Evolution of technology for molecular genotyping in blood group systems

Ajit Gorakshakar¹, Harita Gogri¹ & Kanjaksha Ghosh²

¹Department of Transfusion Medicine, ICMR- National Institute of Immunohaematology, Mumbai & ²Surat Raktadan Kendra & Research Centre, Surat, India

Received June 10, 2016

The molecular basis of the blood group antigens was identified first in the 1980s and 1990s. Since then the importance of molecular biology in transfusion medicine has been described extensively by several investigators. Molecular genotyping of blood group antigens is one of the important aspects and is successfully making its way into transfusion medicine. Low-, medium- and high-throughput techniques have been developed for this purpose. Depending on the requirement of the centre like screening for high- or low-prevalence antigens where antisera are not available, correct typing of multiple transfused patients, screening for antigen-negative donor units to reduce the rate of alloimmunization, etc. a suitable technique can be selected. The present review discusses the evolution of different techniques to detect molecular genotypes of blood group systems and how these approaches can be used in transfusion medicine where haemagglutination is of limited value. Currently, this technology is being used in only a few blood banks in India. Hence, there is a need for understanding this technology with all its variations.

Key words ABO alleles - India - microarrays - molecular genotyping - red blood cell antigens - single nucleotide polymorphisms

Introduction

Since the time Karl Landsteiner discovered ABO blood groups, agglutination was the method of testing for detecting the presence of blood group antigens and antibodies. Apart from this, adsorption-elution, serum inhibition and anti-human globulin test are some other techniques routinely used in transfusion medicine. The first major breakthrough for blood group genotyping at molecular level occurred when GYP A, the gene for the MN blood group system was cloned in 1986¹. This was followed by cloning of the genes for ABO and Rh blood group systems in 1990 and 1992 respectively²,³. Subsequently, the genes for the other blood group systems were also cloned. After studying these genes carefully, it was observed that single nucleotide polymorphism (SNP) is the main cause of variation in these genes. One or more SNPs in particular blood group system can help to identify specific alleles of that system. Apart from this, various other causes responsible for variation seen in different blood group alleles at the molecular level include deletion of a gene or an exon or a nucleotide(s) (e.g. whole gene deletion seen in case of Rh system, point deletion(s) in case of ABO, Kell, Duffy, Dombrock blood group systems), sequence duplication plus a nonsense mutation (e.g. inactive RHD gene), formation of hybrid genes
In the ABO blood group system, initially, the DNA sequence of ‘A’ group specific transferase was partially sequenced from human lung tissue\(^4\). Subsequently, various A, B and O alleles were cloned and sequenced, and sequence variations among them were identified\(^5\)-\(^8\). These variations were based on the presence of different SNPs as well as due to insertion or deletion of single nucleotides. This prompted the scientists to develop molecular techniques to identify these alterations to characterize various alleles. The main advantages of these techniques were: (i) small amount of DNA was required, and (ii) an individual’s genotype could be determined without doing laborious and time-consuming family investigations or without detecting blood group specific molecules on the surface of red blood cells (RBCs).

Today, 35 blood group systems comprising of more than 300 specific antigens are known [International Society of Blood Transfusion (ISBT), http://www.isbtweb.org]. At molecular level, more than 1200 alleles have been identified while 50 genes are involved in the expression of blood group antigens\(^9\). Several review articles on blood group genotyping covering various aspects have been published so far\(^10\)-\(^12\). In the ABO blood group system, several alleles encoding each antigen are identifiable and this has tremendous application in forensic science, chimerism, etc. Similarly, such heterogeneity may also throw some light on hitherto unexplainable or partially explainable phenomenon of ABO isoimmunization. For antigens from other blood group systems, this technology helps to identify several alleles belonging to these systems and type in alloimmunized patients\(^11\).

In the present review, initial development of DNA-based technology for the detection of molecular genotypes has been discussed in the context of the ABO blood group system along with the evolution of these technologies from low to medium to high throughput for genotyping of other blood group antigens as well. This field is currently at the crossroads, bringing in new perspectives and techniques to replace a century-old practice of haemagglutination-based cross-matching in transfusion medicine.

