AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

Pearl Title: HLA testing for Solid Organ Transplantation

Name of Presenter: Idoia Gimferrer

Affiliation: BloodworksNW

DOI: 10.15428/CCTC.2019.306688

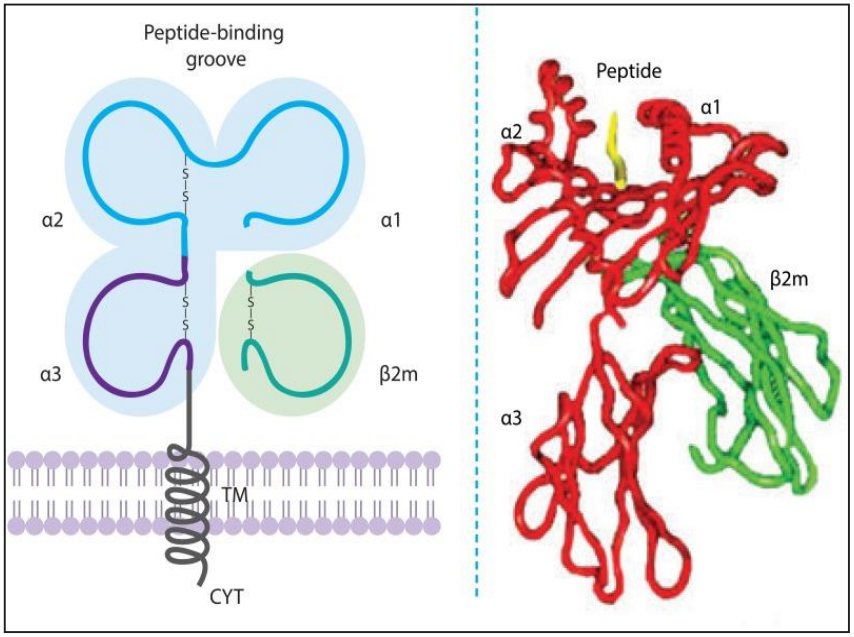
What is HLA?

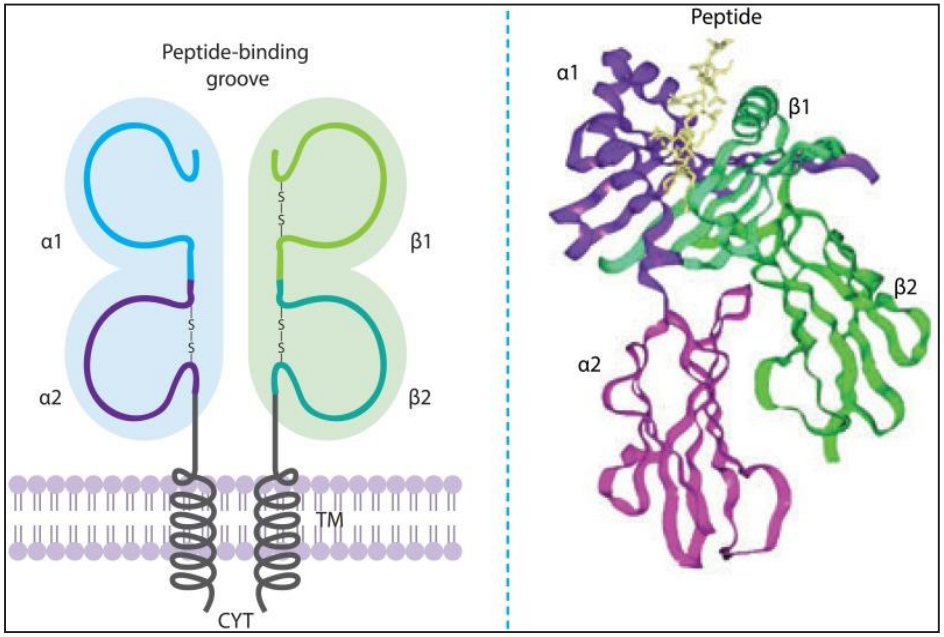
- Human Leukocyte Antigen (HLA) is a gene complex located in chromosome 6 that codifies for a group of very polymorphic proteins.

- There are two major HLA classes:
  - Class I (A, B, C).
  - Class II (DR, DQ, DP).

- The role of both HLA classes is to present protein peptides to T cells. “Self-peptides” (derived from self-proteins) will maintain the homeostasis of the immune system. On the contrary, peptides derived from modified proteins (such cancer cells) or foreign organisms (such viruses) will activate the T cells and trigger immune responses.

