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Neuberg DIAGNOSTICS | CENTER FOR GENOMIC MEDICINE

Transplant Immunology & Immunogenetics

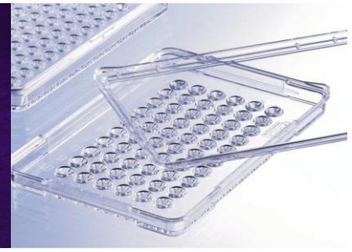
Lymphocyte Cross-Matching (LCM-CDC)

Type of Lymphocyte cross-matching

- Total Lymphocyte cross-matching
- T cell lymphocyte cross-matching-AHG*
- B cell lymphocyte cross-matching—AHG*
- Auto patient's cross-matching
- Auto donor cross-matching

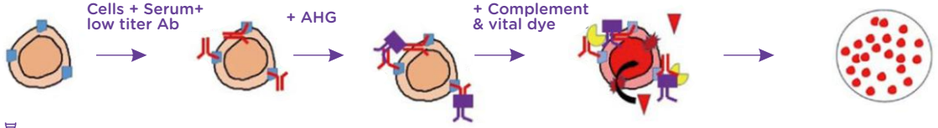
DTT treated serum cross-matching

*anti-human globulin (AHG) crossmatch"



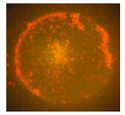
CDC (Complement-dependent cytotoxicity)Crossmatch

Lymphocyte Cross-matching CDC-AHG

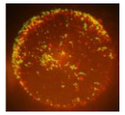


Phase contrast microscopy observation

Positive



Negative



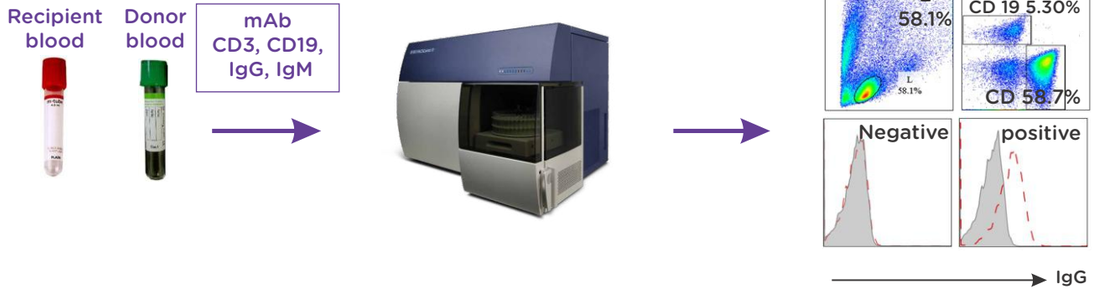
Cross Match Interpretation

Score	Interpretation	%Dead Celles
0	Not Readable	
1	Negative	0-10
2	Doubtful Negative	11-20

Score	Interpretation	%Dead Celles
4	Weak Positive	21-50
6	Positive	51-80
8	Strongly Positive	81-100

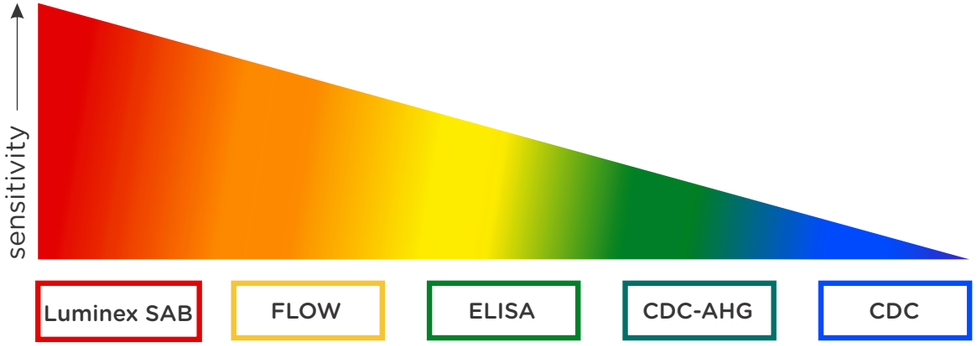
The complement-dependent microcytotoxicity assay ends with a complement incubation followed by the addition of a vital dye. In the wash technique, following the recipient serum-donor cell incubation, a wash step is added to remove nonspecifically bound antibodies and increase specificity. In the AHG technique, following the wash step, the cells are incubated with AHG. T-cells do not have significant immunoglobulin on their membranes; therefore, T-cells that have not bound recipient anti-bodies will not bind AHG but T-cells that have bound recipient antibodies will bind AHG. The bound AHG is more effective at binding complement than the bound recipient antibodies, thus increasing the sensitivity of the assay.

