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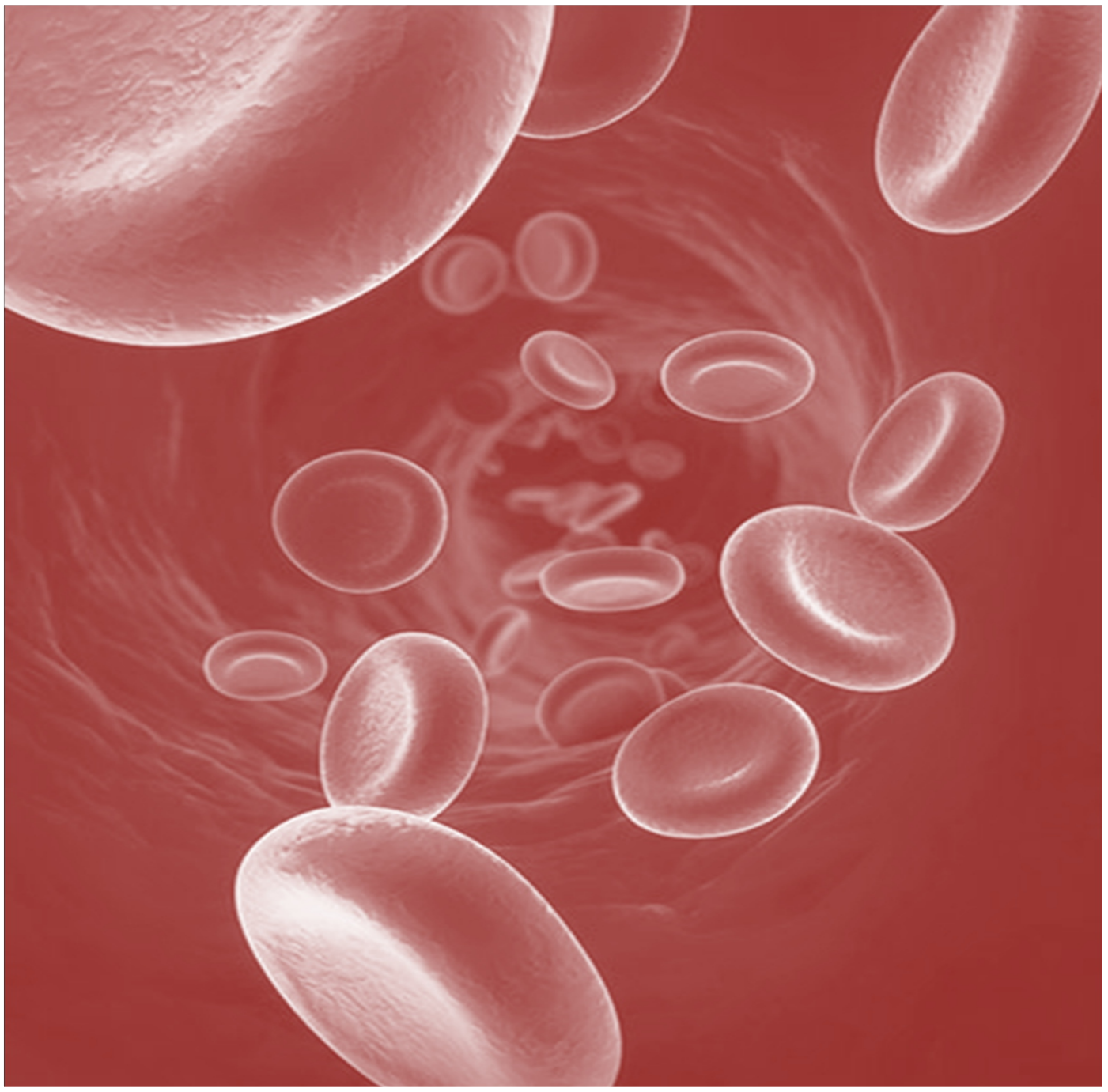

World Federation of Hemophilia IHTC Training Programme

Dr M B Agarwal, Hematologist receiving Dr H M Bhatia Oration

Foundation Day Celebration of the Institute

Molecular genotyping of blood group antigens

Harita Gogri & Swati Kulkarni

Summary

The International Society of Blood Transfusion (ISBT) has identified 35 blood group systems consisting of more than 290 antigens of which less than fifty blood group antigens are polymorphic. Most of these blood group systems were discovered when antigen-specific antibodies present in the human sera were identified by agglutination-based methods. Though these serology-based techniques are simple and inexpensive they have certain shortcomings. Over the past 20 years, the molecular basis of most of the blood group antigens has been identified and has led to the development of various low to high-throughput DNA-based techniques for blood group genotyping. These techniques have immense applications in transfusion and prenatal setting. Molecular genotyping will play an important role in complementing serological techniques and will contribute to increased quality of red cell reference laboratory work. In future, it should be implemented in a stepwise manner in routine blood banking for better patient care.