HLA structure:





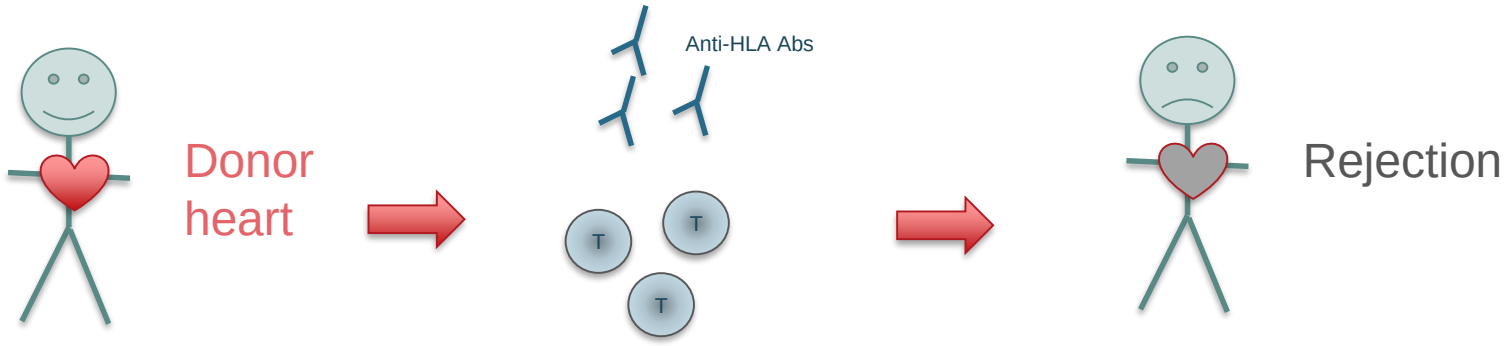
HLA-Class I **HLA-Class II**

Cruz-Tapias P, Castiblanco J, Anaya JM. Major Histocompatibility complex: Antigen processing and presentation. In: Anaya JM, Shoenfield Y, Rojas-Villarraga A, Levy RA, Cervera, editors. R. *Autoimmunity from Bench to bed side. 1st Ed. Bogota D.C. El Rosario University Press; 2013. p. 169-183 (*Reproduced with permission).

Solid Organ transplant

- In solid organ transplantation, a patient receives a graft that usually carries different HLA antigens.
- These allogeneic-HLA will be recognized by patient's T-cells as foreign and will trigger cellular and/or humoral allo-immune reactions.
- Some recipients have pre-formed antibodies against HLA antigens due to previous sensitizing events (pregnancy, transfusion or previous transplant). Donors carrying these HLA specificities should be avoided.
- Patients must be immunosuppressed to prevent graft rejection.

Solid Organ transplant



The goal is to find an immunologically compatible organ for a given patient, based on donor and recipient HLA types and recipient's HLA antibody profile.

HLA Testing in Solid Organ Transplantation

The main role of the HLA laboratory is to assess and monitor the immunological risk of a donor-recipient pair by performing:

HLA typing:

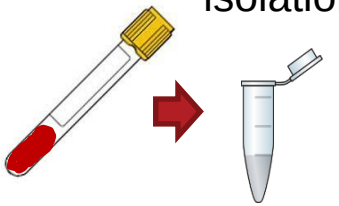
- Molecular typing required by ASHI and UNOS
- Low resolution or antigen level
- Patients and donors
- Fast turnaround time (TAT) for deceased donors

HLA Antibody testing:

- Solid phase antibody (Ab) detection and identification
- Crossmatch (XM)

HLA Molecular Typing Techniques

Genomic DNA isolation



PCR-setup

DNA amplification

Gel electrophoresis

Automated multicapillary electrophoresis

SSP-PCR
Visualization of amplified products

rSSOP
Probe hybridization

Visualization

Real Time PCR

Melting curve

Sequencing
Sanger/NGS/Nanopore

Chromatograms

HLA Molecular Typing

Sequence Specific Primer -Polymerase Chain Reaction (SSP-PCR):

- Primers specific for polymorphic positions that allows to differentiate the HLA antigens or alleles of interest
- Fast (few hours)
- Depending on the tray design allows from full HLA low resolution typing or locus specific high resolution typing

Real time-PCR:

- PCR-SSP based
- Melting curve
- Fastest (less than 2 hours)
- Commercially available kits allow for low resolution only

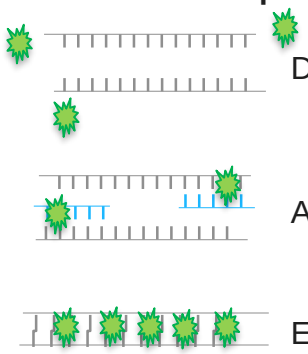
Reverse Sequence Specific Oligonucleotide (rSSO):

- Initial PCR to amplify the HLA loci to be typed
- Luminex technology: Beads carry oligonucleotides complementary to the sequence of interest for hybridization
- Allows intermediate resolution HLA typing
- Efficient for high throughput

Dunckley H. HLA typing by SSO and SSP methods. Methods Mol Biol 2012;882:9-26.