**Structure of ABO gene and alleles**

The ABO gene is located on ‘q’ arm of chromosome 9 (9q34). It encodes a glycosyltransferase which catalyses the addition of a monosaccharide onto a carbohydrate sequence expressing the H antigen. N-Acetyl-galactosamine is a specific sugar molecule responsible for expression of ‘A group’ whereas D-galactose is a specific sugar molecule responsible for ‘B group’ expression. The entire locus spans over 18 kb and consists of seven exons\(^13\). Fig. 1 gives a schematic representation of ABO gene and Table I depicts the sizes (bp) of exons and introns. Exons 6 and 7 of the gene encode for 77 per cent of the full coding region of the glycosyltransferase and 91 per cent of the catalytically active soluble transferase protein\(^13\). Therefore, initially, techniques were developed to identify ABO alleles by screening SNPs from exons 6 and 7 of the gene.

A101 is considered as a reference allele. B101 allele differs from A101 allele in seven positions. Four of these changes [nucleotide (nt) 526, 703, 796, 803] result in amino acid substitution. There are two common A alleles responsible for A\(^1\) group; one is A101 reference allele and the other one is A102. The latter has single nucleotide substitution at position 467. The O alleles are divided into two categories: deletional and non-deletional. The O01 is the most common type of deletional allele, and it differs from the A101 allele by a single nucleotide deletion at nt261. The O\(^1\) variant i.e. O02 allele also from the deletional category shows nt261 deletion

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**Table I. Sizes of the exons and introns of the ABO gene**

| Exons | Size (bp) | Introns | Size (bp) |
|-------|-----------|---------|-----------|
| 1     | 28        | 1       | 12,982    |
| 2     | 70        | 2       | 724       |
| 3     | 57        | 3       | 1451      |
| 4     | 48        | 4       | 1686      |
| 5     | 36        | 5       | 554       |
| 6     | 135       | 6       | 1052      |
| 7     | 688       |         |           |

*Source: Ref 14*
and nine single base substitutions as compared to A101 allele. The O03 allele is an allele from non-deletional category and shows four single nucleotide substitutions when compared with A101 reference allele. The detailed SNP positions occurring in the seven common ABO alleles are shown in Fig. 2.

**Low-throughput techniques**

**ABO genotyping:** Techniques were first developed to discriminate between O and non-O groups using Kpn I/Bst EII enzymes to determine the presence or absence of G at nucleotide position 261 specific to the O allele. Polymerase chain reaction (PCR) followed by allele-specific restriction fragment length polymorphism (RFLP) using BssHII/NarI and HpaII/AluI restriction enzyme pairs were used to distinguish between A and O alleles. Initially, 14 individuals of different blood groups were analyzed by Southern Blot technique and the results were compared with those of PCR-RFLP technique. This allowed the homo/heterozygous detection of SNP at this position.

Later on, techniques were developed to identify common ABO alleles by characterizing minimum number of SNPs. For example, normal A and B allele polymorphisms are present at seven positions. Of these, four sites namely, 526, 703, 796 and 803 are crucial. Hence, techniques were developed to identify A and B alleles by analyzing these four sites. G261 deletion for detection of O allele and G703A substitution specific for B allele were taken into account by some researchers for differentiating these alleles. A similar approach using C526G polymorphism instead of G703A to detect B alleles was used by Stroncek et al. However, they found an anomalous A allele which showed all polymorphisms as a normal A allele except for C526G polymorphism. This clearly showed that more than one polymorphism were required to differentiate between A and B alleles. In 1996, PCR-RFLP was developed to detect polymorphisms at four sites, namely, 261, 526, 703 and 796. This helped to identify A, B and O alleles. The use of NarI which has a cleavage site at nt 526 helped them to differentiate between O01 and O03 alleles. A clinically applicable and simple genotype screening technique based on previously undescribed HpaII site in 3’ untranslated region of the ABO gene was developed. The polymorphism G1096A was found in A101 and O01 alleles but not in B101 and O03 alleles. This polymorphism abolishes the HpaII site and is valuable marker in identification of ABO alleles. The same enzyme was found to be useful in identifying polymorphisms associated with alleles such as A201 (C467T), B101 (G703A) and O03 (G1096A). Direct sequencing of the PCR amplified fragment for ABO genotyping was then developed in 1997.