Introduction

The International Society of Blood Transfusion (ISBT) has recognized 339 antigens on human red cells of which more than 290 are clustered within 35 blood group systems [1]. ABO and Rh the two major clinically important blood group systems in transfusion medicine. However, apart from these, there are 33 other minor blood group systems. Of these, less than fifty of the blood group antigens are polymorphic which can stimulate production of clinically significant antibodies, and play an important role in blood transfusion and in pregnancy. Fig. 1 shows some of the minor blood group system antigens along with ABO and Rh located on RBC membrane [2]. Clinically significant antibodies against blood group antigens are capable of causing mild to severe hemolysis following blood transfusion and pregnancy [3] Fig. 2 illustrates the clinical importance of blood group antibodies [4].

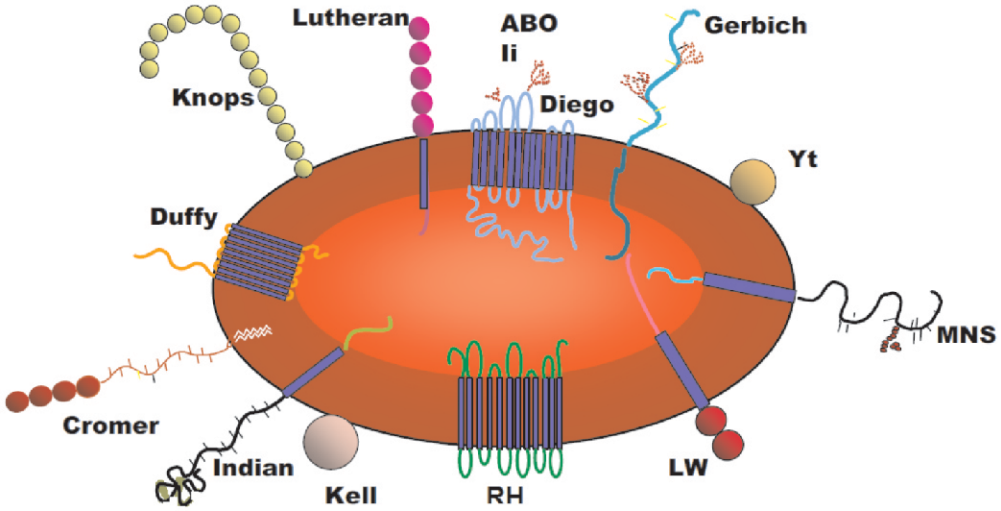

Fig. 1: Diagrammatic representation of different blood group systems on the RBC membrane
(Adapted from Storry J; 2011)

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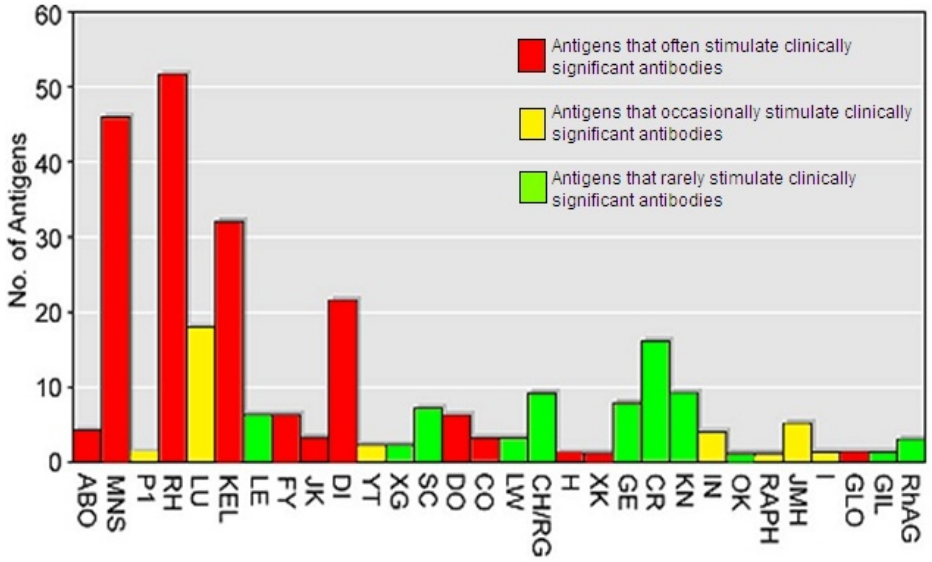

Fig. 2: Clinical importance of different blood group specific antibodies
(Adapted from Anstee DJ; 2009)

Most of these blood group systems were discovered when antigen-specific antibodies present in the human sera were identified by agglutination-based methods. Until 1990s, the determination of blood groups was mainly based on principle of hemagglutination. These serology-based techniques are simple and inexpensive and when correctly performed, had specificity and sensitivity appropriate for the clinical care of the majority of patients. However, haemagglutination methods have certain limitations. It is a subjective test, is labor intensive and time consuming. It does not reliably predict a fetus at risk of hemolytic disease of the fetus/newborn (HDFN). Haemagglutination technique has restricted ability to predict RhD zygosity, define weaker variants of ABO and Rh, red cell phenotyping in multi-transfused and DAT (Direct Antiglobulin Test) positive patients. Except for routine ABO and RhD grouping, potent antisera for extended phenotyping are very costly, weakly reacting and/or unavailable for some blood group system like Dombrock, Colton etc. For some blood group antigens, the antisera prepared from polyclonal human serum are poorly standardized and also available in limited stocks.