Real Time-PCR

1- PCR Step



DNA denaturalized

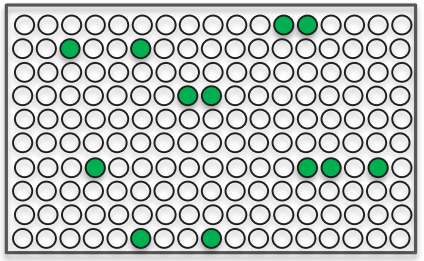
Annealing

Extension

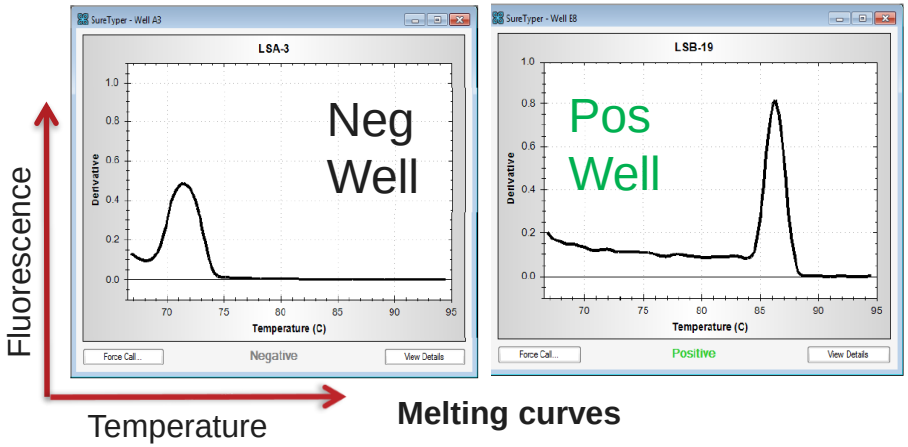
SYBR green binds to double string DNA

2- Melting curve

3- Interpretation



Well with amplicon (positive)



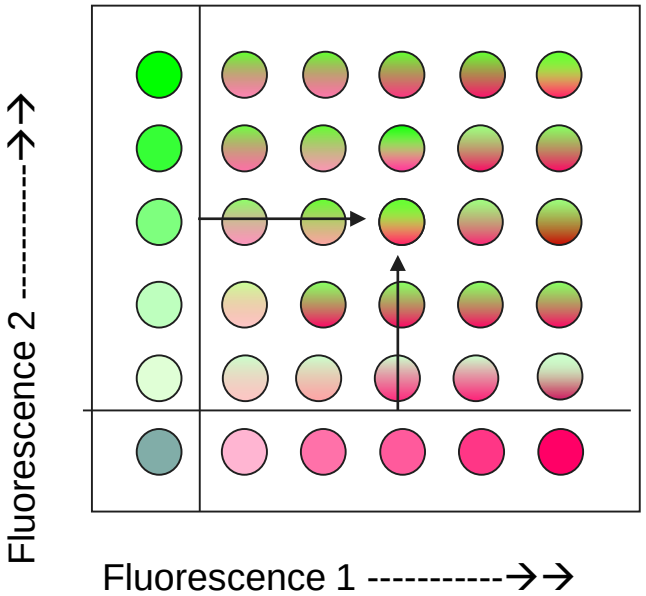
Melting curves

group	allele	antigen
A*02	A*02: 01:01:01, 01:01:02Le-01:03e, 01:04, 01:05e-01:08e, 01:10e-01:13e, 01:15e, 01:18e-01:35e, 01:37e-01:51e, 01:53e-01:73e, 01:75e-01:115e, 02:01, 02:02e, 04-05:01, 05:02e, 05:04e, 05:06e, 06:01, 06:02e-06:06e, 06:08e-06:11e, 06:13e-06:18e, 07:01, 07:02e-07:07e, 08-09, 11:01, 11:03e-13d, 14, 16d, 17:01, 17:02e-18d, 20:01, 20:02e-21d, 22:01, 22:02e, 24:01, 24:02e-25d, 29d, 31e, 34d, 41e-42e, 49d, 59e, 66e-68e, 70e-72e, 74d, 77e-79:01e, 89e-90e, 97e, 105e	A2
	A*02: 03:05e	A203
	A*02: 15Ne, 32Ne, 43Ne, 53N, 82Ne-83Ne, 88Ne, 94Ne, 113Ne, 125Ne	Null
	A*02: 01:14Qe, 26e-28e, 30, 33d, 36d-37e, 40:01e-40:02e, 44d-45d, 47e, 51e, 54e, 56:01e, 57e-58d, 60:01, 60:02e-64d, 69e, 75e-76:01e, 80e, 84d-86d, 91e-92e, 96d, 99e-102e, 104e, 106e-109e, 111e, 115e-116e, 118e-121e, 123d, 126e-128e, 130e-134e, 137d-147e, 149e-151e, 153e-155e, 157e-168e, 170e, 172e-184e, 186e-194e, 196e-229e, 231e-236e, 238e-241e, 243:01e, 246e-252e, 254e-257e, 259e-263e, 265e-278e, 283e-299e, 301Ne-303e, 305Ne-308e, 310e-314Ne, 316e-333e, 335e-337e, 340e-344e, 346e-354e, 356Ne-369e, 371e-408e, 410e-411e, 413e-426e, 428e-430e, 432e-446e, 448e-452e, 455e-462e, 464e-465e, 467e-479e, 481e-503e, 506Ne-526e, 528e, 530e-543e, 545e-555e	-
A*11	A*11: 01:01:01, 01:01:02e-01:06e, 01:08e-01:27e, 01:29e-01:66e, 02:01, 02:02e-03d, 04, 05d, 07e, 09d, 12d-15:02e, 19d	A11
	A*11: 21Ne	Null
	A*11: 06e, 11e, 16e-18e, 20e, 22e-23e, 27e-30d, 32e, 33:02e-34e, 36e-43e, 45e-49e, 51e-93e, 95e-117e, 120e-129e, 131e-138e, 140e-157e, 159e-182Qe, 184e-190e, 192e-198e, 200e-208Ne, 210Ne	-

