In The Name of GOD

HLA Typing


By: M. Farzanehkhah
NoAvaran MAYA Teb Co.
Haplotypes:

An HLA haplotype is a series of HLA “genes” (loci-alleles) by chromosome, one passed from mother and one from the father.
We inherit a set (or haplotype) of HLA-A, B and DR Ags from each parent.

For each full sibling, a patient has a one in four (25%) chance of a full match.
HLA Nomenclature

Serotyping:

**SeroLogic Types (Antigen Names)**

- **B62 (15)**

  - Protein
  - (Broad specificity)*

  Serologic type in order discovered, broad or split

*Not required*
### Original Broad Specificities

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>A9</td>
<td>A23, A24</td>
</tr>
<tr>
<td>A10</td>
<td>A25, A26, A34, A66</td>
</tr>
<tr>
<td>A19</td>
<td>A29, A30, A31, A32, A33, A74</td>
</tr>
<tr>
<td>A28</td>
<td>A68, A69</td>
</tr>
<tr>
<td>B5</td>
<td>B51, B52</td>
</tr>
<tr>
<td>B12</td>
<td>B44, B45</td>
</tr>
<tr>
<td>B14</td>
<td>B64, B65</td>
</tr>
<tr>
<td>B15</td>
<td>B62, B63, B75, B76, B77</td>
</tr>
<tr>
<td>B16</td>
<td>B38, B39</td>
</tr>
<tr>
<td>B17</td>
<td>B57, B58</td>
</tr>
<tr>
<td>B21</td>
<td>B49, B50</td>
</tr>
<tr>
<td>B22</td>
<td>B54, B55, B56,</td>
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</table>

### Splits and Associated Antigens

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>B40</td>
<td>B60, B61</td>
</tr>
<tr>
<td>B70</td>
<td>B71, B72</td>
</tr>
<tr>
<td>DR2</td>
<td>DR15, DR16</td>
</tr>
<tr>
<td>DR3</td>
<td>DR17, DR18</td>
</tr>
<tr>
<td>DR52</td>
<td>DR11, DR17, DR18 DR12, DR13, DR14</td>
</tr>
<tr>
<td>DR53</td>
<td>DR4, DR7, DR9</td>
</tr>
</tbody>
</table>
HLA Nomenclature

Genotyping:

- Hyphen used to separate gene name from HLA prefix
- Suffix used to denote changes in expression

```
HLA-A*02:101:01:02N
```

- Field 1: allele group
- Field 2: specific HLA protein
- Field 3: used to show a synonymous DNA substitution within the coding region
- Field 4: used to show differences in a non-coding region

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# Alleles Diversity (update 2016)

**Class I**

<table>
<thead>
<tr>
<th>HLA</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3,356</td>
</tr>
<tr>
<td>B</td>
<td>4,179</td>
</tr>
<tr>
<td>C</td>
<td>2,902</td>
</tr>
</tbody>
</table>

**Class II**

<table>
<thead>
<tr>
<th>HLA</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1</td>
<td>1,978</td>
</tr>
<tr>
<td>DQB1</td>
<td>900</td>
</tr>
<tr>
<td>DPB1</td>
<td>630</td>
</tr>
</tbody>
</table>

- (10,437)
- (3,508)
Graph showing numbers of alleles named by year from 1987 to end of June 2015.
How Common is Common?

99.9% of all HLA types are common

- 415 common alleles
- 707 WD alleles

12945 rare alleles

Number of CWD alleles

Frequency of common HLA types

- around 0.1% types including rare alleles
- around 99.9% common types (C;C)
Crossing Over:
(Genetic Linkage and Recombination)
HLA typing

How is HLA typing performed?

Typing is performed by two different methods:

**Sero logical testing:** Where the white cells are used

**Molecular Methods:** Where DNA extracted from the white cells is used. (SSP, SSO, SBT)
HLA Serology
Serologic HLA typing

**Principle:**

**Lymphocyte microcytotoxicity test**

- originally was utilized in the mouse by Gorer & Amos
- And later modified by Terazaki use in the Human system
- Has been used since the 1960s
**CDC:**
Complement-Dependent Cytotoxicity

![Diagram showing the process of Complement-Dependent Cytotoxicity](image)
Cell injury is determined by the uptake of dye. Analyse by microscope. 

