

# HAEMATOGENETICS

Investigations on unexplained Hemolytic Anemia and Neonatal Jaundice cases for other red cell genetic abnormalities.

Eighty-four cases of unexplained hemolytic anemia and neonatal jaundice were referred for evaluating the cause of hemolysis with special reference to red cell enzyme defects, RBC membrane abnormalities or unstable hemoglobins.

The following abnormalities were detected

1. Pyruvate kinase deficiency (PK) -----	2
2. RBC Membrane Disorders ----- (hereditary spherocytosis hereditary elliptocytosis)	11
3. Unstable Hemoglobins-----	4
4. PNH -----	0
5. Pyrimidine 5 Nucleotidase -----	0
6. Phosphofructo kinase (PFK), -----	0
7. Phosphoglycerate kinase (PGK), -----	0
<b>8. Glucose phosphate isomerase (GPI) -----</b>	
<b>2</b>	
9. Hexokinase (HK) -----	0
10. Aldolase -----	0
11. 6-Phosphogluconic Dehydrogenase (6-PGD), -----	0
12. Glyceraldehydes-3-Phosphate Dehydrogenase (GA3PD)	0

### Investigations of cases with cyanosis.

Five cases were referred with a history of cyanosis, bluish decolorization of the nail beds, lips and tongue for methemoglobin estimation. Three of them had increased methemoglobin levels varying from 11.13% to 17.35% and one case showed a deficiency of NADH-Methemoglobin Reductase in which cyanosis was the only problem.

### Screening for G6PD deficiency

One thousand two hundred and eighty four cases (860 males and 425 females) were referred from various hospitals for screening of G6PD deficiency. They included patients with non-spherocytic hemolytic anemia, neonatal jaundice; drug induced

hemolysis as well as individuals requiring to start anti-malarial treatment or Dapsone therapy. Screening was done using the DPIP dye decolourization test and female samples were confirmed by enzyme assay spectrophotometrically. Fifteen males and twelve females carriers showed intermediate enzyme activity.

## Identification of Thalassemias and other Hemoglobinopathies

The total number of cases screened for thalassemia and other hemoglobinopathies were 1893 which included referred patients as well as couples and families coming for screening and prenatal diagnosis.

### Hb abnormalities detected were as follows:

$\beta$ Thalassemia trait	- 602
$\beta$ Thalassemia homozygous	- 216
Hb S trait	- 169
Hb S homozygous	- 78
Hb S $\beta$ Thalassemia	- 60
Hb S – D disease	- 1
Hb E trait	- 12
Hb E homozygous	- 7
Hb E $\beta$ Thalassemia	- 2
Hb D trait	- 5
Hb Q – Thalassemia	- 1
$\beta$ Thal intermedia	- 4
↑ Hb F	- 33
Uncharacterized	- 2

### Molecular Characterization of $\beta$ Thalassemia and first trimester prenatal diagnosis

#### Characterization of mutations in $\beta$ Thalassemia and other abnormal hemoglobins:

The heterozygotes identified during population screening, family studies and prenatal diagnosis were taken for mutations analysis. These samples included individuals from different ethnic groups and different regions in the country. Mutation analysis was undertaken using a combination of methods like reverse dot blot hybridization ARMS and GAP PCR.

The following mutations were detected in 405 heterozygotes.

IVS 1-5 (G→C)	- 209
619 bp del	- 25
Codons 8/9 (+G)	- 20
IVS 1 – 1 (G→T)	- 11
Codons 41/42 (-CTTT)	- 17
Codons 15(G→A)	- 46
Codon 30(G→C)	- 13
Cap site +1 (A→C)	- 8
25 bp del	- 2
CD 5 (-CT)	- 5
IVS II – 837 (T→G)	- 1
Codons 16 (-C)	- 6
Hb S	- 40
Hb E	- 2

The mutations characterized in 109 homozygotes or double heterozygotes were as follows :