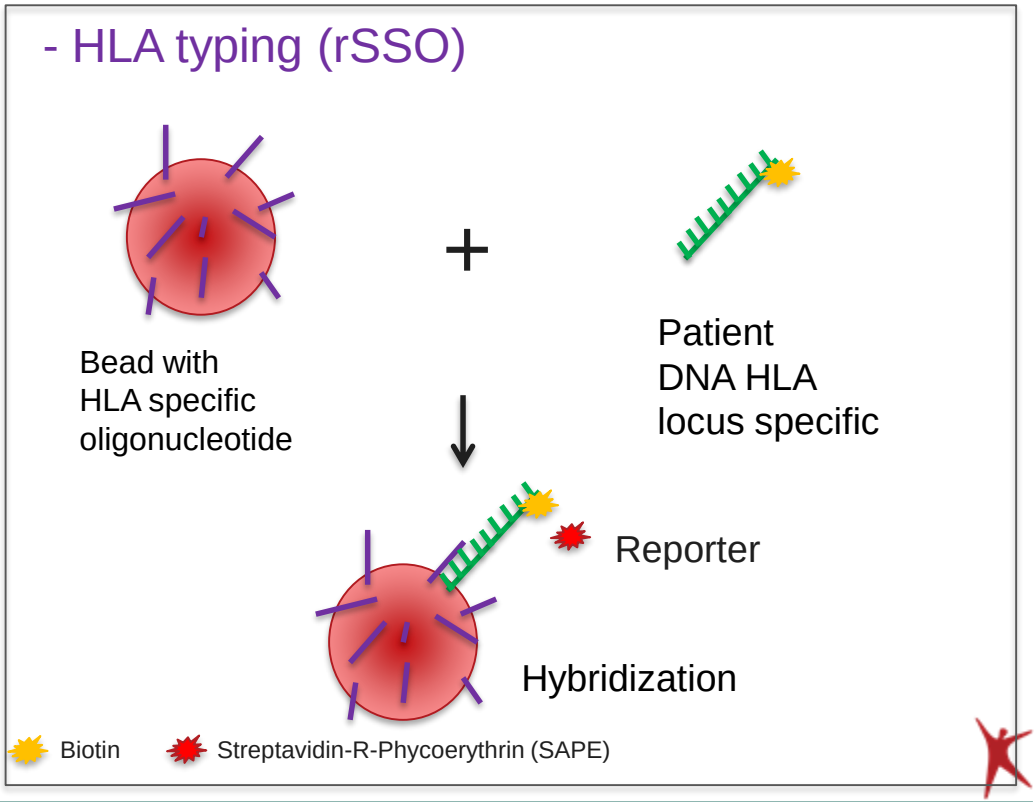
Ponchel F BMC Biotechnol 2003

Luminex Technology

The Luminex Technology is based on internally dyed beads. Different concentrations of red and infrared fluorophores were used to create distinct microsphere sets.