<table>
<thead>
<tr>
<th></th>
<th>+ve Reaction</th>
<th>-ve Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HLA antiserum (Ab) + lymphocyte suspension (Ag)</strong> (Ag - Ab reaction)</td>
<td><img src="image1.png" alt="Diagram" /></td>
<td><img src="image2.png" alt="Diagram" /></td>
</tr>
<tr>
<td><strong>Complement dependent cell lysis</strong></td>
<td><img src="image3.png" alt="Diagram" /></td>
<td><img src="image4.png" alt="Diagram" /></td>
</tr>
<tr>
<td>Cell injury is determined by the uptake of dye Analyse by microscope</td>
<td><img src="image5.png" alt="Diagram" /></td>
<td><img src="image6.png" alt="Diagram" /></td>
</tr>
</tbody>
</table>
Terasaki plate & Hamilton syringe
PBMC isolation:

Centrifuge at 1000 RCF (g) for 10 min at RT (18-25°C)

Brake OFF
PBMC isolation:
The procedure of test

- Add 1 ul Lymphocyte suspension to HLA plate wells
- Incubate 30 min at RT on Rotator
- Add 5 ul Rabbit complement
- Incubate 90 min at RT
- Add 2 ul Eosin Y (5%) staining solution
- Add 10 ul Formaldehyde (12%) to the stop reaction
- Evaluation the results by Invert Microscope
The evaluation of the reaction:

Live cells               Dead cells
The evaluation of the reaction:

Estimating the percent of cell death as follow:

- 0-10% : Negative
- 10-25% : I don’t know but I think it is Negative
- 25-50% : I don’t know but I think it is Positive
- 50-80% : Positive
- 80-100% : Strong positive
Notice:

• The interpretation of serologic reactions requires skill & experience & knowledge, therefore careful QC are required.

• At least 1 HLA-A, 1 HLA-B, & 1 HLA-C & no more than Two HLA for each must be assigned for every HLA typing.

• Cross reactivity
Disadvantage

- For some rare HLA-antigens no antiserum available not detected
- Cross-reactivity
- Serologic assay requires live cells; therefore delayed in sample delivery & typing can produce some errors
- Many alloantisera are derived from sensitized persons & the Ab response changes over time
- Obtain sufficient number of appropriate cells from some patient is very difficult.

However, due to HLA polymorphism and many disadvantage as mentioned, suggesting that serologic methods are not adequate for identifying all HLA antigens relevant to BMT, assays focused on HLA typing by molecular methods.
HLA PCR-SSP
HISTO TYPE SSP is a fast and well established HLA typing method based on: **Sequence Specific Primers.**

It is ideal for smaller numbers of samples and confirmatory tests.

It represents an easy to use and robust method for low resolution HLA typing.
1.) DNA Extraction

2.) Preparation of the PCR Mastermix

3.) Aliquoting the PCR Mastermix, the Taq Polymerase and the DNA samples in the plate

4.) Sealing the plate with adhesive PCR film

5.) PCR reaction
Principle of Sequence-Specific-Primer-(SSP) PCR

Perfect match → Amplification (specific Allel)

Mismatch → no Amplification (unspecific Allel)

PCR: Denaturation 94°C

(Andy Vierstraete 1999)
Electrophoresis

1. Mix agarose and buffer
2. Microwave to boil
3. Cool to 65°C and pour into mold
4. Comb to make wells
5. Finished gel

DNA separation using electrophoresis
Evaluation Diagram
The HISTO SPOT® SSO system is based on the hybridization of amplified DNA to Sequence Specific Oligonucleotides. It is a fast, safe and very convenient method for HLA typing.
A simple, fast and fully automated system for HLA-typing

• **Manual Application:**
  • Set up of the PCR
  • Loading of the **Mr.SPOT** instrument
  • Transfer of data to interpretation software

• **Automation:**
  • **Mr.SPOT**:  - complete hybridization assay
    - photo of the result
  • **Software**:  - Control of the set-up and the instrument,
    - results interpretation

• **Time-to-result**:  ~ 3 hrs (comparable to SSP)

• **Hands-on time**:  ~0,5 hrs

• **Flexibility**:  1 to 96 samples per run

• **Resolution**:  Low resolution in the beginning, 4D at Now, and
  high resolution is an option for the next generation of tests
HISTO SPOT
Workflow for Mr.SPOT

- Blocking of typing well for 5 min
- Denature samples for 5 min
- Aspirate blocking buffer
- Add hybridisation buffer to sample
- Transfer sample to typing well
- Hybridization at 50°C for 15 min
- 3 x Stringency wash
- Add conjugate and incubate for 15 min
- 3 x wash buffer
- Add substrate and incubate for 15 min
- Wash with water
- Dry for 5 min
- Internal camera takes images of typing wells
- Transfer of images to external PC

1 hr 15 min for 8 samples
2 hrs 20 min for 96 samples
Mr.SPOT Processor

- Pipetting arm
- CCD camera hidden in the backspace
- Touchscreen
- Holder for amplicon tray
- Buffer reservoir
- Incubation chamber
- Holder for teststrips
HISTO SPOT – Test Results

- Spots are gridded by the software
- The intensity of the spots is measured and compared to the intensity of the background
- Cut-offs are defined in relation to the intensity of a control probe
Software Analysis
SBT
Sequence Based Typing

NGS
Next Generation Sequencing
‘Sanger sequencing’ has been the only DNA sequencing method for 30 years but hunger for even greater sequencing throughput and more economical sequencing technology.

NGS has the ability to process millions of sequence reads in parallel rather than 96 at a time.

Objections: fidelity, read length, infrastructure cost, handle large volume of data.
Thank You