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Transplant Immunology & Immunogenetics

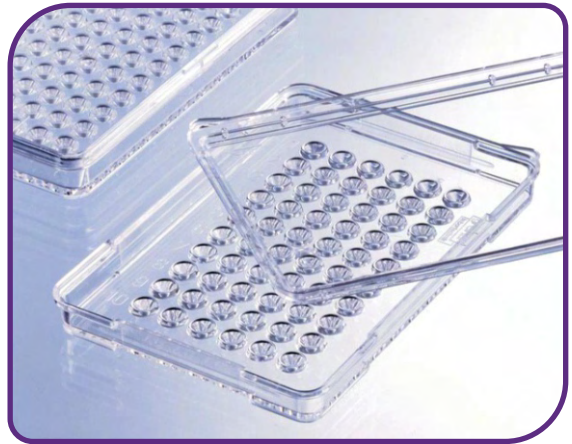
Serial number : 005 Edition : 1. 2022

Lymphocyte Cross-Matching (LCM-CDC)

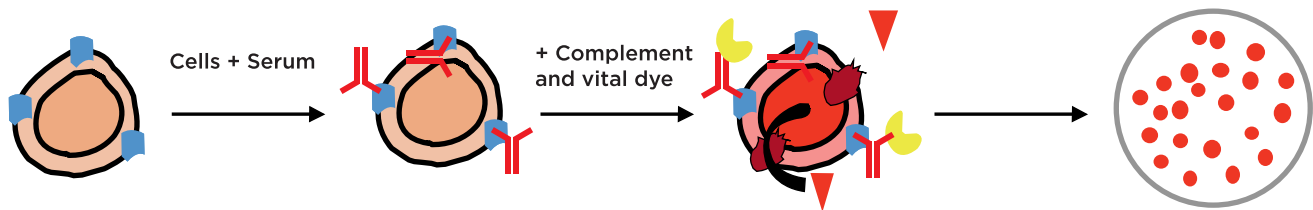
Type Of Lymphocyte cross-matching

- ▶ Total Lymphocyte cross- matching
- ▶ T-cell lymphocyte cross-matching
- ▶ B-cell lymphocyte cross-matching
- ▶ Total Lymphocyte AHG* cross- matching
- ▶ T-cell cross-matching - AHG*
- ▶ B-cell cross-matching - AHG*
- ▶ DTT treated serum cross- matching
- ▶ Auto patient's cross- matching
- ▶ Auto donor cross-matching