Over the past 20 years, the molecular basis associated with most of the blood group system has been identified. This knowledge has led to the development of various DNA-based techniques for blood group genotyping [5].

Molecular basis of blood group expression

Different mechanisms responsible for the molecular diversity of the blood group systems such as nucleotide substitutions, crossing over, alternative RNA splicing, deletion/insertion of nucleotides, exons or genes, and exon duplication events (Table 1) have been reported [6]. The various genetic variations that encode different blood group antigens are documented in the Blood Group Antigen Gene Mutation Database (dbRBC) (http://www.ncbi.nlm.nih.gov/gv/mhc/xslcgi.cgi?cmd=bgmut/home) [7]. However, most blood groups are encoded by single nucleotide polymorphisms (SNPs), which means that a single nucleotide difference in the whole gene determines which blood group antigen is expressed on the red cell. SNPs can alter the antigen expressed by a certain blood group molecule and also modify the number of copies expressed in the RBC membrane.

1. ABO blood group system

ABO blood group system is the most clinically important blood group system. The ABO gene, located on chromosome position 9q34.1-q34, encodes for the glycosyltransferases that catalyze

Table 1: Different molecular events giving rise to blood group phenotypes (Adapted from Hillyer CD 2007)

Event	Mechanism	Blood group phenotype
Promoter mutation	Nucleotide change in GATA box	Fy(b-)
Alternative spicing	Nucleotide change in splice site	S-s-, Gy(a-)
Premature stop codon	Deletion of nucleotides	Fy(a-b-), Rh null, Ge2, Ge3, Ge4, K0
	Insertion of nucleotides	D-, Co(a-b-)
Amino acid change	Missense mutation	D-, Rh null, K0
Hybrid genes	Crossover	GP.Vw, GP.Hil
	Gene conversion	GP.Mur, GP.Hop, D --
Interacting protein	Absence of RHAG	Rh null
	Absence of Kx	Weak expression of Kell antigen
Modifying gene	IN(LU)	LU(a-b-)

synthesis of ABO antigens. It consists of seven exons ranging from 28 to 688 bp and six introns of which, the transcribed mRNA of exons 6 and 7 codes for 77% of the catalytic domain of ABO glycosyltransferase [8]. The A, B and O alleles differ by only a few nucleotide substitutions in exons 6 and 7. ABO*B101 (B group) is distinguishable from ABO*A101 (reference allele) at seven nucleotide positions namely, 297, 657, 930, 526, 703, 796, and 803. The A2 blood group is caused due to nucleotide substitution at position 467 and a single base deletion (1061delC) in exon 7, which results in disruption of the stop codon and an A-transferase product with an extra 21 amino acid (AA) residue at the C-terminus. The nucleotide sequence of ABO*O01 (O group) differs from that of ABO*A101 by a single base deletion at position 261 in exon 6 which shifts the reading frame, thus generating a premature stop codon. ABO*O01 is either silent or translated into a truncated and catalytically inactive peptide. Apart from these several other allelic forms of this gene are also reported [8].

2. Rh blood group system

The Rh blood group system is encoded by two closely linked and highly homologous RH genes (RHD and RHCE), present at chromosome location 1p36.1. Both RHD and RHCE genes are inherited together and consists of ten exons each that encode for 417 amino acid protein which forms a transmembrane protein with six extracellular loops. High degree of homology between RHD and RHCE genes strongly favors the formation of hybrid alleles of RHD-CE-D or RHCE-D-CE type.

The RHD gene is responsible for the production of D antigen. The loss of D antigen expression can be caused by multiple genetic events. Deletion of RHD gene is the major cause D negativity in Caucasians. However, in Africans, the presence of RHD pseudogene (RHDψ) and RHD-CE-Ds hybrid gene along with RHD deletion are the main causes of D negativity [9].

RhD variants are mainly of two types partial D and weak D. Partial Ds (qualitative D variants) are mainly

caused by presence of RHD/RHCE hybrid alleles or missense mutations in the extracellular protein segments. Weak Ds (quantitative D variants) mostly arise due to missense mutations in intracellular parts of the RhD protein [10]. Very weak D phenotype "DEL", most commonly encountered in eastern Asia, results from a silent mutation in RHD gene [11].

The RHCE gene is responsible for production of C/c and E/e antigens on the same protein. RHCE exists in four allelic forms, and each allele determines the expression of two antigens in Ce, ce, cE, or CE combination. The C/c polymorphism is caused by six nucleotide substitutions resulting in four amino acid changes (Cys 16 Trp, Ile 60 Leu, Ser 103 Pro, Ser 68 Asn). E/e polymorphism is caused by single nucleotide substitution causing the amino acid change Pro 226 Ala.

3. Other Blood Group Systems

Apart from ABO and Rh blood group systems, most of the antigens of other blood group systems are encoded by SNPs. These antigens are encoded by different alleles of the gene, and their diversity will determine the number of different antigens in the system. These SNPs can be either silent (do not alter the amino acid sequence), or missense (cause change of one amino acid to another), or nonsense (change the codon to stop codon) or may alter splice sites. The different SNPs responsible for formation of common blood group antigens of clinically important blood group systems are enlisted in dbRBC [7] and shown in Table 2.