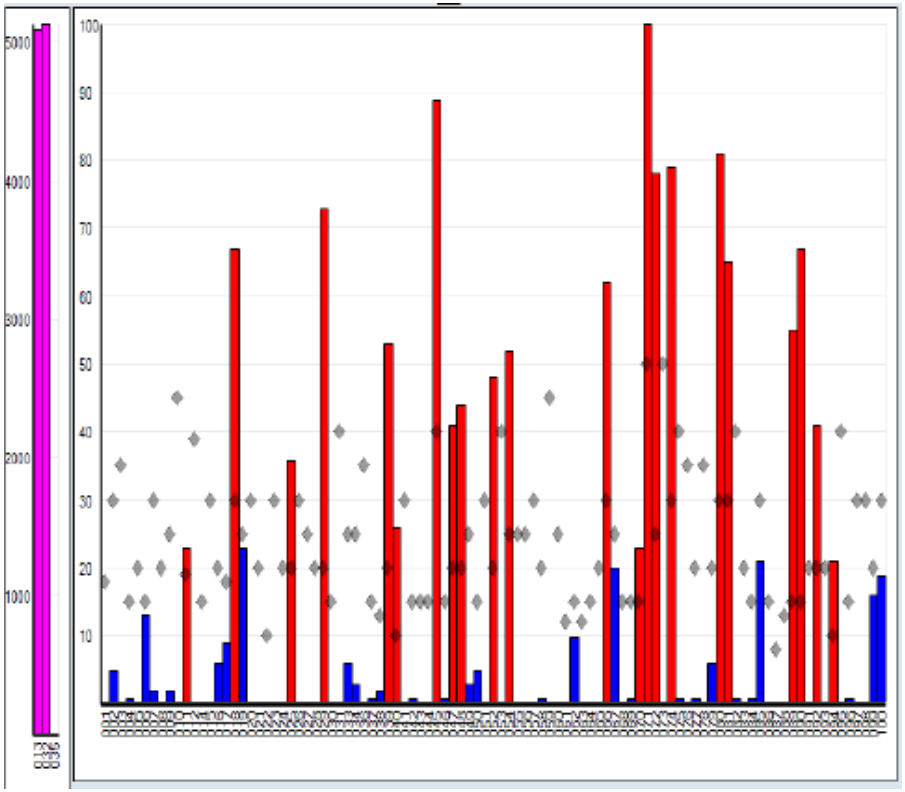


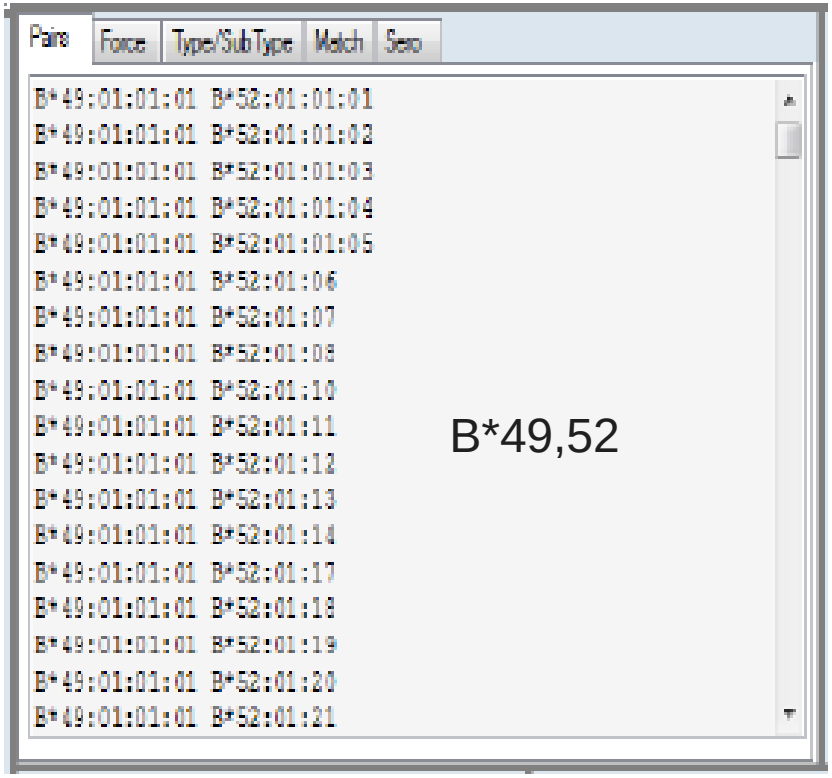
- Luminex system can be used for both:
  - HLA typing
  - HLA antibody testing



Reslova N. Front Microbiol 2017;8:1-17.

Real Time-PCR





HLA Antibody Testing

Solid Phase:

- ELISA. Few labs use it.
- Luminex Technology
  - Detection
  - Identification
  - Complement-binding

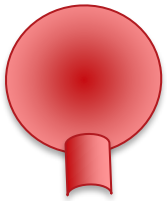
Crossmatch:

- CDC
- Flow Cytometry
- Virtual XM

Tait BD, Hudson F. Tissue Antigens 2010;76:87- 95.
Tinckam K. Transplant Rev 2009;23:80-93.

Luminex Technology

- HLA Antibody testing



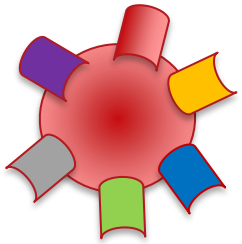
Bead with HLA proteins

Patient serum with HLA Abs

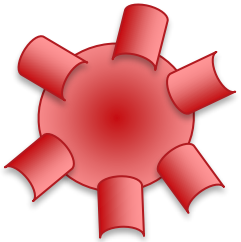
Anti-Human IgG PE

PE: R-phycoerythrin

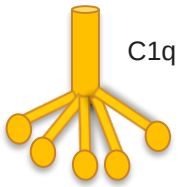
- Types of beads



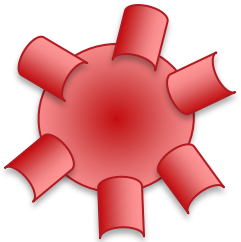
Multiple HLA antigens per bead
- Used for screening