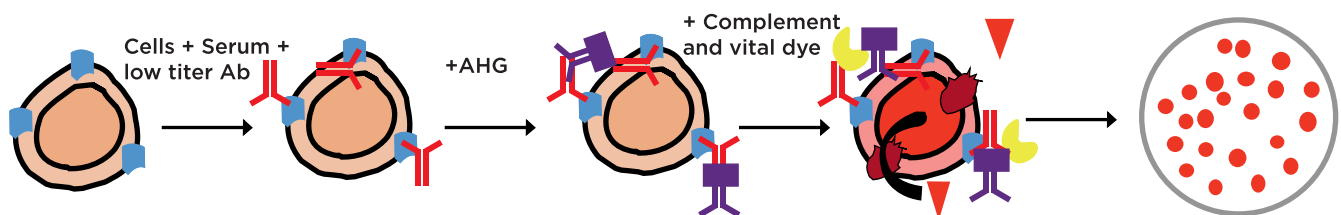
*Anti human globulin



Lymphocyte Cross-matching LCM-CDC

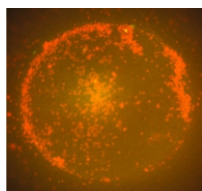


Lymphocyte Cross-matching CDC-AHG

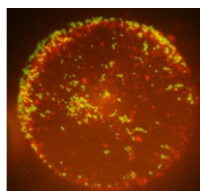


Observation : Phase contrast microscopy

Positive



Negative

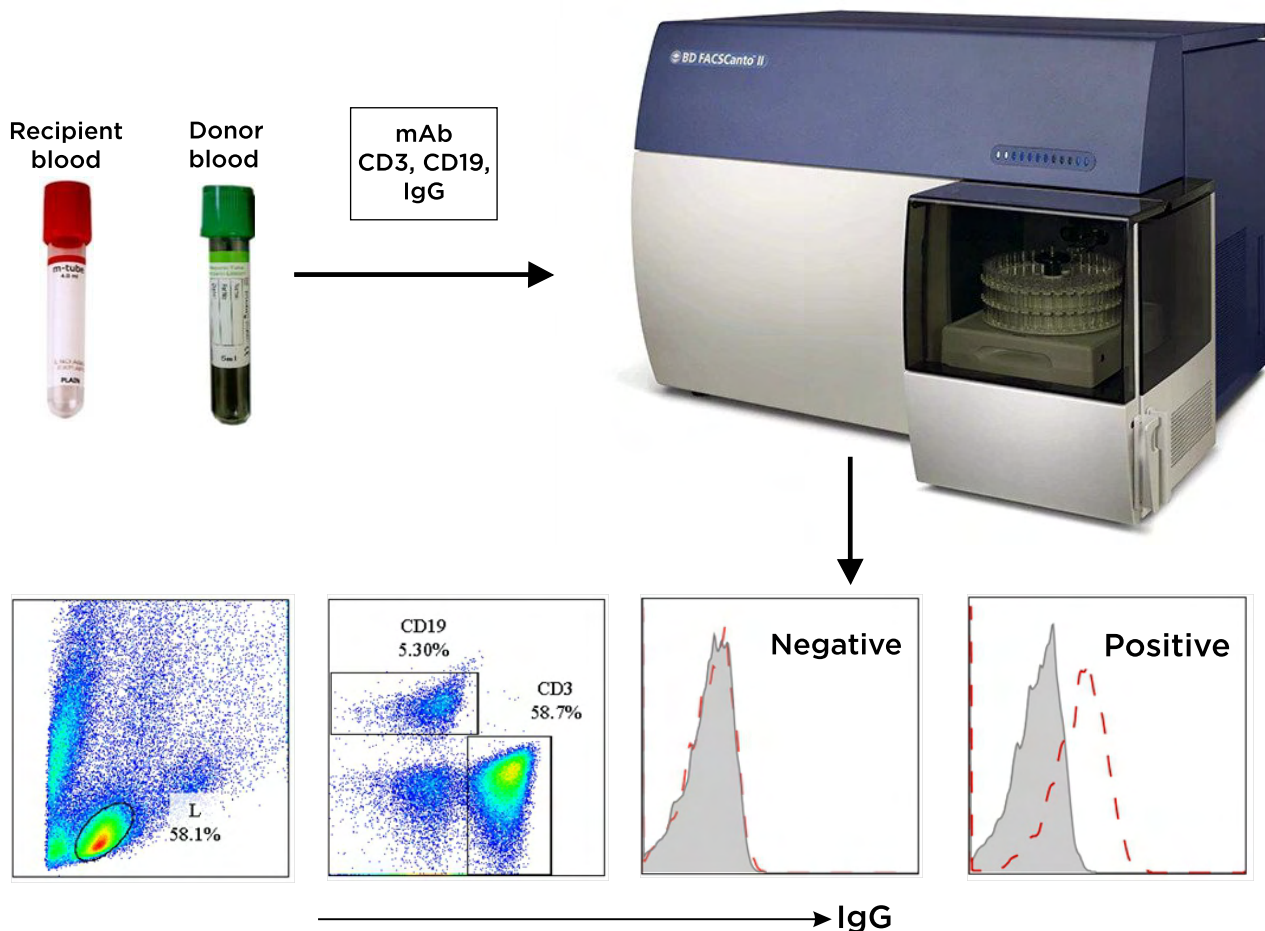


LCM-CDC Cross Match Interpretation

Score	%Dead Cells	Interpretation
0		Not Readable
1	0-10	Negative
2	11-20	Doubtful Negative
6	21-50	Weak positive
6	51-80	Positive
8	81-100	Strongly Positive

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.