The second approach which was simultaneously developed was PCR using allele-specific primers (PCR-ASP). The main advantage of this method was that it did not require post-amplification treatment with restriction enzymes thus reducing the processing time. However, in case of ABO blood group system, more than one SNP is required to be identified to characterize the alleles. Hence, more than one set of primers are required. Initially, ASP incorporated with P32 were developed to characterize common ABO alleles. However, due to radioactivity extra care was required while using this technique. PCR-sequence specific oligonucleotide method developed in 1996 was based on specific sequence of the allele using an oligonucleotide probe hybridization reaction. To detect three alleles (O01, O02 and O03) within O group, ten probes were developed. Alternatively, other researchers developed methods that required seven or eight PCR reactions to identify the common ABO alleles. Later on, the reactions were multiplexed, and thus, only two PCR reactions to identify the common ABO alleles were required. One of the drawbacks of this approach was that homo- or heterozygosity at each SNP could not be detected.

Initially, all the SNPs covered by ASPs to identify common alleles were from exons 6 and 7 of the ABO
gene. Later on, primers were redesigned to screen all exons, two regulatory regions and introns except intron 1. This study revealed several unknown polymorphisms in coding as well as non-coding regions. Based on this, and other similar studies, various ABO alleles were reanalyzed and renamed, and database of these alleles has been developed (dbRBC, www.ncbi.nlm.nih.gov/projects/gv/mhc/xslcgi.cgi?cmd=bgmut/systems).

Some investigators developed another approach which involved combined use of PCR-RFLP and PCR-ASP techniques to detect hybrid alleles as well as weaker variants of A and B.

Due to extremely heterogeneous nature of ABO gene and the possibility of identifying new alleles based on the SNPs that were not detected earlier, some researchers tried to use mutation scanning techniques such as denaturing gradient gel electrophoresis (DGGE) or single strand conformation polymorphism (SSCP) to detect ABO alleles. Johnson and Hopkinson identified four different O alleles and two B alleles by amplifying 250 bp fragment of ABO gene and by running DGGE for 19 h at 61°C. Akane et al. could identify four common ABO alleles by SSCP analysis of a single PCR product covering exon 6 of the ABO gene. Ogasawara et al. further developed this technique and analyzed four PCR products amplified from exons 6 and 7. Thirteen different alleles (common as well as rare) were identified. This approach was further developed by multiplexing three PCRs in a single tube and analyzing the three amplified products by SSCP in a single lane. This could identify polymorphisms at nine positions in exons 6 and 7 (nt 261, 297, 467, 526, 646, 657, 681, 1059 and 1096). Based on these SNPs, seven common ABO alleles could be differentiated using a ‘single tube-single lane format’. Initially, a catalogue of various patterns corresponding to different ABO genotypes has to be prepared. For this, genotypes of the samples should be determined by PCR-RFLP technique. The same samples are then analyzed by SSCP to develop a catalogue. This method can also identify new alleles based on unknown SNPs in the three amplified fragments. This technology has been used to identify common ABO alleles in the Indian population.

To genotype three major alleles (A', B and O') of the ABO blood group system simultaneously, inverse PCR technique was developed. In this technique, sequence (about 1.7 kb) from exons 6 and 7 of each allele was amplified, both the termini of the fragment were then ligated and allele typing was performed by the inverse PCR-RFLP and inverse PCR-ASP techniques. In a modified assay, labelled primers were used and the alleles were identified by measuring the excess radioactivity present in the amplified reaction mixture.

A multiplexed single base primer extension reaction which allows the simultaneous determination of six SNPs (nt 261, 297, 681, 703, 802, 803) has also been described to detect common ABO genotypes. The evolution of the molecular genotyping techniques for characterization of ABO alleles is illustrated in Table II.