Evolution of HLA Antibody using Flow cytometry (FCM)



Value Indication

Range MCS (Median channel sift)	T cell-Flow Cross Match
<50	Negative flow cross-match
>50	Positive cross matching
Range MCS (Median channel sift)	**B cell-Flow Cross Match**
<150	Negative flow cross-match
>150	Positive cross matching



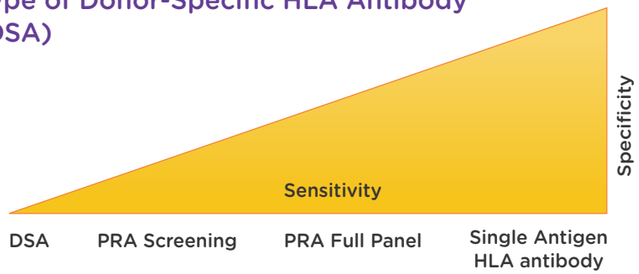
Panel Reactive Antibodies (PRA)

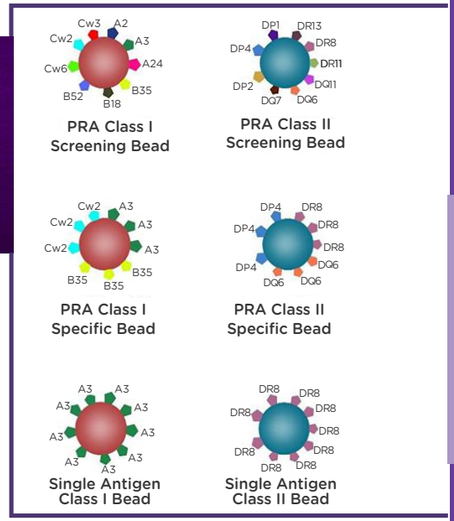
- A screening mechanism to determine the HLA antibody profile of potential transplant recipients.
- Periodic screening (monthly/quarterly) of recipient sera with a panel of HLA typed cells.
- Sensitization of the recipient is expressed as the percentage of serum reactivity with the total panel. Typically, high PRA is indicative of a highly sensitized recipient- one who is at risk for early graft loss
- Historically, PRA has been antigen-nonspecific

Type of Solid Phase Assay by Luminex

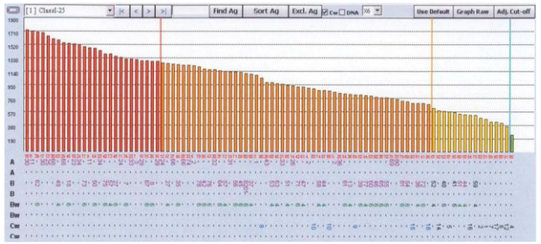
- > Virtual cross matching by Luminex
- Anti-HLA antibody screening By Luminex
- Panel reactive antigen HLA-Class I and HLA-Class II (PRA) By Luminex
- Single antigen panel for HLA-Class I and HLA-Class (SAP) By Luminex

Type of Donor-Specific HLA Antibody (DSA)