Single HLA antigen per bead
- Used Ab identification



\+

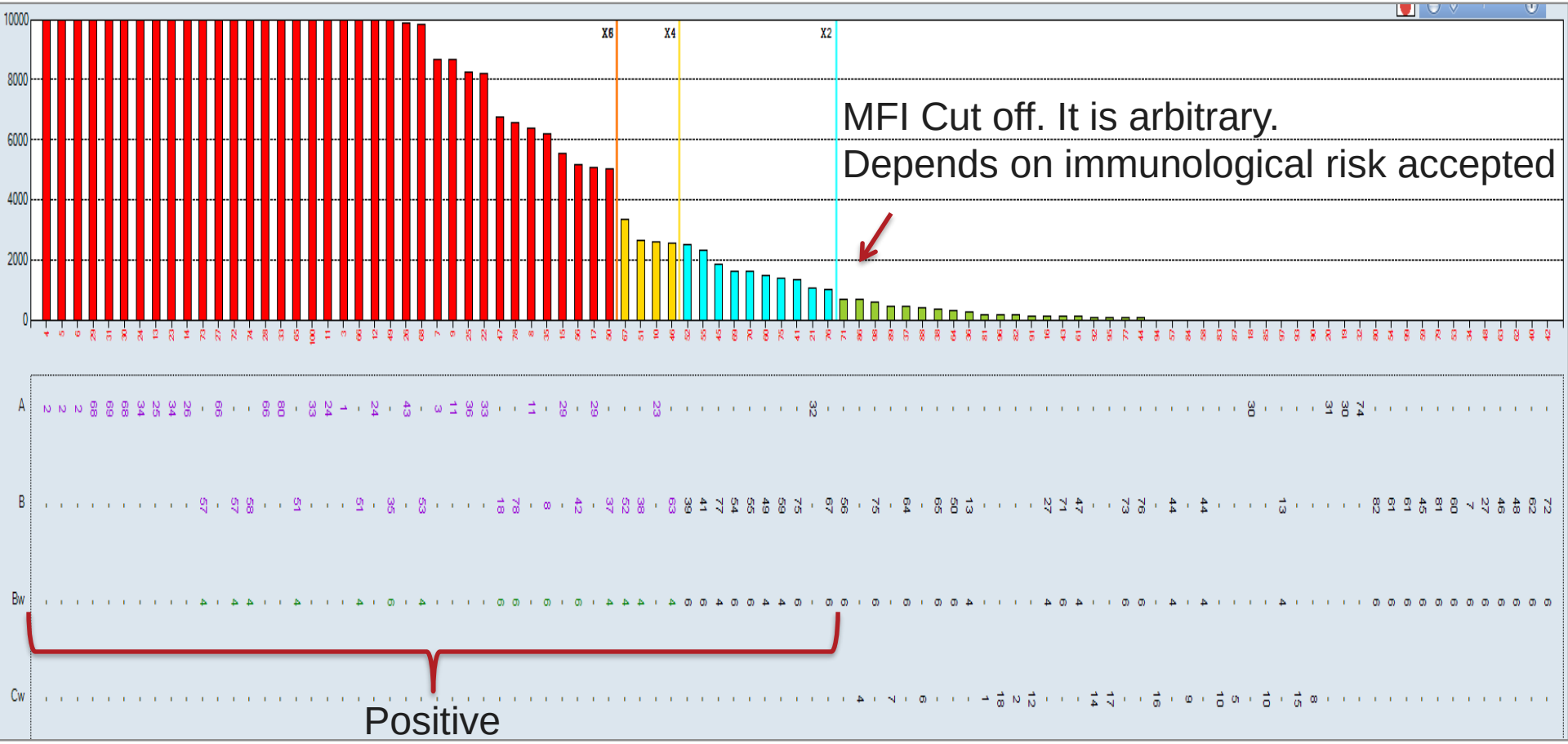


(or C3d)

Identification of HLA Complement binding antibodies

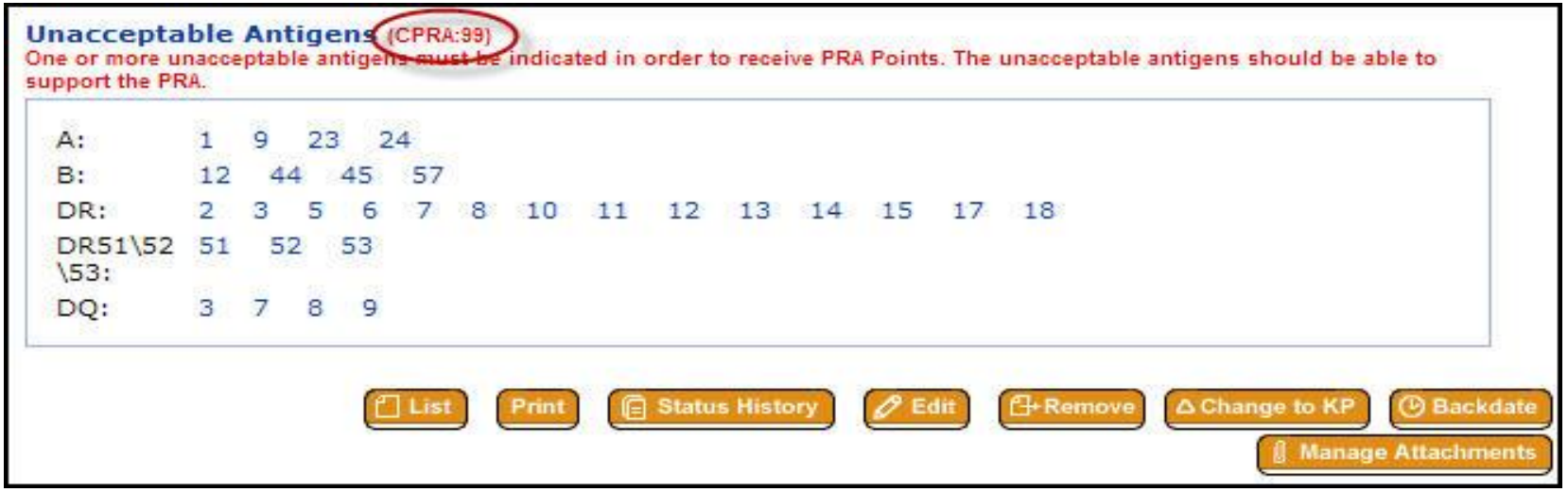
CCTC Clinical Chemistry Trainee Council

Luminex Technology



MFI: Mean fluorescence intensity. Semiquantitative measurement of the antibody bound to the beads.