The technology was developed to extract DNA from various tissues and characterize the ABO alleles. This was a very sensitive technique as ABO genotype was determined using only 0.1 ng of genomic DNA. In addition, the ABO genotype could also be detected from the tissues obtained from bones, muscles, teeth, nails, semen contaminated vaginal fluid, etc. where the conventional serological technique could not be used. Lee et al. developed ABO genotyping technique using four reactions of allele-specific multiplex PCRs to detect five common ABO alleles. In this, whole blood without extracting DNA was used. Here, ‘AnyDirect’ PCR reaction buffer was used. It conserved the enzyme activity of DNA polymerase for effective use in direct PCR from whole blood which contained PCR inhibitors. The specificity and sensitivity of the novel buffer used in this reaction was good. This is a rapid and convenient technique and has many applications in forensic medicine. ABO genotyping using fresh blood, hair, body fluids, etc. without extracting DNA has also been described where, a fast PCR instrument and optimized Taq polymerase were used. The amplified products were analysed by GeneScan programme after capillary electrophoresis. For amplifications, ASP was used. This technique saved lot of time.

A kit based on PCR-SSP technique has been developed for detection of common ABO alleles as well as for some weaker variants such as Aⅰ, AS, B and B. The kit also contains ASP to detect common alleles of Kell, Kidd and Duffy blood group systems.

Genotyping of Rh and other minor blood group systems: After ABO, Rh is the second clinically important blood group system. It is encoded by two genes, namely, RHD and RHCE, which are closely linked and highly homologous and located on chromosome 1, exact location being 1p36.1. Both these genes are inherited together. The RHD gene
is responsible for the expression of the D antigen. Multiple genetic events may be responsible for the absence of D antigen on RBCs. Among Caucasians, D negativity is associated with deletion of \( RHD \) gene between upstream and downstream of Rhesus boxes, while D negativity with intact \( RHD \) gene has been observed in many other populations.

Shao et al. studied 76 RhD-negative cases and 26 Del cases from China and found as many as five alterations responsible for affecting the expression of D antigen on RBCs. At phenotypic level, 54 antigens have been detected while at molecular level 493 alleles have been illustrated. Thus, Rh is a very complex system and to develop a strategy like PCR-SSP for detecting various Rh alleles in a particular population group or geographical area, knowledge about the profile of alterations in the RH gene in that particular group or region is essential.

Very few studies have reported alterations in RHD at molecular level. As it is very difficult to design a generalized technique to detect Rh alleles in different populations, only a few investigators have included detection of RH alleles in genotyping platforms.

Subsequently, molecular basis of other blood group systems was investigated. MNS (46 antigens), Diego (22 antigens) and Kell (35 antigens) are some of the blood group systems where many antigens are recognized phenotypically, and 59, 91 and 92 alleles have been identified among these blood group systems, respectively. In majority of the cases, alleles have been identified on the basis of SNPs. Hence, PCR-SSP technique was developed to detect these alleles.

### Table II. Evolution of different techniques for molecular genotyping with reference to ABO blood system

| Year | Author          | Method             | Nucleotide position analyzed                  | No of PCR reactions | Reference No. |
|------|-----------------|--------------------|-----------------------------------------------|---------------------|---------------|
| 1990 | Yamamoto et al  | PCR-RFLP           | 261, 526, 703, 796                            | 4                   | 2             |
| 1992 | Ugozoli and Wallace | PCR-ASP using P32 labelled primers | 261, 526, 703 | 1 | 21           |
| 1992 | Lee and Chang   | PCR-RFLP           | 258, 700                                      | 2                   | 15            |
| 1992 | Johnson and Hopkinson | PCR-DGGE    | 261, 297                                      | 1                   | 29            |
| 1993 | O’Keefe & Dobrovic | PCR-RFLP          | 261, 703                                      | 1                   | 16            |
| 1995 | Stronccek et al | PCR-RFLP           | 261, 526                                      | 2                   | 17            |
| 1995 | Olsson and Chester | PCR-RFLP      | 261, 467, 703, 1096                           | 1 (multiplex)       | 19            |
| 1996 | Mifsud et al    | PCR-RFLP           | 261, 526, 703, 796                            | 4                   | 18            |
| 1996 | Gassner et al   | PCR-ASP           | 261, 802, 803, 1059                           | 2                   | 23            |
| 1996 | Mifsud et al    | PCR-SSO           | 261, 297, 526, 802                            | 2                   | 22            |
| 1996 | Akane et al     | PCR-SSCP          | -                                             | 1                   | 30            |
| 1996 | Ogasawara et al | PCR-SSCP          | 261, 297, 467, 526, 703, 796, 802, 1061        | 4                   | 31            |
| 1997 | Nata et al      | Direct sequencing  | All positions encompassing the amplified product |                      | 20            |
| 1997 | Procter et al   | PCR-ASP           | 261, 467, 706, 802, 1061                       | 7                   | 24            |
| 1998 | Pearson & Hessner | PCR-ASP         | 261, 706, 802, 1061                           | 2                   | 25            |
| 2000 | Yip             | PCR-SSCP          | 261, 297, 467, 526, 646, 657, 681, 1059 and 1096 | 4                   | 32            |
| 2000 | Kobayashi and Akane | IP-RFLP, AISP | 261, 796, 803                                 | 2                   | 34            |
| 2001 | Watanabe et al  | CASPA              | 261, 796, 803, 1065                           | 1                   | 35            |
| 2003 | Seltsam et al   | PCR-SSP           | 52 known polymorphisms and remaining rare       | 102                 | 26            |
| 2004 | Doi et al       | Multiplex single-base primer extension reaction | 261, 297, 681, 703, 802, 803                  | 1                   | 38            |