Table 2: Different single nucleotide polymorphisms responsible for formation of blood group antigens

ISBT No.	Name	Symbol	Chromosome Location	Nucleotide change	Amino acid change	Specificity
002	MNS	MNS	4q31.21	59C>T; 71G>A; 72T>G	Ser20Leu, Gly24Glu	M>N
				143T>C	Thr48Met	S>s
005	Lutheran	LU	19q13.32	230A>G	His77Arg	Lu^a>Lu^b
006	Kell	KEL	7q34	578T>C	Met193Thr	K>k
				841T>C	Trp281Arg	Kp^a>Kp^b
				1790C>T	Pro597Leu	Js^a>Js^b
008	Duffy	FY	1q23.2	125G>A	Gly42Asp	Fy^a>Fy^b
009	Kidd	JK	18q12.3	838G>A	Asp280Asn	Jk^a>Jk^b
010	Diego	DI	17q21.13	2561T>C	Leu854Pro	Di^a>Di^b
011	Cartwright	YT	7q22.1	1057C>A	His353Asn	Yt^a>Yt^b
013	Scianna	SC	1p34.2	169G>A	Gly57Arg	Sc1>Sc2
014	Dombrock	DO	12p12.3	793A>G	Asn256Asp	Do^a>Do^b
015	Colton	CO	7p14.3	134C>T	Ala45Val	Co^a>Co^b
023	Indian	IN	11p13	137C>G	Pro>Arg	In^a >In^b

Molecular methods

The molecular genetics was revolutionized in 1983 with the advent of PCR (polymerase chain reaction), which allows the amplification of DNA and analysis of genes [12]. It is now possible to predict blood group phenotypes by performing tests on genomic DNA, with a high degree of accuracy. The various molecular methods used for red cell typing has been reviewed by Monteiro et al [13] and summarized in Table 3.

Table 3: Different techniques used in blood group genotyping

	Low throughput	Medium throughput	High throughput
Technique	PCR-SSP, PCR -RFLP, Nested PCR, Multiplex PCR etc.	PCR-Sequencing, Quantitative PCR etc.	Microarrays, Mini sequencing etc.
Applications	Genotyping common antigens of Rh, Duffy, Kell, Kidd etc., weaker variants etc.	Identification of weaker variants of ABO & Rh, non-invasive fetal RhD typing, Rh zygosity testing, SNP genotyping etc.	Genotyping of multiple blood group and platelet antigens
Advantages	Simple and easy, Cost effective	More sensitive, no post PCR processing required	Large scale donor typing for many antigens in a single test, automated

1. Low throughput methods:

Today conventional PCR applications are predominantly SSP-PCR (PCR using sequence-specific primers). This technique is used do differentiate antithetical blood group antigens differing by a SNP and requires prior knowledge of the target DNA sequence, including differences between alleles. On the other hand, PCR-RFLP (restriction fragment length polymorphism) technique is based on the introduction or loss of a restriction enzyme site by a SNP of interest. PCR-SSP and PCR-RFLP testing platforms have been used for determining antigens of ABO, Rh, Duffy, Kell, Kidd blood group systems [14, 15]. These techniques enable genotyping to a very low cost, as the reagents and the equipment are not expensive.

Multiplex PCR enables simultaneous amplification of many target alleles or regions of DNA in one reaction by using multiple primer pairs. This allows a reduction in the number of different assays performed, which saves time, reagents and cost. Based on the technical platform and design of the process, multiplex PCR can be set-up as a low or medium-throughput assay. This assay has been used for identifying common partial RHD variants. Common antigens of various minor blood group systems can also be detected simultaneously [16, 17].

However, methods like PCR-SSP, Multiplex PCR etc. require additional post PCR steps (agarose-gel electrophoresis) to detect the amplified products and interpret the results. Also, these techniques are

labour intensive, with some discrepancies that require extensive serologic and molecular investigations [18-20].

2. Medium throughput methods:

In contrast to conventional PCR-based methods, Real-Time PCR, DNA sequencing are more robust methods for genotyping. Real-Time PCR method is based on detection of fluorescence signals in the PCR cycler. It measures the amplified DNA quantitatively in real time i.e. as the reaction progresses. This method eliminates the need to perform post PCR steps. Non-specific fluorescent dyes which intercalate with double-stranded DNA (i.e. SYBR_ Green) or specific dyes, which anneal within a region of target DNA sequence amplified in presence of specific primers (i.e. Taqman assay), can be used in Real time PCR. Real-time PCR applications can be set-up as low or medium-throughput methods. This method has been implicated in determination of RhD zygosity and in noninvasive prenatal RHD typing. Melting curve analysis which involves the assessment of the dissociation-characteristics of double-stranded DNA(Tm) during heating in the presence of fluorophore has been applied to study various blood group polymorphisms [21-24].

Sanger sequencing is being widely used in blood group genotyping. This method of DNA sequencing is based on the selective incorporation of chain-terminating dideoxynucleotides (ddNTPs) by DNA polymerase during in vitro DNA replication. It gives the sequence of nucleotides in the target sequence. Pyrosequencing is a DNA sequencing method based on detecting pyrophosphate release rather than on strand termination. It has been successfully used for red cell genotyping, but presently limited to only few blood group systems [25].