IVS 1- 5 (G→C) homozygous	- 51
Codons 41/42 (-CTTT) homozygous	- 3
Codon 30 (G→C) homozygous	- 3
IVS 1 – 1 (G→T) homozygous	- 2
Codon 5 (-CT) homozygous	- 1
IVS 1-5(G→C) + Hb S	- 8
IVS 1-5 (G→C) + Codon 8/9(+G)	- 1
IVS 1-5 (G→C) + Codon 15(G→A)	- 2
IVS 1-5 (G→C) + Codon 30(G→C)	- 1
IVS 1-5 (G→C) + Codon 41/42 (-CTTT)	- 1
IVS 1-5 (G→C) + Codon 16(-C)	- 1
IVS 1-5 (G→C) + Codon 5 (-CT)	- 1
IVS 1-5 (G→C) + IVS II – 837 (T→G)	- 1
IVS 1-5 (G→C) + Hb E	- 2
Codons 8/9 (+G) + 619 bp del	- 4
Codons 41/42 (-CTTT) + 619 bpdel	- 1
Codon 5 (-CT) + 619 bp del	- 2
IVS 1-1 (G→T) 619 bp del	- 4
Codon 15 (G→A) homozygous	- 3

Codons 8/9 (+G) + Capsite +1 (A→C)	- 1
Codons 8/9 (+G) + Codon 30 (G→C)	- 1
Codon 15(G→A) + Hb S	- 3
619 bp del + Hb Q	- 1
Hb S homozygous	- 8
Hb E Homozygous	- 2
δβ thal Indian deletion homozygous	- 1

### First trimester prenatal diagnosis of thalassemia syndromes.

One hundred and nineteen couples were referred for first trimester prenatal diagnosis of thalassemia from different parts of the country. Among them, 100 were at risk of having a child with β Thalassemia major, 14 with sickle cell anemia, 3 with Hb S thalassemia and 2 with Hb E thalassemia.

CVS was done between 9.5 to 14.0 weeks gestation. CRDB and ARMS were used to detect the mutations. Diagnosis was possible in 115 couples. 4 couples were called for cordocentesis in the second trimester because of uncharacterized mutations in the parents. Twenty four fetuses were found to be affected ( 17 with β Thalassemia major, 6 with sickle cell disease and 1 with Hb E Thalassemia). Counselling was given to these couples and they were given the option to terminate the pregnancies.

### The mutations in the affected fetuses were as follows:

IVS 1-5 (G→C) homozygous	- 11
Codon 41/42 (-CTTT) + Codon 15 (G→A)	- 1
Codon 5 (-CT) homozygous	- 1
Codon 30 (G→C) homozygous	- 1
IVS 1 – 1 (G→T) + 619 bp del	- 1
Codons 8/9 + 619 bp del	- 1
IVS 1 – 5 (G → C) + FS 16 (- c)	- 1
IVS 1 – 5 (G →C) + Hb E	- 1
Hb S homozygous	- 6

### Second Trimester Prenatal Diagnosis of Hemoglobinopathies.

Cordocentesis and fetal blood analysis was done in 24 couples who came late for prenatal diagnosis. (18 to 22 weeks) or where both the parental mutations could not be identified. Among these couples, 21 were

at risk for  $\beta$  Thal major, 3 for Hb S – thalassemia. Only one fetus was affected ( $\beta$  Thal major) and the couple was given the option to terminate the pregnancy.

## HAEMOSTASIS

Thrombophilia workup in cases of deep vein thrombosis

**In the current year we have investigated 373 cases with thrombosis for the 4 common thrombophilia markers – Protein C, Protein S, Antithrombin III and factor V Leiden mutation. They are clinically classified based on the sites of thrombosis as follows**

<b>Superior sagittal sinus thrombosis</b>	<b>- 61</b>
<b>Portal vein thrombosis</b>	<b>- 52</b>
<b>Buddchiari syndrome</b>	<b>- 26</b>
<b>Pulmonary embolism</b>	<b>- 20</b>
<b>DVT of lower and upper limbs</b>	<b>- 65</b>
<b>Bad obstetric history (bolt)</b>	<b>- 36</b>
<b>Others</b>	<b>- 113</b>
	-----
	<b>373</b>
	-----

Results are tabulated as follows:

Parameters Studied	Number deficient/abnormal	Percentage
<b>Protein C</b>	<b>29</b>	<b>7.8</b>
<b>Protein S</b>	<b>33</b>	<b>8.8</b>
<b>Antithrombin III</b>	<b>7</b>	<b>1.9</b>
<b>Factor V Leiden</b>	<b>11</b>	<b>2.9</b>
<b>Total</b>	<b>80</b>	<b>21.4</b>

**Approximately one fifth of the patients with thrombosis could be explained by any one of these genetic risk factors.**

1. Routine Coagulation factor assays

**In the current year a total of 803 patients were referred for diagnosis of an underlying bleeding disorder. Following were the various assays performed as and when required to offer a final diagnosis.**

- 1) Screening coagulation assays (PT, APTT, TT, Screening for inhibitors)**
- 2) Factor assays ( Factor I, II, V, VII, VIII, IX, X, XI, XII, XIII).**
- 3) Platelet aggregation studies (Ristocetin, ADP, Collagen, epinephrine, arachdonic acid)**
- 4) Platelet receptor studies by flow cytometry (Gp 1b – IX, GpIIb, Gp IIIa, P-Selectin, fibrinogen)**
- 5) Bethisda Assay for quantitation of inhibitors.**
- 6) Estimation of Von Willebrand factor (VWF) antigen by immunoelectrophoresis.**
- 7) Platelet antibody quantitation by flow cytometry.**

**Final diagnosis offered are as follows.**

<b>Factor I deficiency</b>	<b>- 1 Severe</b>
<b>Factor V and VII def</b>	<b>- 2 moderate</b>
<b>Factor VII</b>	<b>- 1 severe</b>
<b>Factor VIII</b>	<b>- 134 (Severe – 80 , Moderate – 32, Mild –22).</b>
<b>Factor IX def.</b>	<b>- 35 ( Severe 15, Moderate – 8, Mild 12.</b>
<b>Factor X def</b>	<b>- 5 Severe</b>
<b>Factor XIII def .</b>	<b>- 2</b>
<b>Von Willebrand disorders</b>	<b>- 30</b>
<b>Glanzmann’s thrombosthemia</b>	<b>- 9</b>
<b>Factor VIII inhibitors</b>	<b>- 7</b>
<b>Anti platelet antibodies</b>	<b>- 45.</b>

**3. Carrier detection in hemophilia A and by RFLP analysis.**

**Sixty nine women belonging to hemophilia A and nineteen hemophilia B families were referred for the detection of carrier status. All the analysis were done by both direct and indirect linkage analysis. In case of factor VIII gene analysis the strategy was to first to screen for Intron 22 inversions followed by intron 1 inversion by PCR. The linkage analysis was performed by using Bcl 1 , Hind III, Intron 1 GT repeats. In case of factor IX gene Taq 1, Xmn1, Dde 1, Mnl1 and Mse 1 were used for RFLP analysis.**

**Results of RFLP analysis are provided in table.**

Information RFLP Markers							
Haemophilia A (n=69)	<b>Int 22</b>	<b>Int 1</b>	<b>Bcl 1</b>	<b>Hind III</b>	<b>IVS13</b>	<b>IVS22</b>	<b>ST14</b>
	<b>21</b>	<b>1</b>	<b>25</b>	<b>7</b>	<b>6</b>	<b>4</b>	<b>41</b>
Haemophilia B (n=19)	<b>Dde 1</b>	<b>Mnl 1</b>	<b>Hha 1</b>	<b>Taq 1</b>	<b>Xmn 1</b>	<b>Mse 1</b>	
	<b>-</b>	<b>8</b>	<b>6</b>	<b>1</b>	<b>-</b>	<b>8</b>	

**In case of hemophilia A , two were noninformative for inversions and RFLP markers (2/69) whereas in case of haemophilia B a diagnosis of carrier / non carriers could be offered to all the women referred.**