RFLP, restriction fragment length polymorphism; DGGE, denaturing gradient gel electrophoresis; SSO, sequence specific oligonucleotide; SSCP, single strand conformational polymorphism; ASP, allele specific PCR; IP-RFLP, inverse PCR- restriction fragment length polymorphism; AISP, allele specific inverse PCR; CASPA, consumed allele-specific primer analysis.
et al\textsuperscript{53} developed this technique to detect various alleles of Duffy blood group system, while Hessner et al\textsuperscript{54} designed sequence-specific primers to detect alleles of Kidd blood group system. Yan et al\textsuperscript{55} used this technique to identify alleles of eight blood group systems (ABO, Rh, MNS, Kidd, Duffy, Cartwright, Scianna and Colton) among Chinese; while Touinssi et al\textsuperscript{56} developed this approach to screen French Basques to detect the alleles of six blood group systems (Kell, Kidd, MNS, Dombrock, Colton and Cartwright).

Medium-throughput techniques

Assays with medium throughput include real-time PCR, Sanger DNA sequencing and Pyrosequencing. In real-time PCR, amplified DNA is detected as the reaction progresses\textsuperscript{57}. Three methods are used for the detection of the products\textsuperscript{58} (i) Non-specific fluorescent dyes like SYBR green which intercalates with any double-stranded DNA; (ii) TaqMan probes; and (iii) Hybridization probe protocol involving fluorescence resonance energy transfer (FRET). Sanger DNA sequencing involves the principle of termination of growing DNA chain after inclusion of dideoxynucleotide triphosphates with fluorochrome labelled bases. SNPs can be identified after reading the sequence of the gene. In pyrosequencing, a pyrophosphate molecule is detected on nucleotide incorporation. Pyrosequencing technique has been used to detect alleles of Kell, Kidd and Duffy blood group systems\textsuperscript{59}.

High-throughput techniques

Assays with high throughput involve the use of microarray technology. Majority of the alleles of various blood group systems can be identified by detecting one or two SNPs. Microarray technology can identify large number of SNPs at the same time from genomic DNA. This technology is generally used to detect the extended genotype of a donor. The Blood Chip (Progenika Biopharma, Spain), HEA BeadChip (IMMUCOR, USA), GenomeLab (Beckman Coulter USA), Progenika IDcore+ (Progenika Biopharma, Spain) and The Bead Chip (Bioarray Solutions, USA) are some of the microarray platforms available for molecular genotyping. Glass slides or beads or microtitre plates are used to attach SNP specific DNA probes. The ‘on-chip’ test is based on the hybridization of targets which were amplified earlier by multiplex PCR followed by a detection step allowing the simultaneous identification of many SNPs involved in detection of various alleles of different blood group systems which in turn help to determine the extended genotype.