3. High-throughput methods:

High throughput methods like BeadChip array, Genome lab SNP Stream, Blood chip etc. are microarray based methods that integrate thousands of reactions in a single test and can be used to test multiple blood group antigens at different loci simultaneously [26-29]. Fluidic microarray system, Luminex XMAP system, is a microsphere-based technology that is internally dyed with two spectrally distinct fluorochromes. It can analyze up to 100 different analytes in a single reaction using a flow cytometer. Karpasitou et al. [30] adapted the technology to type for JKA/JKB, FYA/FYB, S/s, K1/K2, KPA/KPB/JSA/JSB, COA/COB, and LUA/LUB antigens.

Taqman openarray method is based on real-time PCR using taqman probes. The array consists of collection of 3072 reaction through-holes in the plate and provides genotyping and gene expression analysis of hundreds to thousands of samples and assays per day [31].

MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) technique has the capacity to discriminate DNA fragments that differ in a single nucleotide. It is a high-throughput, qualitative, and quantitative method that can analyze 36–40 multiple SNPS in a single reaction that takes approximately eight hours. This method has been used for genotyping of some of the Kell blood group system genes and for platelet antigens [32]. Using the Sequenom platform, a prenatal screening test for fetal RHD typing has been reported [33].

Applications of red cell genotyping in transfusion medicine

Molecular genotyping is being increasingly used in immunohaematology reference laboratories in western countries and has now become more efficient and cost-effective. Some of the important applications of molecular blood group genotyping in transfusion setting and in prenatal diagnosis include:

- ☒ Identification of variants of ABO and RHD gene
- ☒ Fetal RHD typing
- ☒ Determining the actual antigen profile of recently transfused patients
- ☒ Determination of RhD Zygosity
- ☒ Screening for rare donor blood types
- ☒ Determining antigen profile in patients with AIHA
- ☒ Differentiate alloantibody from autoantibody
- ☒ Quality assurance of reagent red cell panels

Though, DNA-based methods have great value in Immunohaematology laboratories, they have certain limitations if performed alone. As many genetic events give rise to the same blood group phenotype, an apparent discrepancy between phenotyping and genotyping test results may be seen. The predicted antigen negativity by DNA testing has to be confirmed by hemagglutination. For e.g., D-- phenotype results from at least three different mechanisms: reduced transcriptional activity, internal gene deletion, and homologous recombination [34-37], but phenotypically show the same expression. It is serologically easy to detect D-- RBCs with anti-Rh17 (or -C and -c). Similarly, it is also easy to detect Fy (a-b-) with anti-Fy3, to detect Jk (a-b-) with anti-Jk3 or by urea lysis test [38] etc.

The molecular basis associated most of the blood group system antigens have been reported. However, in many studies the analysis has been restricted to a relatively small number of individuals and not all alleles in all ethnic populations are known. Before recommending DNA typing for blood group antigens in clinical practice, population studies from a variety of ethnic backgrounds should be analyzed to establish the correlation between serology and DNA testing results [39]. DNA genotyping is technically challenging, as it requires specialized laboratories with skilled personnel to interpret the results. New high-throughput technologies for blood group genotyping are unaffordable for many blood centers at the moment.

Experience at NIIH

Department of Transfusion Medicine at National Institute of Immunohaematology, Mumbai is a reference centre where samples are referred from all over the country for resolving problems in blood grouping and cross matching. Currently, the department is also involved in molecular blood group genotyping and the findings of our studies are as follows:

- ☒ **Solving ABO blood group discrepancies:** Discrepancies in ABO blood groups among donors and patients arise when cell grouping and serum grouping results do not match. This may often be due to expression of variant ABO allele. We identified nine rare alleles affecting the normal expression of A and B antigens of which two were Aw06, one A209, one Ax20, two O05, one O49, one O56 and one O19 alleles [40].
- ☒ **Antigen typing when antibody-based typing reagents are unavailable or only weakly reactive:** Agglutination tests depend upon the availability of specific reliable antisera. Blood group typing reagents are not available for phenotyping of common and clinically important antigens of some blood group systems like Indian, Colton, Dombrock etc. We have developed a simple PCR-SSP for genotyping common antigens of Indian blood group system and the frequency of Ina antigen was found to be 2.9% [41].
- ☒ **Identification of weaker and partial variants of RHD gene:** Serological testing with two anti-D reagents in blood banks may not identify D variants and also will not distinguish between partial D from weak D [42]. Our study has shown that only 63.4% of RhD variants could be classified by partial D kit (panel of six monoclonal anti-Ds) using serology. However, Multiplex PCR could characterize 76.6% of partial D variants [43].
- ☒ **Typing of blood group antigens in recently**

transfused patients: Molecular typing of antigens can help in determining the actual antigen profile of a recently transfused patient and also the additional blood group antigens to which the patient may get sensitized. Blood samples from 100 multi-transfused thalassemia patients were studied by us using hemagglutination and PCR-SSP for common antigens/ alleles of Rh, Kell, Kidd and Duffy blood group system. The genotyping was concordant with the serological red cell phenotype in only 21% cases for five antithetical antigen pairs in four blood group systems [44].