4. First trimester antenatal diagnosis in haemophilia A and B families by chorionic villus sampling procedure.

**Forty eight women belonging to hemophilia families were referred for antenatal diagnosis in the first trimester of their pregnancies. Using the same strategy described above for carrier detection, diagnosis was offered in all cases except one in haemophilia A family who was subsequently offered a diagnosis by cord blood sample analysis. Twenty seven families were normal while twenty were affected.**

5. Cord blood sampling analysis for antenatal diagnosis in haemophilia families.

**Nine families belonging to hemophilia A and three to hemophilia B were referred for antenatal diagnosis. Out of the 12 families 6 were noninformative with the available technique while remaining 6 were referred late in second trimester of the pregnancy. After analysis the cord blood samples for both factor VIII/ IX :C and factor VIII / IX : Ag levels, a diagnosis was offered in all the 12 fetuses. Five were normal while 7 were affected.**

## STEM CELL BIOLOGY

### Leukemia Immunophenotyping

During the year, 139 cases of Leukemia were referred for immunophenotyping. Out of these, 110 (79.1%) cases were diagnosed as Acute Leukemia and 29 (20.8%) cases were diagnosed as Chronic Leukemia based on the peripheral blood and bone marrow examination for morphology, cytochemistry and immunophenotypic studies.

Immunophenotypic characters were assessed by multi-parameter analysis based on Triple colour immunophenotypic study of the surface antigens and cytoplasmic antigens using combinations of Phycoerythrine (PE), Fluorescein Isothiocyanate (FITC) and PerCP conjugated monoclonal antibodies. The following antibodies were used

- Myeloid markers** : CD11c, CD13, CD14, CD 15, CD 33, CD41a,  
Gly A and cMPO
- Lymphoid markers** : CD2, CD3, CD4, CD5, CD 7, CD8, CD10, CD19,  
CD20, CD22, CD23 and Cd79a
- NK Cell markers** : CD16 and CD56
- Non lineage markers** : CD34, CD45, and HLA-DR

**Table 1: Classification of 29 cases of CLL on the basis of immunophenotyping**

Diagnosis	Number of Cases	Percentage (%)
B-CLL	24	82.8
FCL	1	3.4
PCL	1	3.4
HCL	3	10.3
	29	

**Table 2: Classification of 110 cases of acute leukemia on the basis of immunophenotyping**

Diagnosis	Subtype	Number of Cases	Percentage (%)
AML	AML-M0/M1	16	14.5
	AML-M2	17	15.5
	AML-M3	14	12.7
	AML-M4	12	10.9
	AML-M5	6	5.5
	AML-M6	4	3.6
	AML-M7	1	0.9
ALL	B-ALL	28	25.5
	T-ALL	10	9.1
Biphenotypic	AML + BALL	2	1.8
Total		110	

### PNH studies by flow cytometry

During the year, 63 cases were referred to us for PNH studies by flow cytometry. Out of these, 15 (23.8%) cases were diagnosed as PNH based on immunophenotypic studies.

Immunophenotypic characters were assessed by multi-parameter analysis based on Dual colour immunophenotypic study of the surface antigens using combinations of Phycoerythrine (PE) and Fluorescein Isothiocyanate (FITC) conjugated monoclonal antibodies. The antibodies for the GPI linked protein, i.e. CD55 and CD59 were used for the study. The presence of the antigen was seen on the neutrophils as well as RBCs.

Table 3: Results of screening of patients with different haematological disorders for evidence of PNH clone by Flow Cytometry.

Cases	AA (%)	MDS (%)	Haemolytic anaemia with suspected PNH (%)	DVT (%)
Screened	30 (47.6)	11 (17.4)	5 (7.9)	17 (27.0)
PNH Positive	8 (12.7)	2 (3.1)	5 (7.9)	0 (0)

## CYTOGENETICS

During one year period , one thousand and two hundred sixty patients including genetic diseases (672) were referred for cytogenetics investigations. Among 588 hematological malignancies, cytogenetics abnormality was detected in 227(38.61%) patients (Table 1). Out of 672 patients suspected with genetic defects, chromosomal abnormality was detected in 192 (28.57%) Cases (Table 2).