Classification of Antibodies using Single Antigen Reagents & MFI Values



- **HLA typing data output in different methods**
- **HLA-A2 Serology**
- **HLA-A*02 Allele group (SSP)**
  Main specificity/serological equivalent
- **HLA-A 02:01 Allelic subtypes: (Luminex)**
  Differences in 3rd and 4th digit - different protein
- **HLA-A 02:01:01 (Sequence Based typing-SBT)**
  Differences in 5th and 6th digit - different nucleotides, identical protein Silent variation
- **HLA-A*02:01:01:01 (Next Generation Sequencing-NGS)**
  Differences in 7th and 8th digit
  variation outside coding regions
  Intron variation, splice sites,
  promoter

NGS Workflow

NGS gives you genotyping results with minimal editing,no additional efforts for new sequences and >95% unambiguous results.

1. Statistics 2. Change of locus 3. Nucleotide position 4. Alleles
5. Jump to mismatch 6. Genomic position

Transplant Immunology & Immunogenetics Services

Name of The Test	TAT	Sample Requirement
HLA typing by Luminex A, B, C, DRB1, DQB1	3 days	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA - DRB 3,4,5	3 days	5 ml EDTA blood
HLA - G (NGS)	7 days	5 ml EDTA blood
HLA typing by Next generation sequencing based typing (High resolution) A, B, C, DRB1, DQB1 (DPB - if required)	5-7 days	8 ml EDTA blood (2 to 3 Purple Vacutainer)
Compliment dependent cross-matching (CDC cross-matching) Total Lymphocyte cross-matching T cell lymphocyte cross-matching- AHG* B cell lymphocyte cross-matching –AHG* Auto patient's cross-matching Auto donor cross-matching DTT treated serum cross-matching *anti-human globulin (AHG) crossmatch”	2 days	Recipient - 4 ml plane tube/ECD tube serum sample (Red top or yellow top) Donor – 18 ml (4 to 5 Heparin green vacutainer)
Flow cytometery cross-matching: T cell lymphocyte B cell lymphocyte	2 days	Donor – 18 ml (4 to 5 Heparin green vacutainer) Recipient - 4 ml plane tube (red top)
Virtual cross matching by Luminex **Donor Specific Antibodies (DSA)**	3 days	Donor – 18 ml (4 to 5 Heparin green vacutainer) Recipient - 4 ml plane tube (red top)
HLA-Class I and HLA-Class II Antibody screening (By Luminex):	3 days	Recipient- 4 ml plane tube (red top)
Panel reactive antigen HLA-Class I and HLA-Class II(PRA) (By Luminex):	3 days	Recipient- 4 ml plane tube (red top)
Single antigen panel for HLA-Class I and HLA-Class (SAP) (By Luminex):	3 days	Recipient- 4 ml plane tube (red top)
Single antigen panel for MICA Antibody (By Luminex):	3 days	Recipient- 4 ml plane tube (red top)
Chimerism analysis (Pre and Post stem cell transplant)	5 days	8 ml EDTA blood (2 to 3 Purple Vacutainer)
DNA profiling for patient and donor relationship establishment (STR Analysis)	5 days	8 ml EDTA blood (2 to 3 Purple Vacutainer)

Disease Association

Test Description	Specimen Requirements
HLA-A 2901/2902 for birdshot retinopathy	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA-B*27 for ankylosing spondylitis	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA-B*51 for Behcet's disease	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA-B*5701 for abacavir sensitivity	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA-B*5801 for allopurinol induced Stevens-Johnson syndrome risk	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA-DQA 05/DQB1*02, DQA*03/DQB1*0302 for Celiac disease risk	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA-DQB1*0602 for narcolepsy	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA-DRB1*1501/1502 for anti-glomerular basement membrane disease	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA*15:02 (Carbamazepine)	8 ml EDTA blood (2 to 3 Purple Vacutainer)

HLA Typing - Customized

Test Description	Specimen Requirements
Molecular Typing-Single Locus (specify) Locus: _____ Resolution _____	8 ml EDTA blood (2 to 3 Purple Vacutainer)

* All biological samples to be stored for further investigations/diagnosis/research.

Your PARTNERS in HEALTH

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