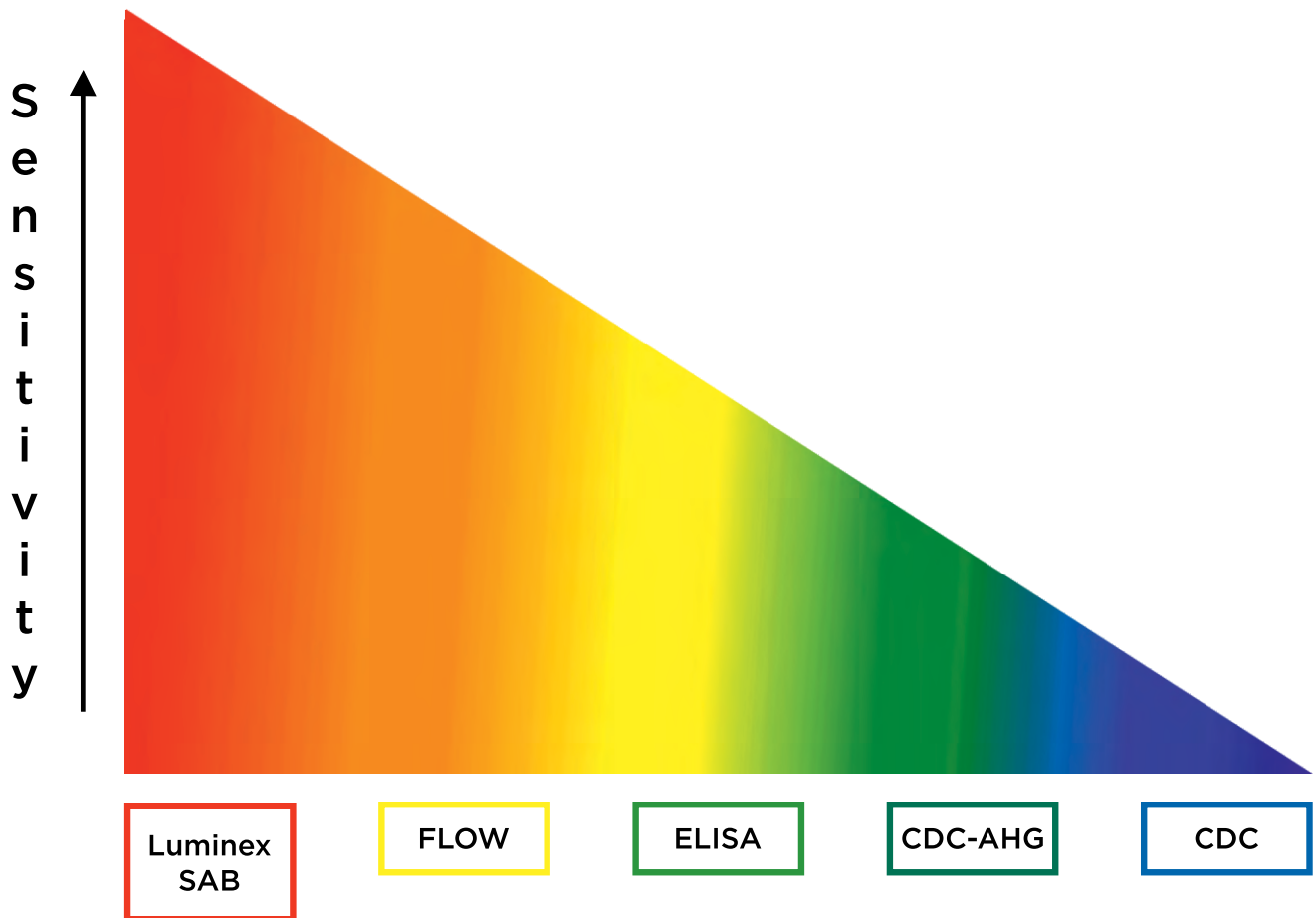
Flow cytometry cross match (FCM/FCXM)



Value Indication

Range MCS (Median Channel Shift)
<50
>50
Range (Median Channel shift)
<150
>150

T cell- Flow Cross Match
Negative
Positive
B cell-Flow Cross Match
Negative
Positive

