Neuberg DIAGNOSTICS

● India ● UAE ● South Africa ● USA

# New INSIGHTS

# Transplant

## Immunology & Immunogenetics

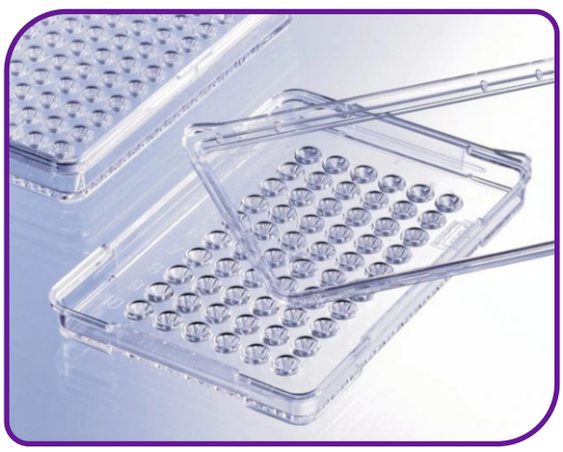
Serial number : 005 Edition : 1. 2022

# Lymphocyte Cross-Matching (LCM-CDC)

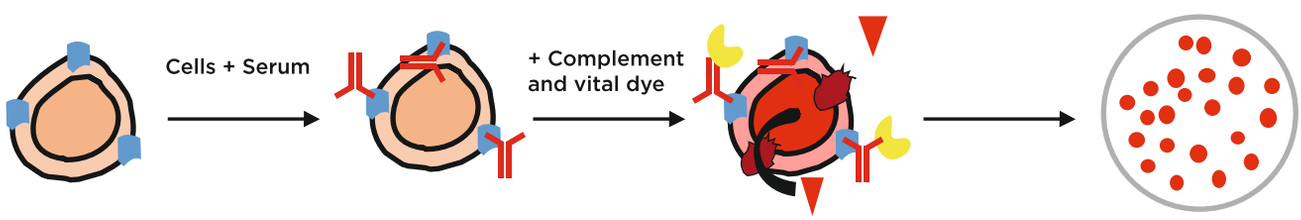
## Type Of Lymphocyte cross-matching

- 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

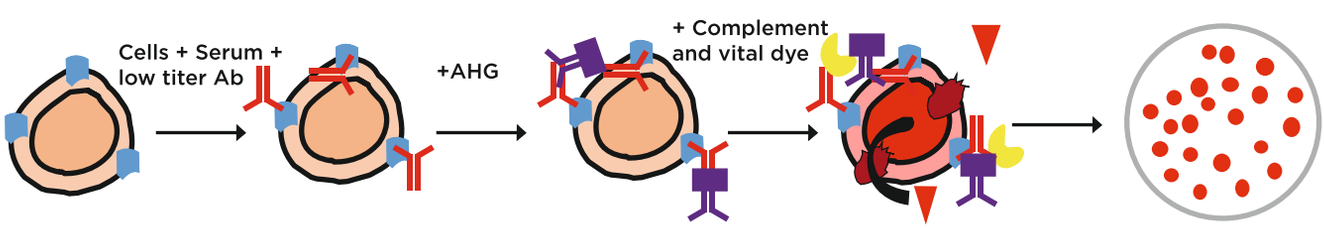
*Anti human globulin



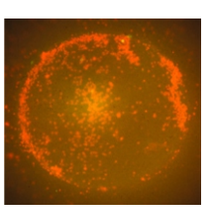
### Lymphocyte Cross-matching LCM-CDC

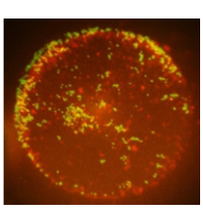



### Lymphocyte Cross-matching CDC-AHG




### Observation : Phase contrast microscopy

Positive 