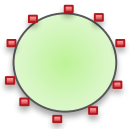
Calculated Panel of Reacting Antibodies (CPRA)



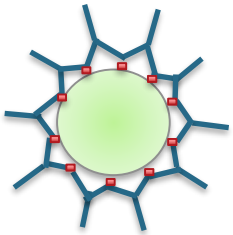
- cPRA is calculated based on the “frequency” of these unacceptable HLA Ag in the population. Therefore, **cPRA relates to the frequency of the Ag in the donor population, does not relation to the antibody titer!**

Crossmatches

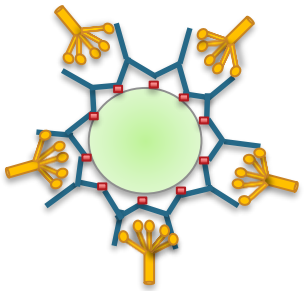
CDC



Donor Lymphocytes (T OR B) with HLA proteins



Patient serum with anti-HLA Abs

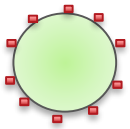


Rabbit Complement added

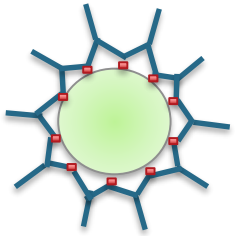
Activation Complement cascade. Cell death

Positive XM

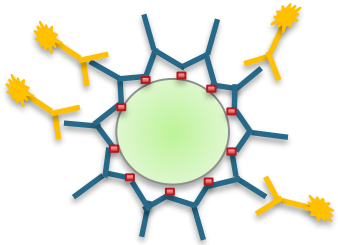
Flow Cytometry



Donor Lymphocytes (T AND B) with HLA proteins

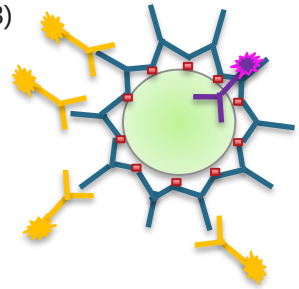


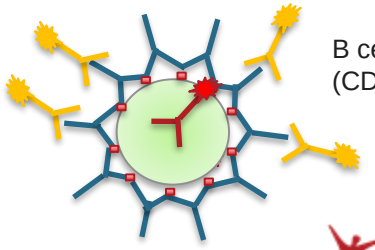
Patient serum with anti-HLA Abs



Anti-human IgG-fluorochrome

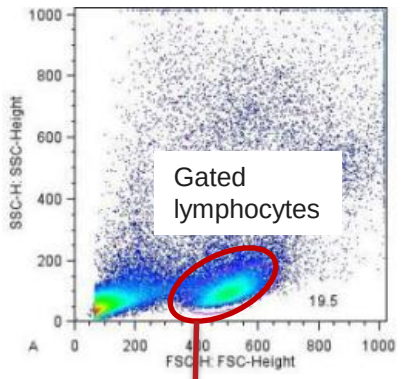
T cell Marker (CD3)



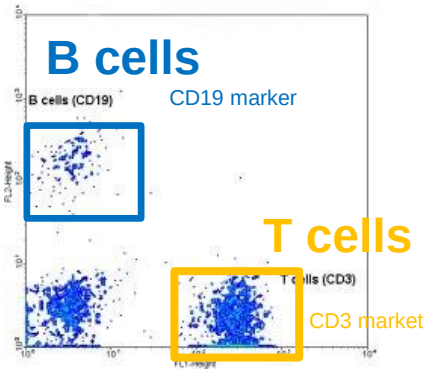


B cell Marker (CD19)