Three studies describing molecular genotyping of various blood group systems and platelet antigens using SNP platforms specific for blood group genotyping were published simultaneously in 2005\textsuperscript{50,60,61}. All the three groups used multiplexed PCR based assays with visual endpoints. Hashmi et al\textsuperscript{60} identified 18 SNPs describing 36 alleles of more than 11 blood group systems. Denomme and Van Oene\textsuperscript{50} screened 372 samples for 12 SNPs detecting several blood group and platelet antigens. Beiboer et al\textsuperscript{61} detected various platelet antigens in 92 blood donors. Subsequently, several investigators reported\textsuperscript{52,62-67} their results of molecular genotyping using various platforms of microarray technology, the details of which is summarized in Fig. 3.

Fluidic microarray system (Luminex xMAP) is a microsphere-based technology used for blood group genotyping. In this method, microspheres are dyed with two spectrally distinct fluorochromes. Using precise amounts of each of these fluorochromes, an array is created. The system detects PCR amplified targets involving various SNPs by direct hybridization to microspheres which are coupled to allele (SNP) specific oligonucleotides. Using this technology, Karpasitou et al\textsuperscript{64} analysed alleles of seven different blood group systems. Later on, this technique was validated by the same group using biotinylated PCR product. This method involves two multiplex PCRs for screening of 16 antigens of Kell, S, Duffy, Kidd, Lutheran and Colton blood group systems\textsuperscript{58}.

Nanofluidic open array system has been used to genotype 32 SNPs for 42 blood group antigens in more than 40,000 donors\textsuperscript{66}. The results were confirmed by phenotyping before release of blood unit. This helped them to get antigen-negative blood units. Hence, they abandoned the screening by serology. These results are encouraging as these help to shift to molecular genotyping from serological techniques, provided that the platform for molecular genotyping is well established.

Other techniques of high-throughput technology

Mini sequencing or the SNAPSHOT assay: This method involves SNP analysis to detect the exact base and computer-assisted visualization of the specific alteration/polyorphism. Fluorescently labelled dideoxynucleotides are used with multiplex PCR product as a template. After the hybridization and extension steps, the fluorescent signals from the array are measured and the genotypes are determined by
cluster analysis. This technique has been successfully used to detect the most common alleles of the ABO blood group system\textsuperscript{69}. The SNaPshot platform has been used to detect the alleles from 12 different blood group systems\textsuperscript{70}. Using this platform, nine SNPs defining 16 blood group alleles from five blood group systems were simultaneously identified among blood donors from Brazil\textsuperscript{71}.

**Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS):** This technique can discriminate two DNA fragments which differ in a single nucleotide. The technique involves two parts: first, laser-induced desorption/ionization of matrix molecules and second, separation and analysis of these molecules on the basis of their intrinsic physical properties. It is a quantitative as well as qualitative technique and can analyse several SNPs in a single reaction which takes about eight hours. This technique has been used to detect some alleles of Kell blood group system\textsuperscript{72}. Two groups from Switzerland also used this system to identify several blood group alleles among Swiss blood donors\textsuperscript{73,74}.

**Limitations of molecular blood group genotyping:**
Low-throughput techniques such as PCR-SSP and PCR-RFLP were initially developed to detect various alleles of ABO blood group system. However, many SNPs need to be identified to characterize a particular ABO allele. Furthermore, considering the pace of discovery of new alleles in ABO as well as other blood group systems, it became difficult to develop a low-throughput method to detect all the alleles. These techniques require post-PCR analysis and may give false- or false-negative reactions with certain hybrid alleles. There is a preferential amplification of only one allele when present in heterozygous condition.

Medium-throughput techniques such as real-time SNP assay and DNA sequencing are time-consuming and complex techniques. Further, in DNA sequencing, large data are generated which are difficult to store and analysis of the data requires bioinformatic tools. Second, the sample to results takes days to weeks which later on decreased due to automation and optimization of workflow\textsuperscript{74}.