**Table 1 : Cytogenetic abnormality in haematological malignancies.**

Sr. No.	Diagnosis	No. of Cases	Chromosomal Abnormality no.	Abnormality %
1.	CML	125	113	90.4
2.	AML	92	32	34.78
3.	ALL	80	26	32.5
4.	Aplastic Anemia	157	38	24.30
5.	CLL, Lymphoma, Myeloma etc.	134	18	13.43
	<b>Total</b>	<b>588</b>	<b>227</b>	<b>38.61</b>

**Table 2 : Frequency of chromosomal abnormality in patients suspected with genetic defects.**

<b>Sr. No.</b>	<b>Diagnosis</b>	<b>No. of . Cases</b>	<b>Chromosomal abnormality No.</b>	<b>Abnromality %</b>
1.	Down Syndrome	86	84	97.67
2.	Mother	86	7	8.14
3.	Father	86	3	3.49
4.	Dysmorphic features	62	12	19.35
5.	Primary Amenorrhea	53	15	28.30
6.	Sex abnormals	36	28	77.77
7.	RSA/BOH			
	Females	76	13	17.10
	Males	76	8	10.52
8.	Other, Ataxia, Short Stature, MR, MCA, MS , POF etc.	111	22	19.82
		<b>672</b>	<b>192</b>	<b>28.57%</b>

## **RED CELL SEROLOGY**

### **Confirmation of ABO and Rh Blood grouping**

During the year, 30 blood samples were referred from different blood banks and hospitals for confirmation of ABO grouping and 15 samples for confirmation of Rh grouping. Confirmation of ABO blood grouping on the samples referred showed the following results :

Bombay phenotype	-	15	(Oh Rh positive 14 and Oh Rh negative 1)
Weaker variant of A (Aw)	-	3	
ABw	-	1	
AwB	-	2	
A group with very low titre anti B	-	4	
B group with weak anti A in serum	-	1	
A <sub>2</sub>	-	1	
O group	-	2	
A <sub>1</sub> group	-	1	

Out of the 15 samples referred for Rh grouping, 11 were confirmed as partial D, 2 were Rh negative and 2 were Rh positive.

### **Identification of antibodies :-**

During the year, 40 samples were referred for detection of atypical antibodies. These samples were from patients who had either shown some grouping or cross matching problems or were from multitransfused patients for intentional antibody screening. 15 samples showed the presence of red cell atypical antibodies and testing with panel of red cells identified the specificities as – C(1), -c(1), -Jk<sup>a</sup>(1), -Fy<sup>a</sup>(1), -M(2), -N(3), -c + JKb(1), -Le<sup>a</sup>(1), -c+Jka (1), A<sub>2</sub> with anti-A<sub>1</sub>(1), -I (2).

A total of 260 Rh negative antenatal women were referred from different hospitals for detection of Rh antibodies in serum. Out of this, 169 women were referred for Rh antibody titre only once, 60 women were referred twice, 24 women were referred thrice and 7 women were tested four times or more during their antenatal period. Rh antibodies

were detected and identified in 17 Rh negative women with their Rh antibody titre ranging from 1:16 to 1:2048.

One hundred and twenty three cases were referred for routine direct and indirect antiglobulin tests. Nineteen of these cases were found to show direct and indirect antiglobulin test positive with three showing anti-e, 3 anti I and one - D specificities. The rest did not show any clear specificity.

## AUTOIMMUNE DISORDERS

During the year 2005-2006, 3083 patients suspected to be suffering from various collagen vascular autoimmune disorders referred by various hospitals in Mumbai were investigated for ANA (anti-nuclear antibody) by the indirect immunofluorescence test using a broad spectrum anti-human globulin serum conjugated to FITC.

Totally 772 patients were found to be positive for ANA and these samples were further investigated for autoantibodies to double stranded DNA (anti-dsDNA), as these are immunodiagnostic markers that are considered with the ARA clinical criteria for diagnosis of SLE. On the basis of these parameters, 105 patients were diagnosed as SLE and the strength of their reactions (Titre) was also determined. All autoantibody positive SLE cases were followed up at every three months interval.