- **Quality control in Reagent red cells:** We have prepared in-house reagent red cell panel by screening O group regular donors for clinically important antigens and have confirmed the antigen status by DNA-based typing method, to provide greater quality assurance of reagent RBCs used for screening and identification of alloantibodies [45].

- **Fetal RhD typing:** The prenatal determination of fetal RhD status helps in management of RhD alloimmunised pregnancy. We have evaluated the usefulness of fetal RhD typing by PCR using chroinic villus tissue DNA [46]. These conventional techniques carry significant risk of complications including pregnancy loss and also increased maternal sensitization. The discovery of cell free fetal DNA in maternal plasma has opened up new possibilities for noninvasive prenatal diagnosis. We have recently standardized Real Time PCR method for noninvasive fetal RhD typing [47]. This test will now be used diagnostically for early management of antenatal alloimmunized women and also in nonimmunized pregnant women for restricting use of Rh Immunoglobulin prophylaxis.

- **Determination of RhD Zygosity:** The RhD zygosity determination is very important in RhD negative pregnant women to predict the possible RhD status of the fetus. RhD negative pregnant women can be reassured and managed less intensively if RhD negative status of fetus was confirmed. RhD zygosity in RhD positive individual is commonly inferred from the serological phenotype expressed and the most probable genotype (MPG) is deduced and thus the D zygosity. This approach has been a simple guess and is therefore not very accurate or reliable. The correct RhD zygosity can be predicted by molecular studies. Our study among the Indian donors showed 18% discrepancy in D zygosity between serological MPG and hybrid box PCR [48].

Conclusion

The knowledge of molecular basis of most of the blood group systems has led to development of various low to high-throughput molecular biology techniques which will have immense applications in transfusion and prenatal setting. Micro-array based methods are now commercially available to genotype large number of donors and patients for multiple blood group antigens simultaneously.

Genotyping for RBC polymorphisms will play a more important role in routine blood banking in future. Although it will not replace serological methods, especially for ABO and RhD grouping and antibody detection and identification nevertheless, it will support decision making in serologically complex situations and will contribute to increased quality of red cell reference laboratory work. The widespread use of blood group genotyping would lead to provide safer and effective blood transfusions. With advances in high throughput molecular typing, if a situation can be attained where all donors and patients are fully genotyped, it should be possible to allocate safe blood for transfusion without further compatibility testing. All matchings could thus be computerized, and chronically transfused patients would be most benefitted from this development.

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DEPARTMENT OF LIBRARY & INFORMATION SCIENCE 2016

Vijay Padwal, ALIO

GENERAL INFORMATION

In the present information age, electronic information resources are very much essential for learning. They play vital role for the growth of society. Now the printed sources of information are outdated due to the high cost of their purchasing and delay in dissemination. Therefore it is time for the researcher to educate themselves and also their students in electronic information resources. The emergence of internet, particularly the World Wide Web, as a new medium of information storage and delivery in the 21st century has changed the entire concept of library profession. The phenomenon of consortia or group of libraries buying e-information together has become very important in the last few years. In order to avail e-information and e-resources at our door-step, ICMR our parent body is taking care to promote better, faster and cost effective ways of providing electronic information sources to the information seekers.

To facilitate end users, ICMR has renewed the subscription for the e-journals consortia like JGate Plus, NML-ERMED consortium and e-journals like Nature, Science, Lancet and NEJM for the year 2016.

Digitalization of NIIH Library

The NIIH library has started digitalizing their resources by implementing the Digital Library

Database from Techfocuz for retrieving the CD & Image administration with multiple accessibility and administrative management systems. Initially we have taken the digitalization work of the old rare photographs and manuscripts of the Institute. NIIH Subscribed e-journals portals are imported in the database for easy retrieval of full text articles to the readers.

Automation of Library Resources

The NIIH Library started automation of library function in the year 2005 with GLAS Automation software and built a complete database of over 1200 books, 50 current journals and 1500 bound volumes. Computerisation of Back Volumes, Theses, Reports and other audio visual is ongoing process. The GLAS software was upgraded with an advanced library automation software SLIM21 in the year 2014 and the House Keeping Activities such as Acquisition, Cataloguing, Circulation, Serial Control, OPAC, Statistics for over all purposes of management and Information services such as Current Awareness Service (CAS), Selective Dissemination of Information Service (SDI), Web OPAC, Internet Services.We have online services provided to users through our Local Area Networking (LAN) system. The Online Public Access Catalogue (OPAC) is used to carry out online searches of library database by Author, Title, Publisher, Keywords and Accession Numbers which enables to provide good reference service to staff, researchers and students. In 2016 the library started issuing books with computer system with barcode technology. The barcoded library cards are issued to all the Institute staff for easy Issue/Return of the books and journals. All modules i.e. Acquisition, Cataloguing, Serial Control and OPAC are working with SLIM 21 software in the Library.

Facilities

Library is having high speed unlimited WIFI connectivity for Internet access.

Digital Color Multipurpose Photocopier for Scanning and Printing.

Electronic Document Delivery Services from nearby Medical Colleges, Hospitals and Research Institutions.

Audio-visual and Multimedia facilities for Doctors, Scientists and Students.

Access to New Library field from NIIH website www.niih.org.in

Publications

Annual report of the Institute for the year 2015-2016.