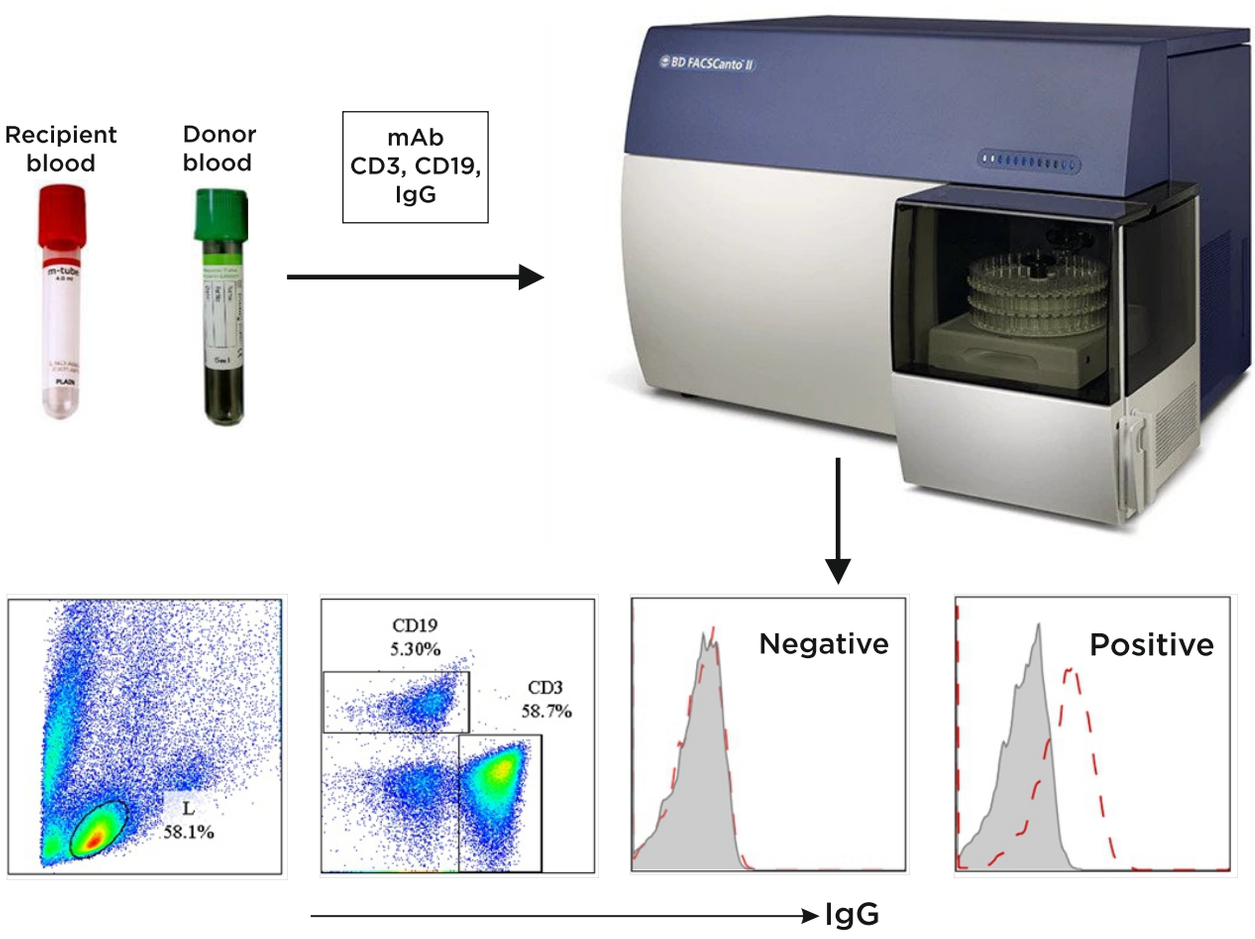
Negative 

## 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 |

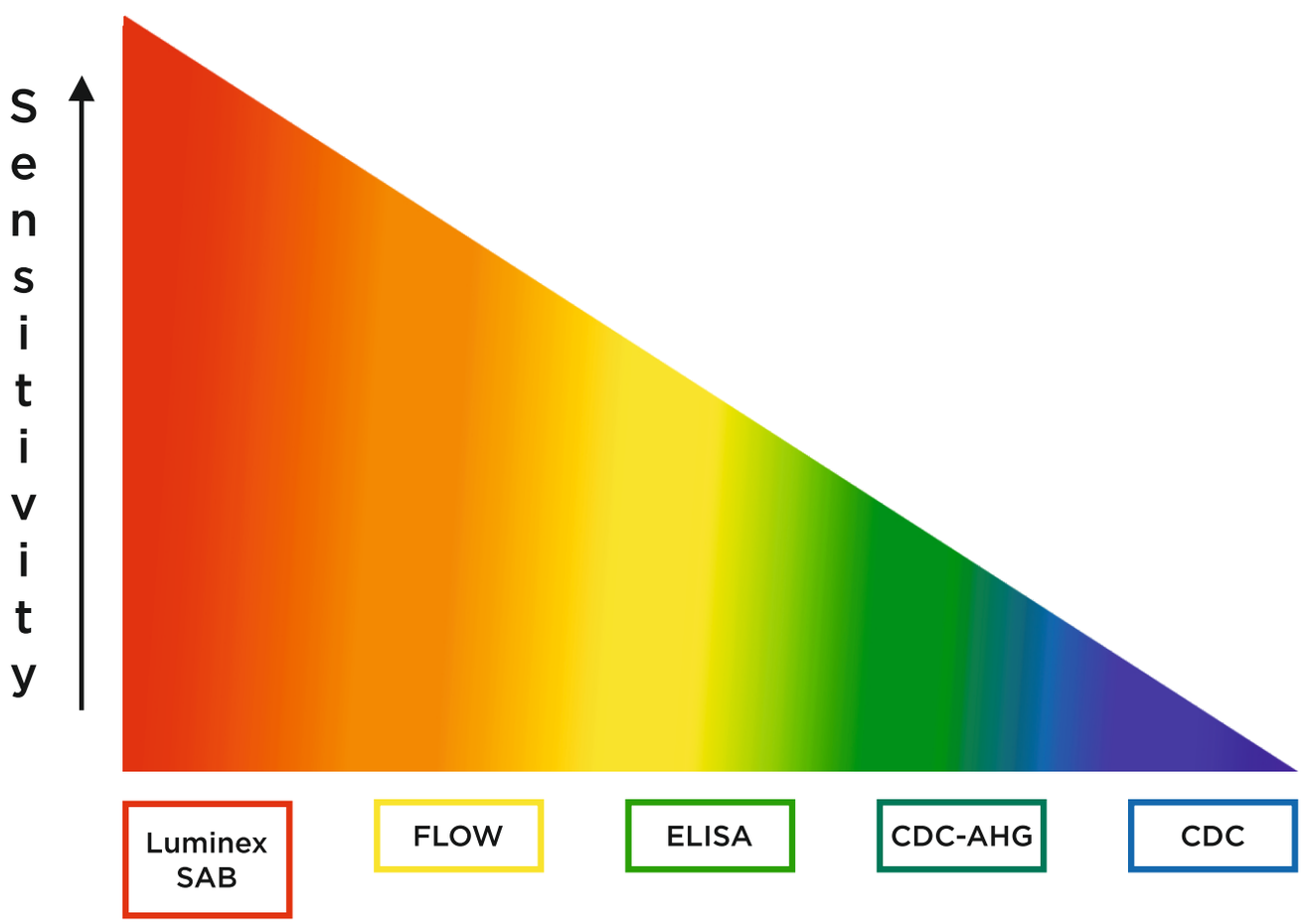
The complement-dependent microcytotoxicity assay ends with a complement incubation followed by the addition of a vital dye. In the wash technique, following the recipient serum- donor cell incubation, a wash step is added to remove nonspecifically bound antibodies and increase specificity. In the AHG technique, following the wash step, the cells are incubated with AHG. T-cells do not have significant immunoglobulin on their membranes; therefore, T-ceels that have not bound receipient anti-bodiees 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) | T cell- Flow Cross Match |
|---|---|
| <50 | Negative |
| >50 | Positive |
| **Range (Median Channel shift)** | **B cell-Flow Cross Match** |
| <150 | Negative |
| >150 | Positive |

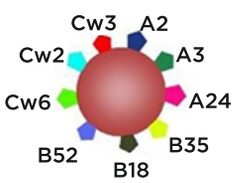


# Panel Reactive Antibodies (PRA)

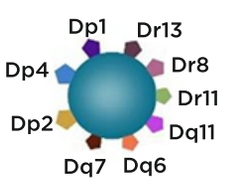
- A screening mechanism to determine the HLA antibody profile of potential transplant recipients.
- Periodic screening (monthly/quarterly) of recipient sera with a panel of HLA antigens
- Sensitization of the recipient is expressed as the percentage of serum reactivity with the total panel of antigens. 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