Six hundred and four samples suspected to be suffering from different types of glomerulonephritis and ANCA associated vasculitis (AAV) were also referred from nephrology and rheumatology departments for ANCA (anti-neutrophil cytoplasmic antibodies) testing. ANCA was detected using ethanol and formalin fixed cytospun preparations of PMN by indirect immunofluorescence assay. One hundred and twenty eight samples were found to be positive for ANCA. The breakup of these autoantibodies detected is as shown in table 1.

Platelet associated immunoglobulins (PAIgG) were tested in 50 suspected cases of idiopathic thrombocytopenic purpura referred by hematology department. Of these cases twenty nine patients showed raised levels of PAIgG by indirect ELISA as compared to normals (1.8 to 19.8 ng/ 10<sup>6</sup> platelets) and these patients had platelet counts ranging from 50,000/ $\mu$ l to 1,50,000/ $\mu$ l.

<b>Autoimmune Disorders</b>	<b>Number tested</b>	<b>ANF positives</b>	<b>Anti-dsDNA positive</b>	<b>ANCA positive</b>
SLE	781	375	105	20
DLE	76	17	0	0
MCTD	138	12	0	15
RA	675	125	0	10
Renal	757	110	0	55
Heamatological	150	28	0	20
Others	506	105	0	8
<b>Total</b>	<b>3083</b>	<b>772</b>	<b>105</b>	<b>128</b>

## TRANSFUSION TRANSMITTED DISEASE.

### A) Western Blot Analysis.

During the year a total of 149 sera samples which were reactive or equivocal for

Anti - HIV antibody by ELISA was referred from different HIV screening centers of government and municipal hospitals in Mumbai. Our Western Blot Analysis showed that 83.22 % (124 sera) samples were found to be reactive, 12.08 % (18 sera) samples were indeterminate and 4.69% (7 sera) were non –reactive.

**Table1: Results of Western Blot Analysis**

RISK GROUP	ELISA DONE	WESTERN BLOT		
		REACTIVE	NON-REACTIVE	INDETERMINATE
Heterosexually Promiscuous	5	5	-	-
Heterosexual Single	89	82	3	4
Opportunistic Infections	12	12	-	-
Spouse of HIV Reactive	5	5	-	-
Recipient	-	-	-	-
Antenatal Vertical Transmission	-	-	-	-
Unknown	38	20	4	14
<b>Total</b>	<b>149</b>	<b>124</b>	<b>7</b>	<b>18</b>

### B) External Quality Assurance Scheme by MDACS :

During the year as a routing service we have processed 2726 sera ( 5 % positive and 5 % negative ) from 4 SRL's ( State Reference Laboratory ) under K. E. M. Hospital , J. J. Hospital, Sion Hospital & Nair Hospital of these 13.13 % sera ( 358 samples ) were reactive & 85.8 % sera ( 2339 samples ) were non - reactive by Anti HIV ELISA. However 29 samples from 2 center's J. J. Hospital & K.E.M Hospital were not tested due to insufficient quantity or mislabeling. Accordingly, our results were corresponded to MDACS.

**Table 2: EQAS results from 4 SRLs of Mumbai**

MDACS EQAS

Anti HIV test results among SRL's in Mumbai.

Sr. No	Centre's	No.of Samp	Elisa Reacti	Elisa Non- Reactive	Not Tested
1	K.E.M. Hospital	747	102	635	10
2	J.J. Hospital	688	141	528	19
3	Sion Hospital	1109	83	1026	0
4	Nair Hospital	182	32	150	0
<b>Total</b>		<b>2726</b>	<b>358</b>	<b>2339</b>	<b>29</b>

### C) National EQAS - Sentinel Surveillance for SRL's.

Our Institute as a National Reference Laboratory (NRL) is carrying out the sentinel surveillance for SRL's in NACO project. Accordingly, we receive Anti – HIV all reactive and 5 % non – reactive samples from SRL's in Mumbai, Madhya Pradesh, Chhattisgarh as per NACO directives under the National EQAS programme during the year we studied a total of 1342 samples of which 32.12 % sera (431 samples) were reactive & 67.88 % sera (911 samples) were non – reactive by ELISA.