Some Recent Additions (Apr2015-Mar 2016):

Books		- 7
Journals	- International	- 39
	National	- 24
Bound Volumes		- Nil
CD-ROM/DVD		- 28

NIIH HAPPENINGS

Department of Pediatric Immunology and Leukocyte Biology

Dr Manisha Madkaikar, Scientist F & Director-in-charge

Invited to deliver a lecture entitled "Flowcytometry in diagnosis of Primary immunodeficiency Disorders (PID) and molecular mechanism in PID" at 3rd National Conference on Primary Immunodeficiency (ICPID) held at New Delhi from 12th to 13th March 2016.

Invited to deliver a lecture entitled "Primary immunodeficiency Disorders (PID)" at 13th FIMSA Advanced Immunology Course-2016 held at Post Graduate Institute of Medical Education and Research, Chandigarh from 17th to 19th March 2016.

Ms Aparna Dalvi, Technician

Received Best Oral paper presentation award for the paper entitled "Mendelian susceptibility to Mycobacterial diseases experience at NIIH." At the 3rd National Conference on Primary Immunodeficiency (ICPID) held at New Delhi from 12th to 13th March 2016

Ms Jahnavi Aluri, SRF

Received Best poster presentation award for the paper entitled "Retrospective testing of T cell receptor excision circles in severe combined immunodeficiency disorders and combined immunodeficiency disorders" at the 3rd National Conference on Primary Immunodeficiency (ICPID) held at New Delhi from 12th to 13th March 2016.

Department of Hematogenetics

Dr Malay Mukherjee, Scientist E

Organized Sickle Cell Training Camp for National Rural Health Mission at Chandrapur on 14th February 2016.

Attended "39th Annual Conference of Mumbai Hematology Group" held at Mumbai from 12th to 13th March, 2016 and chaired a session.

Attended "6th Hemoglobin Update Meets" held at Mumbai on 19th April, 2016.

Dr Anita Nadkarni, Scientist E

Attended and chaired a session in "39th Annual Conference Mumbai hematology group" held at Mumbai from 12th to 13th March 2016.

Participated in Pall Forte Bio user meet held at Mumbai on 11th April 2016.

Participated in "6th Hemoglobinopathies Update Meets" by Bio-Rad Laboratories India held on 19th April 2016 at Mumbai.

Dr Prabhakar Kedar, Scientist D

Invited as a Faculty member and delivered a lecture on "Congenital non-spherocytic hemolytic anemia – Indian scenario" at the 39th Annual Conference of Mumbai Hematology Group held at Mumbai from 12th to 13th March 2016.

Following staff and students have attended and presented papers in the International conference on "Celebrating Genetics – The Human way and 41st Indian Society of Human Genetics (ISHG) Annual Meeting" organized by Vision Research Foundation (VRF) held at Chennai from 3rd to 5th March, 2016:

1. **Dr. Khushnooma Italia, RA:** Presented a poster entitled "Can Hydroxyurea also work as an iron chelator in β-thalassemia patients?".
2. **Dr. Madahvi Sawant, PDF:** Awarded 2nd prize for poster presentation for the paper entiltled "HbF induction by hydroxyurea works through MIR96 inihibition in sickle cell anaemia patients."
3. **Priya Hariharan, SRF:** Presented a poster entitled "Hereditary Persistance of Fetal Hemoglobin (HPFH) due to co-inheritance of β-thalassemia with novel Gγ-globin promoter mutation [-ATAAG (-533 to -529)]."

Following staff and students have attended and presented papers in the 39th Annual Conference of MHG held at Mumbai from 12th -13th March 2016:

1. **Dr. Prashant warang, TA:** Presented a paper entitled "Effects of oxidative stress on red blood cell in sickle cell anemia and the protective effect of natural antioxidant - fermented papaya preparation: An in-vitro study."

2. **Pratibha Sawant, TA:** Presented a poster entitled "Identification of five rare haemoglobin variants in India: A careful Investigation."

3. **Dr. Khushnooma Italia, RA:** Presented a poster on "Genetic Determinants for Response to Hydroxyurea Therapy in β-thalassemia Patients".

4. **Dr. Madhavi Sawant, PDF:** Awarded Dr. JC Patel best paper Award for the paper entiltled "Role of MicroRNA in Hydroxyurea mediated HbF induction in Sickle anemia patients."

5. **Dr. Dipti Upadhye, SRF:** Presented a paper entitled "Valproic acid and Angelicin induce gamma globin gene expression in K562 cell lines and in erythroid progenitors of sickle cell anemia patients."

6. **Priya Hariharan, SRF:** Presented a poster entitled "Role of HMOX-I gene promoter on HbF levels in sickle cell homozygous patients."

7. **Pallavi Mehta, TA:** Presented a paper entitled "Hemoglobin Andrew-Minneapolis: A Case report from India."

Following staff and students have attended Workshops / Seminars:

1. **Prtibha Sawant, TA and Dr. Dipti Upadhye, SRF** attended Internal Audit & Quality Management Systems certificate course held at Mumbai from 30th March to 2nd April.

2. **Dr. Madhvi Sawant, PDF and Miss Priya Hariharan, SRF** attended Pall Forte Bio user meeting and seminar on "Label-Free Interaction Analysis" held at Mumbai on April 11th April 2016.