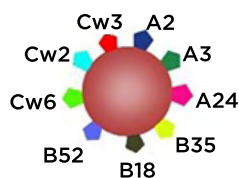


Panel Reactive Antibodies (PRA)

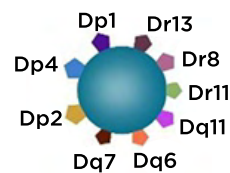
- ▶ A screening mechanism to determine the HLA antibody profile of potential transplant recipients.
- ▶ Periodic screening (monthly/quarterly) of recipient sera with a panel of HLA antigens
- ▶ Sensitization of the recipient is expressed as the percentage of serum reactivity with the total panel of antigens.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

Types of Solid Phase Assay by Luminex

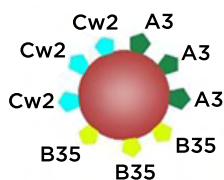
- ▶ Donor specific antibody (DSA) by Luminex
- ▶ Virtual cross matching by Luminex
- ▶ Anti-HLA antibody screening by Luminex
- ▶ Panel reactive antigen (PRA) HLA-Class I and HLA-Class II by Luminex
- ▶ Single bead assay (SAB) for HLA-Class I and HLA-Class by Luminex



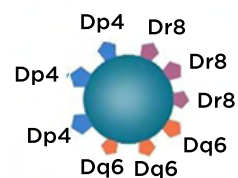
PRA Class I Screening Bead



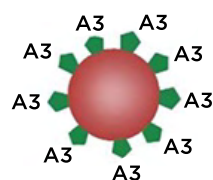
PRA Class II Screening Bead



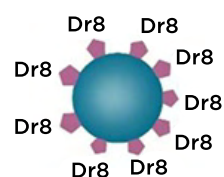
PRA Class I specific bead



PRA Class II specific bead

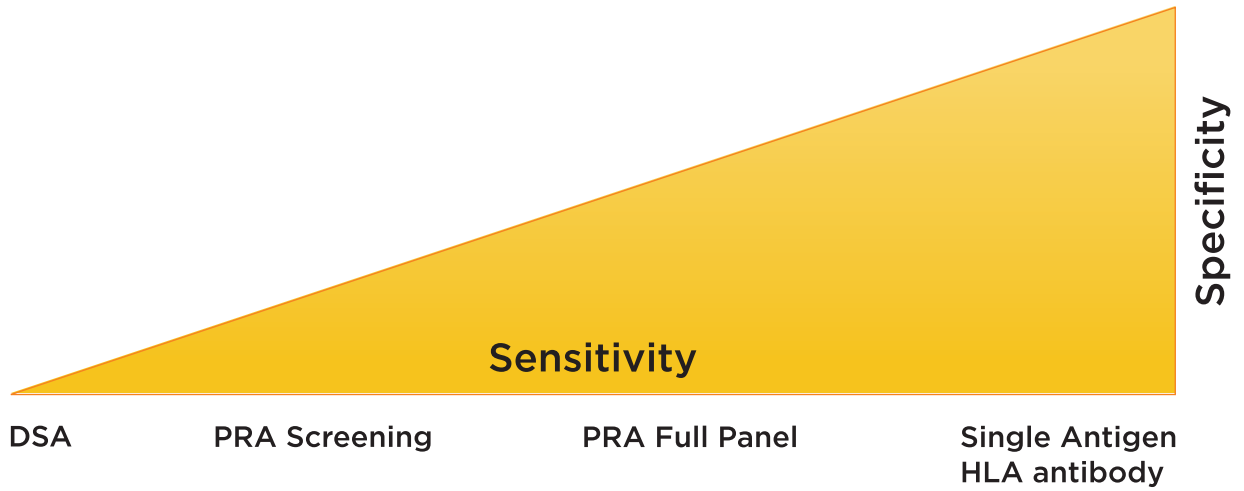


Single antigen class I bead

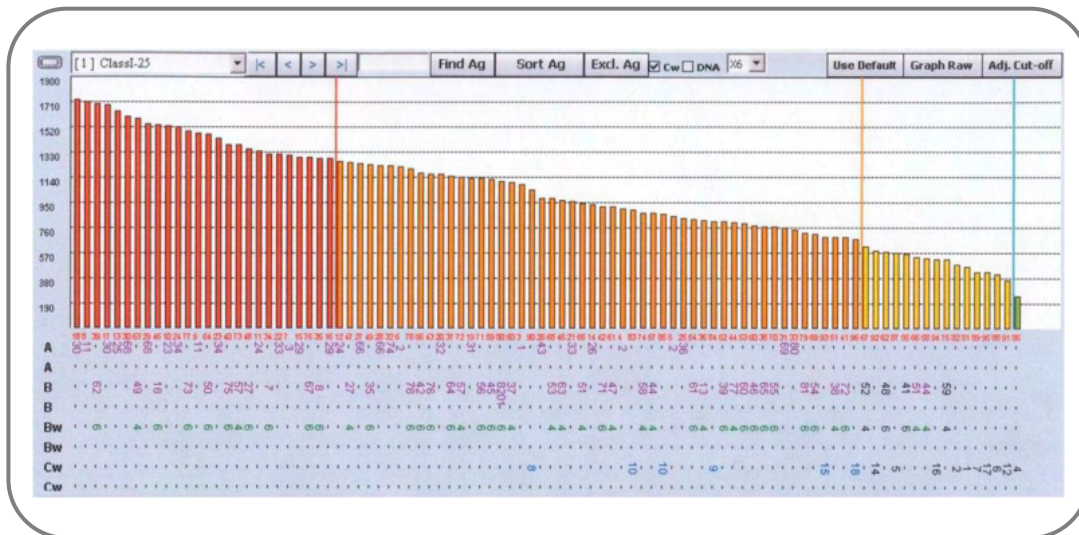


Single antigen class II bead

Type Of Donor-Specific HLA Antibody (DSA)

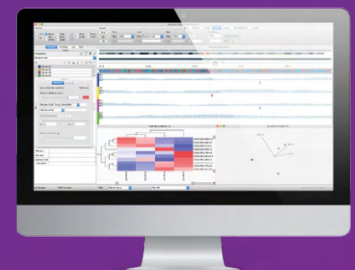
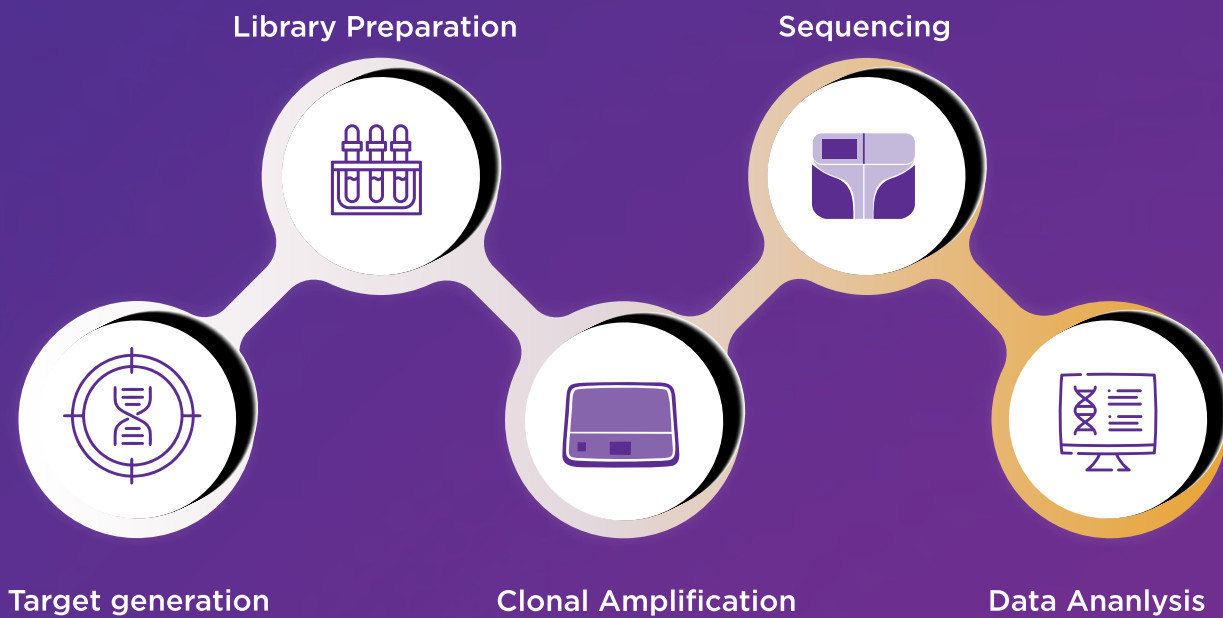