**Table 3 : Sentinal Surveillance results from Mumbai**

Sr. No	Centre's	No.of Samples	Elisa Reactive	Elisa Non-Reactive
1	K.E.M. Hospital	126	73	53
2	Nair Hospital	213	149	64
3	Sion Hospital	147	59	88
4	Choitram, MP	224	14	210
5	Jabalpur, MP	177	31	146
6	Chattisgarh	90	20	70
7	Ahmedabad	330	76	254
8	Kalwa	35	9	26
<b>Total</b>		<b>1342</b>	<b>431</b>	<b>911</b>

### D) Prevalence of Transfusion Transmitted Diseases.

During the year a total of 258 hematologically abnormal patients comprising of Hemophilia

(114 samples), Aplastic Anaemia (75 samples) and Thalassaemia / Sickle cell Anaemia

(69 samples) were studied for their HIV, HCV and HBs Ag reactivity by ELISA. Our results revealed that 3.50 % of Hemophilics (4 patients), 5.79 % of Sickle cell anaemics / Thalassaemics ( 4 patients ) were found to be reactive for Anti – HIV antibodies. None of the patients from APA were found to be reactive for HIV antibody. Further, the HCV reactivity was seen in about 12.28 % of hemophilics (14 patients), 1.33 % of Aplastic

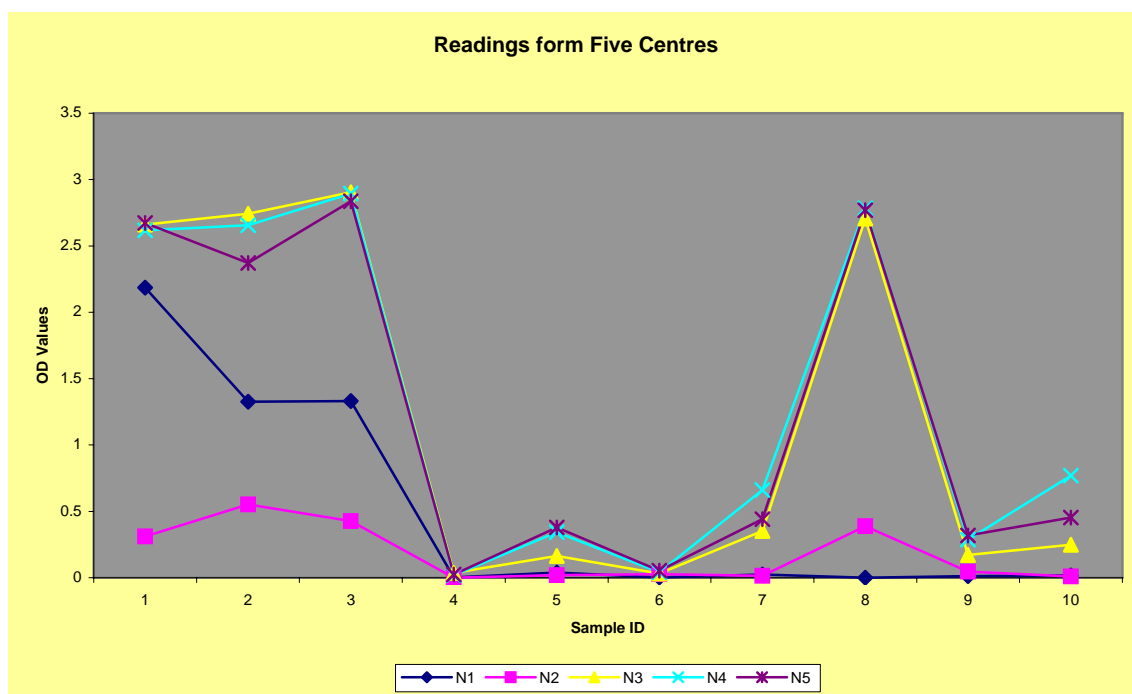
Anaemia patients (1 patient) & 15.94 % of Sickle Cell Anaemia / Thalassaemia (11patients). It was observed that 1.44% of Sickle Cell Anaemia / Thalassaemia patients, 1.33 % of Aplastic anemia patients & 3.50 % of hemophilia patients were reactive for HBs Ag. It was seen that all the HIV, HCV & HBs Ag antigenicity were present in hemophiliacs and Sickle Cell Anaemia / Thalassaemia patients. Whereas, HIV was not identified among Aplastic anaemia Patients in our present investigations.