3. **Priyanka Parab, Lab Technician and Miss. Ritika Deogharkar, Lab Technician** attended "6th Hemoglobin User update meet" held at Mumbai on 19th April 2016.

4. **Priya Hariharan, SRF** attended workshop on Biostatistics and Research methodologies organized by CIENCIA Pvt. Ltd. held at Hyderabad from 22nd to 24th April 2016**.**

Department of Transfusion Medicine

Dr Ajit Gorakshakar, Scientist F

Attended meeting for Bombay Phenotype individuals organized by NGO Think Foundation and State Blood Transfusion Centre on 13th February 2016 and delivered a talk on "Bombay phenotype" and resolved their queries related to blood donation.

Attended pre conference workshops on Thalassemia and Sanger DNA Sequencing, organized during 5th Annual Conference of Molecular Pathology Association of India on 10th March 2016 at PGIMER, Chandigarh and delivered following talks;

1. " Hb electrophoresis and HPLC"

2. "Sanger sequencing: Interpretation of data".

Attended Expert Group meeting for RDB kit held at ICMR, New Delhi on 30th March 2016.

Received **The Bharat Excellence Award** for extra ordinary achievements in the field of bio medical research from Friendship Forum held at New Delhi on 25th April 2016.

Department of Hemostasis

Dr Shrimati Shetty, Scientist E

Invited to talk on "Factor assays-troubleshooting at the 2nd "International Conclave - Total Quality Management and Recent Trends in Transfusion Medicine" held at Kokilaben Dhirubhai Ambani Hospital, Mumbai from 19th to 21st February 2016.

Participated in the Haematology/Haemostasis Analyser's Education Meet at Christian Medical College, Vellore from 26th to 27th of February 2016

Invited by Jammu Haemophilia Chapter and HFI, India to talk on Diagnosis of Hemophilia on 5th of March 2016

Invited to talk on "Genetic Diagnosis of Bleeding Disorders in the 39th Annual Meeting of MHG from 12th to 13th of March 2016

Invited by the University of Pune as an expert in the International Consultation on birth defects, held at Pune from 17th - 18th of March, 2016.

Following students have attended and presented papers in the 39th Annual Conference of MHG held at Mumbai from 12th -13th March 2016:

1. **Anshul Jadli, SRF:** Presented a paper entitled "Circulating microparticles as a novel biomarker for early prediction of preeclampsia and other adverse pregnancy conditions".
2. **Aniket Prabhu Desai, PhD student:** Presented a paper entitled "Heritable and acquired risk factors in cases of recurrent retinal vein occlusion".
3. **Darshana Mirgal, PhD student:** Presented a paper entitled "Modulation of the function of Activated Protein C (APC) to improve the clinical phenotype of Haemophilia".
4. **Rutuja Deshpande, SRF:** Presented a poster entitled "A novel mutation p.Pro353His in antithrombin gene (*SERPINC1)* gene in an extended Omani family".

Department of Cytogenetics

Dr. V. Babu Rao, Scientist E

1. Invited as a chairman for the selection committee at NIRRH, Mumbai on 27th January 2016 to select JRFs.
2. Attended 41st Annual conference of ISHG held at Sankara Netralaya, Chennai, from 3rd to 5th March 2016 and presented a paper entitled "BCR-ABL kinase domain mutations in Imatinib resistant Indian chronic myeloid leukemia patients: Frequency and clinical outcome".
3. Attended Executive committee meeting of Molecular Pathologists Association of India (MPAI) held at SRL laboratories, Mumbai, on 5th April 2016.

Seema Korgaonkar, TO

Attended Internal auditor and quality management systems certificate course conducted by Foundation for Quality (India) held at Seth G. S. Medical College, Mumbai from 30th March to 2nd April 2016.

Following students have attended and presented papers in the International conference on "Celebrating Genetics – the human way and 41st Indian Society of Human Genetics (ISHG) Annual Meeting" organized by Vision Research Foundation (VRF) held at Chennai from 3rd to 5th March, 2016:

1. **Purvi Mohanty, SRF**: Presented a paper entitled "Cytogenetic and Molecular Profile of Indian patients with de-novo Myelodysplastic Syndromes.
2. **Avani Solanki, SRF**: Presented a Paper entitled "Fanconi anemia gene mutations in India: Genotype and Phenotype correlation".

Visitor :

1. **Prof. Minoru Takata,** Director, Radiation Biology Center, Kyoto University, Japan, visited Cytogenetics laboratory under Indo-JSPS programme from 11th to 13th February 2016.

Department of Clinical & Experimental Immunology

Dr. Vandana D. Pradhan, Scientist B

Attended Internal auditor and quality management systems certificate course conducted by Foundation for Quality (India) held at Seth G. S. Medical College, Mumbai from 30th March to 2nd April 2016

Library

Vijay G Padwal, ALIO

Attended Knowledge Resource Management (KRM) 2016 on Digital Contents, Copyrights and Libraries at Tata Memorial Hospital, Mumbai from 28th to 29th Jan 2016.

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NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY (ICMR)

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Swachh Bharat Abhiyan at Parel English High School

Bombay Blood Group donors meet

Inauguration of 39th Annual Conference of Mumbai Hematology Group

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