Classification of Antibodies using Single Antigen Reagents & MFI Values



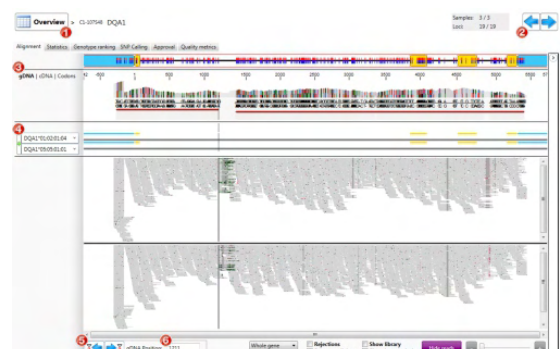
- ▶ HLA typing data output in different methods
- ▶ HLA-A2 Serology
- ▶ HLA-A*2 Allele group (SSP)
Main specificity/serological equivalent
- ▶ HLA-A 02:01 Allelic subtypes:(Luminex)
Differences in 3rd and 4th digit different protein
- ▶ HLA-A 02:01:01 (Sequence Based typing-SBT)
Difference in 5th and 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
4. Alleles
5. Jump of mismatch
6. Genomic position



Transplant Immunology & Immunogenetics Services

Name of The Test	TAT	Sample Requirement
HLA test package for Cadaver recipient (HLA typing, PRA-I & II, SAB-I & II)	5 - 7 days	Recipient blood 8 ml in EDTA vial 4-6 ml in plain vial (red top vial)
HLA typing (High resolution) by NGS (HLA - A, B, C, DRB1, DQB1)	5 - 7 days	8 ml EDTA blood (2 to 3 Purple Vacutainer)
HLA typing (low resolution) by Luminex (HLA - A,B, HLA- 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
Total Lymphocyte cross- matching T-cell lymphocyte cross-matching B-cell lymphocyte cross-matching Total Lymphocyte AHG* cross- matching T cell cross-matching - AHG* B cell cross-matching - AHG* DTT treated serum cross- matching Auto patient's cross- matching Auto donor cross-matching	2 days	Recipient - 4 ml plane tube/ECD tube serum sample (Red top or yellow top) Donor - 10 ml (4 to 5 Heparin green vacutainer)
Flow cytometry cross-matching: (FCM/FCXM) T-cell lymphocyte B-cell lymphocyte	2 days	Recipient - 4 ml plane tube (red top) Donor - 10 ml (4 to 5 Heparin green vacutainer)
Virtual cross matching by Luminex (Donor HLA typing & recipient SAB)	7 days	Recipient - 4 ml plane tube (red top) Donor - 8 ml EDTA blood
Donor Specific Antibodies	3 days	Recipient - 4 ml plane tube (red top) Donor - 10 ml (4 to 5 Heparin green vacutainer)
HLA-Class I and HLA-Class II Antibody screening (By Luminex):	3 days	Recipient - 4 ml plane tube (red top)
Panel reactive antigen (PRA) HLA-Class I and HLA-Class II (By Luminex):	3 days	Recipient - 4 ml plane tube (red top)
Single antigen Bead assay (SAB) for HLA-Class I and HLA-Class (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)

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

Our Services



Inherited Genetic Disorder



Reproductive Genetics



Cancer Genomics



Haemato Oncology



Transplant Immunology



Infectious Disorders



Pharmacogenomics



Research Services

PARTNERS IN HEALTH



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