel Electrophoresis

PCR with Sequence Specific Primers

Genomic DNA

>10^6 copies
Sequence Specific PCR (SSP)

### DR Specificities

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## HLA ANTIGENS

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<th>A</th>
<th>B</th>
<th>C</th>
<th>DR</th>
<th>DQ</th>
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<td>1991</td>
<td>26</td>
<td>35</td>
<td>14</td>
<td>56</td>
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<td>1995</td>
<td>59</td>
<td>122</td>
<td>35</td>
<td>152</td>
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<td>1997</td>
<td>85</td>
<td>188</td>
<td>42</td>
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April, 2004

**Class I alleles:** 1012

**Class II alleles:** 493
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<th>Serology</th>
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<td><strong>Parent</strong></td>
<td><strong>Splits</strong></td>
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<td>A2</td>
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<td>A9</td>
<td>A23</td>
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<td>A24</td>
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<td>B15</td>
<td>B62, B63</td>
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<td>B17</td>
<td>B57</td>
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<td>DR6</td>
<td>DR13</td>
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<td>DR14</td>
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HLA Nomenclature

- Serology
- Molecular
- Proteins
- Genes
- Antigens
- Alleles
- Few
- Many
- Moderately Complex
Clinical Histocompatibility testing

- Class I typing - A, B, C loci
  - DNA
- Class II Typing - DR, DQ
  - DNA, sequence-specific primer amplification by PCR, or Direct Sequencing
- Cross-matching
- Antibody Screening – Pre and Post (DSA)
Antibody Detection Methods

- Cytotoxicity
- Luminex
- Flow Cytometry
COMPLEMENT-DEPENDENT (CDC) LYMPHOCYTOTOXICITY ASSAYS

- CDC
- AMOS
- Antiglobulin
- Modified
- Augmented (AHG)

- Stage 1
  - Cells + Serum
  - Ag-Ab
  - Interaction
  - 30 min
  - Wash
  - Wash

- Stage 2
  - Complement
  - X1
  - X3
  - C’ Mediated
  - X2
  - Add AHG
  - Cell Injury
  - 60 min
  - or
  - 2 min

- Stage 3
  - Vital Stain
  - X3
  - Visualize
  - Injury
LABScreen™ PRA

Alloantibody

PE anti-IgG

Purified Antigen Coated Beads
Each Color-code Is A Precise Blend of Two Colors

Each unique microsphere is color-coded using a blend of different fluorescent intensities of two dyes.
Principle of LABScreen Technology

Substrate

Dual-colored bead
Color-coded Measurements

Luminex measures all-in-one!
Digging Deeper:

Precision fluidics align the microspheres in single file.

Microspheres pass through the laser beams one at a time!
Cross-Matching

- NIH Microlymphocytotoxicity
- Amos wash
- Extended incubation NIH
- 3-wash
- Antiglobulin
- Flow cytometry
FlowPRA™ Screening Test

Data Analysis

% PRA is represented by the percentage of events shifted to the right of the cut-off point.
The cell surface is a jungle!
70% of labs called A34
Several labs commented on antibody to epitope DP DEAV 85-87

DEAV DP1, 3, 5, 9, 10, 11, 14, 17, 19, 20
More labs reported DQA1*0201 (66%) than the highest DQB1 specificity (DQ9 at 34%)
# Cadaver Donor Virtual Crossmatch Worksheet

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<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Date of Birth</th>
<th>Sample Date of most recent Luminex</th>
<th>Antibodies as detected by Luminex</th>
<th>Unacceptable Antigens Listed in UNOS</th>
<th>Notes</th>
<th>Mismatched Antigens</th>
<th>Date updated and Tech. Initials</th>
<th>Current Serum Date</th>
<th>Mis Match</th>
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## Cadaver Donor Virtual Crossmatch Worksheet

**Donor ID:** YHX261  
**Donor Phenotype:** A30, B6, 13; Cw6, 7; DR7, 17; DR52, 53; DQ2, -  
**Tech:** Lauren  
**Date:** 8-24-11

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ALTERNATIVES TO LIVING DONATION

- ABO INCOMPATIBLE TRANSPLANTS

- DESENSITIZATION WITH IVIG/PLASMAPHERESIS FOR THE HIGHLY SENSITIZED PATIENTS

- PAIRED KIDNEY EXCHANGE PROGRAMS
WHO IS THE RECIPIENT AND WHO IS THE DONOR?