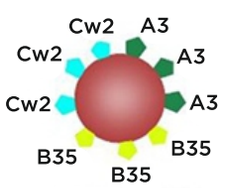
- Donor specific antibody (DSA) by Luminex
- Virtual cross matching by Luminex
- Anti-HLA antibody screening by Luminex
- Panel reactive antigen (PRA) HLA-Class I and HLA-Class II by Luminex
- Single bead assay (SAB) for HLA-Class I and HLA-Class by Luminex



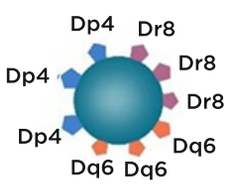

**PRA Class I Screening Bead**



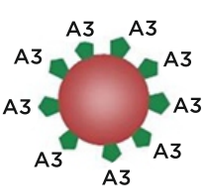

**PRA Class II Screening Bead**



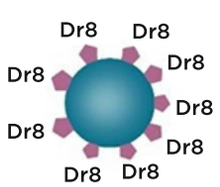

**PRA Class I specific bead**




**PRA Class II specific bead**

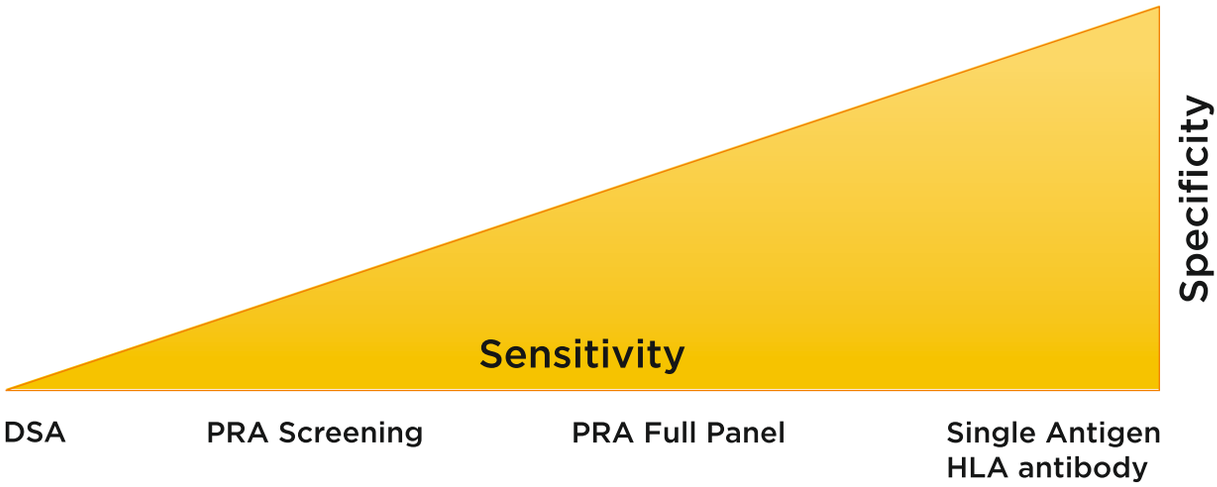



**Single antigen class I bead**

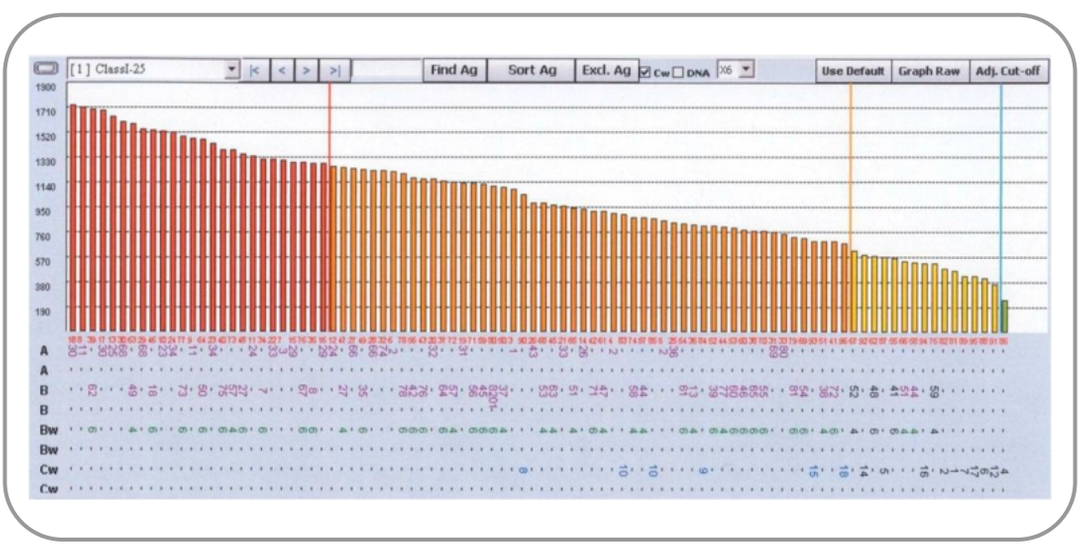



**Single antigen class II bead**

# Type Of Donor-Specific HLA Antibody (DSA)

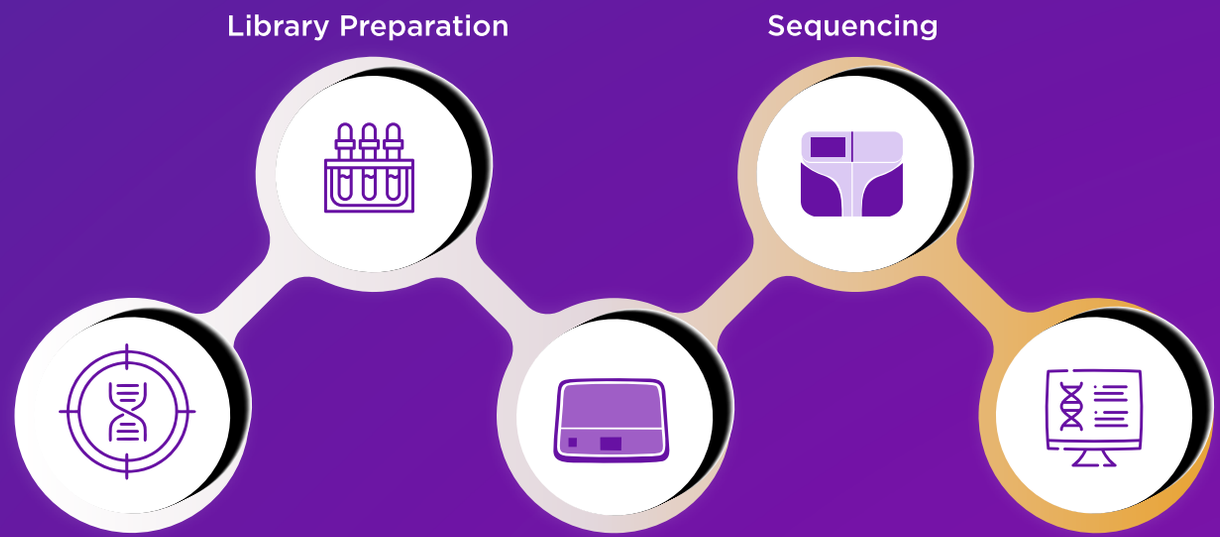


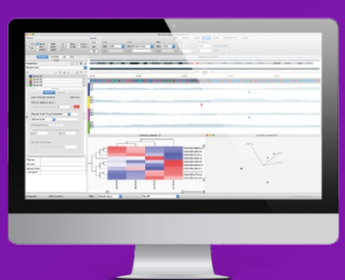

# Classification of Antibodies using Single Antigen Reagents & MFI Values




- 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<br>8 ml in EDTA vial<br>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<br>(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<br>(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<br>T-cell lymphocyte cross-matching<br>B-cell lymphocyte cross-matching<br>Total Lymphocyte AHG* cross- matching<br>T cell cross-matching - AHG*<br>B cell cross-matching - AHG*<br>DTT treated serum cross- matching<br>Auto patient's cross- matching<br>Auto donor cross-matching | 2 days | Recipient - 4 ml plane tube/ECD tube serum sample (Red top or yellow top)<br>Donor - 10 ml (4 to 5 Heparin green vacutainer ) |
| Flow cytometery cross-matching: (FCM/FCXM)<br>T-cell lymphocyte<br>B-cell lymphocyte | 2 days | Recipient - 4 ml plane tube (red top)<br>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)<br>Donor - 8 ml EDTA blood |
| Donor Specific Antibodies | 3 days | Recipient - 4 ml plane tube (red top)<br>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<br>(2 to 3 Purple Vacutainer) |
| DNA profiling for patient and donor relationship establisment (STR Analysis) | 5 days | 8 ml EDTA blood<br>(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

# Notes :

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