**Table 4: HIV, HCV and HBV states among haematological disorders**  
**Haematological Disorder's**

**Reactivity of TTI's in certain Haematological Disorders.**

<b>Haematological Disorder</b>	<b>No. of Samples</b>	<b>HIV Reactive</b>	<b>HCV Reactive</b>	<b>HBs Ag Reactive</b>
Haemophilia	114	4 (3.50%)	14 (12.28%)	4 (3.50%)
Aplastic Anaemia	75	0	1 (1.33%)	1 (1.33%)
Sickle cell Anaemia Thalassaemia	69	4 (5.79%)	11 (15.94%)	1 (1.44%)
<b>Total</b>	<b>258</b>	<b>8 (3.10%)</b>	<b>26 (10.07%)</b>	<b>6 (2.32%)</b>

**Fig. Quality control exercise of 5 different laboratories in EQAS**



### Programme for the EQAS Training at IIH

One day workshop on Proficiency Panel as part of EQAS was held at IIH on Saturday 15<sup>th</sup> October 2005. An elaborative overview of HIV / AIDS was given on Quality Assurance mainly focusing on ELISA. A panel of 10 samples (4 strong positive, 2 negative and 2 equivocal) was given to all 5 centers for practical work by ELISA. All 5 participating centers detected 4 strong positive samples and 2 negative samples. Only 2 of 5 centers could detect 2 indeterminate samples in duplicate. One center found only one indeterminate sample but not in duplicate. The analysis of the results are given below.

Three day Regional Review workshop at IIH: Road map for EQAS next phase  
**7<sup>th</sup> to 9<sup>th</sup> Dec 2005**

Dr. Dimple Kasana from NACO and all the participants from Gujarat, Aurangabad, and Mumbai SRLs, NARI, MDACS and MSACS representatives were involved. The discussion for the road map for the next phase of EQAS was done as well as hands on training for Panel preparation was carried out and the minutes of the proceedings were sent to Apex lab.

## **LEUCOCYTE IMMUNOLOGY**

During the year 616 samples were typed. Among those 13 end stage renal failure patients referred for HLA-Class I tissue typing and /or pretransplant cross match along their prospective donors.

Those 13 prospective donors 54% were parents and 46% were sibs. The mean age of the patient ranged from 30-40 years. Altogether 3 families were referred for HLA-Class I typing along with their family donors for bone marrow transplantation during the period. All the three cases were b- thalassaemia. Further 354 suspected ankylosing spondylitis patients were referred for HLA-B27 allele typing as well as a total 77 couples comprising of normal and recurrent spontaneous abortion were also referred for HLA-typing during the period.

## COLLABORATIVE PROJECTS CYTOGENETICS

### 1. Genomics of Male Infertility.

**This project was started in 2004, in collaboration with National Institute for Research in Reproductive Health (NIRRH). The chromosomal analysis was carried in 50 patients with male infertility. Conventional cytogenetics using G-banding revealed 10% chromosome aberrations. The molecular cytogenetic study will be carried out using AZF locus specific probes.**

### 2. Chromosomal Instability in Glioma cell lines.

**This project is in collaboration with Bombay Hospital. The objective of the project is to understand the tumorigenesis in Glioma patients. The chromosomal analysis was carried out at different cell lines passages and high frequency of chromosome aberrations found in 140 passage stage. The results will be correlated with molecular studies.**

### 3. Genotoxicity studies with Antimicrobial Peptides Nisin, SSP12 and Basant.

**This project is in collaboration with NIRRH. The effect of Nisin was evaluated using different concentrations in peripheral blood cultures. The cell/genotoxicity was observed at high concentration of NISIN in vitro**