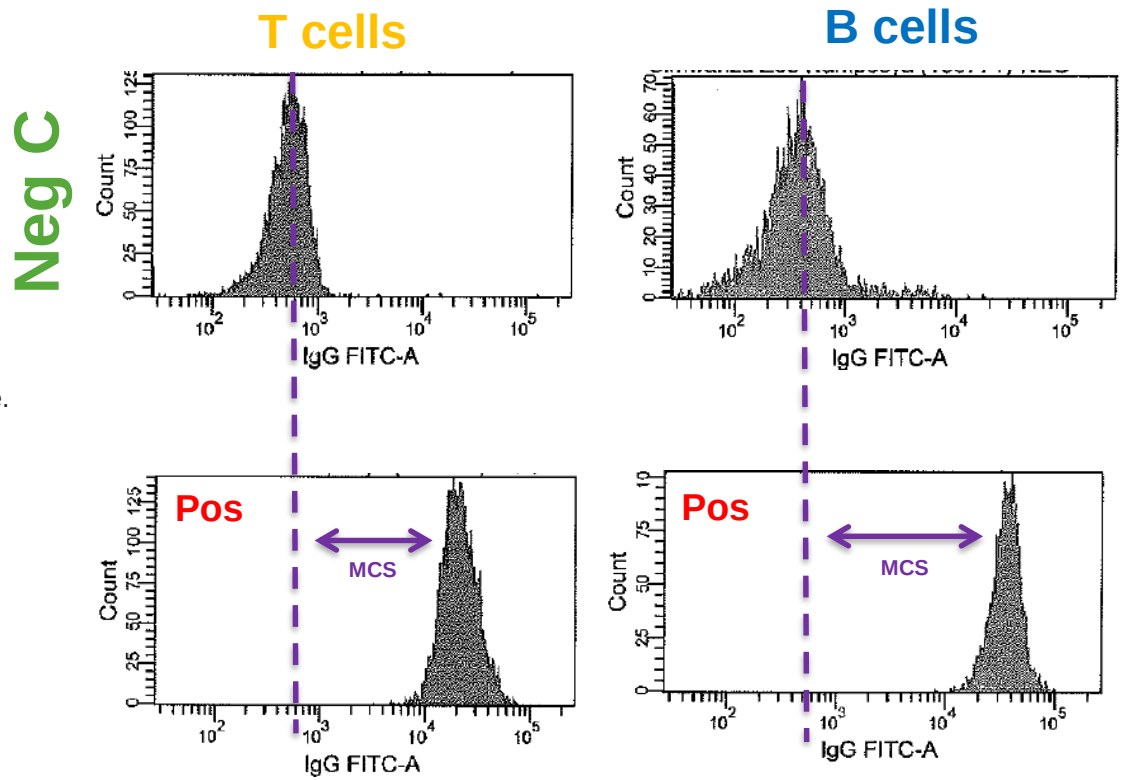
Flow Cytometric XM



T and B lymphocyte are separated by cell surface specific markers labeled with fluorochrome.



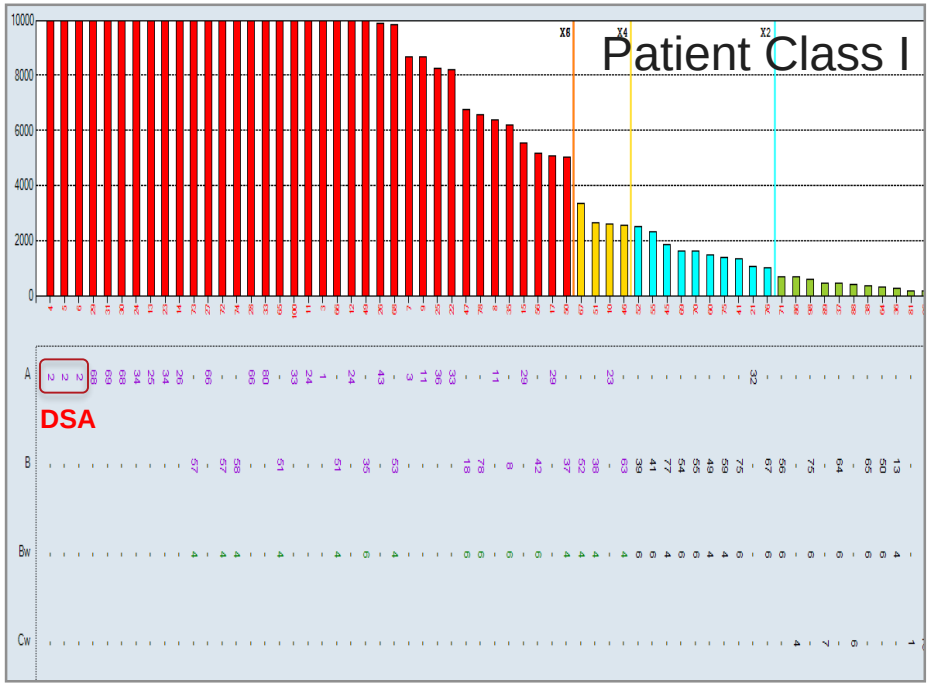
Patient's reactivity is measured in number of channels shifted over Negative control



MCS: Mean Chanel Shift

Virtual XM

- Prediction of the XM results based on donor HLA type and patient HLA ab profile



Donor: A2,3 B44,61 C9,16

- Patient has donor specific Abs (DSA) against HLA-A2.
- XM will be POSITIVE

Ellis TM. Hum Immunol 2010;73:706-710.

Summary

- HLA laboratory testing for solid organ transplantation involves accurate typing for patients and donors and precise patient's HLA antibody profile determination.
- For some organs a physical XM pre-transplant is required (kidney).
- Post transplant, monitoring of DSA-HLA antibodies is done by Luminex technology using single antigen kits.
- The goal of the HLA testing is to provide an immunologic risk for a given patient-donor pair based on HLA typing and HLA Ab profile.

References

1. Cruz-Tapias P, Castiblanco J, Anaya JM. Major Histocompatibility complex: Antigen processing and presentation. In: Anaya JM, Shoenfield Y, Rojas-Villarraga A, Levy RA, Cervera, editors. R. *Autoimmunity from Bench to bed side. 1st Ed. Bogota D.C. El Rosario University Press; 2013. p. 169-183.*
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7. Ellis TM, Schiller JJ, Roza AM, et al. Diagnostic accuracy of solid phase HLA antibody assays for prediction of crossmatch strength. Hum Immunol 2010;73:706-710.

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: No disclosures
- **Consultant or Advisory Role:** No disclosures
- **Stock Ownership:** No disclosures
- **Honoraria:** No disclosures
- **Research Funding:** No disclosures
- **Expert Testimony:** No disclosures
- **Patents:** No disclosures

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