High-throughput donor typing techniques vary considerably in methodology, antigen selection, throughput and cost. Even though several techniques are reported, only a few report this as an ongoing activity and provide data on large number of samples\textsuperscript{51,66,75}. Although these are highly efficient for testing a large number of samples for multiple blood group alleles simultaneously; but these may be suitable for screening only some populations. The new alleles identified

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### Fig. 3. Various platforms of microarray technology used for blood group genotyping. Numerals in parentheses denote reference number.

| Platform | Description | Reference |
|----------|-------------|-----------|
| Bead-based | Beadchip (BioArray Solutions, USA) (61) | • 24 antigens; 18 SNPs  
• Systems: MNS, RhCE, LU, KEL, FY, JK, DI, DO, CO, SC, LW  
• Time: 96 samples in 5h 1h 'hands on time' |
| Chip-based | Bloodchip (Projenika Biopharma SA, Spain) (62) | • 47 antigens; 116 (RH&D:73, ABO:12, others:31) SNPs  
• Systems: ABO, MNS, RH, RhCE, KEL, FY, JK, DI, DO, CO  
• Time: 20 samples, 10h |
| 384 wells plate | Genome Lab SNPstream (Beckman Coulter, USA) (51) | • 19 antigens; 12 SNPs  
• Systems: MNS, RH, RhCE, KEL, FY, JK, DI, HPA1  
• Time: 384 samples, 36h |
| Nanofluidic-based | OpenArray (Biotrove) (64) | • 16 antigens; 17 SNPs  
• Systems: RHCE, FY, JK, LU, KEL, DO |
| Luminex xMAP (Luminex Corp, USA) (65) | • 16 antigens; 8 SNPs  
• Systems: MNS, KEL, FY, JK, CO, LU  
• Time: 2x96 samples in one day per blood group |
| HIFI technology (AXO Science, France) (66) | • 24 antigens; 26 SNPs  
• Systems: KEL, JK, FY, MNS, YT, CO, DO, LU, DI  
• Time: 4.5 h |
| Genome Lab SNPstream (Beckman Coulter, USA) (51) | • 22 antigens; 12 SNPs  
• Systems: MNS, RhCE, KEL, FY, JK, HPA1, HPA2 & HPA5  
• Time: 6,000 genotypes per day |
| OpenArray real-time PCR system (Life technologies) (65) | • 42 antigens; 32 SNPs  
• Systems: MNS, LU, RH, KEL, FY, JK, DI, YT, SC, DO, CO  
• Time: 700 samples / 36 h |
cannot be detected and have to be incorporated into the testing platform. It has then to be validated again before routine use. These technologies are expensive and one has to consider the cost. As per the requirement of the centre, the platform with number of antigens to be screened can be designed (e.g. clinically important antigens, minor antigens, rare alleles, null types) which can also take into account cost per sample.

**Molecular genotyping in India:** Only one study has described molecular ABO genotyping in Indian population\(^3\). In this study, molecular genotyping was done by PCR-RFLP and PCR-SSCP techniques. Totally, 13 common and rare alleles belonging to the ABO blood group were identified. Considering the heterogeneity of Indian population many more alleles are likely to be detected among various population groups.

Identification of rare donors is a critical factor in establishing a rare donor registry\(^7\). It can be done by performing mass screening of donors for clinically important blood group antigens by serological testing or by using gel technology. However, serological typing of large cohorts of donors is labour intensive and expensive exercise and many a times hampered by the lack of reliable antisera. To overcome this, genotyping of various blood group systems will be an important aspect.

There are many reports where healthy blood donors from different cities in India have been screened to look for the frequency of various blood group antigens\(^7\).\(^8\)\(^-\)\(^12\). Similarly, many studies report the prevalence of alloimmunization against various antigens from different parts of India\(^13\)\(^-\)\(^20\). Recently, in a screening among 1221 donors from Mumbai, 261 donors who lacked a combination of clinically important common antigens, were detected\(^9\). All these studies have used serological techniques. At National Institute of Immunohaematology, Mumbai, India, using a PCR-SSP technique, a study has been initiated to detect antigens of Kell, Kidd, Duffy, MNS, Indian, Diego blood group systems among donors\(^10\)\(^11\). Also RhD typing of the foetus of a Rh-negative pregnant woman by using non-invasive DNA-based technique has been established\(^12\).

**Conclusion**

Molecular genotyping is set to play an important role in the routine blood banks in the future. Whether it will replace serology completely or not needs to be seen. ABO genotyping will be useful in ABO-incompatible bone marrow transplant cases and in case of identifying weaker subtypes. However, complex genotyping strategies are required to identify correct ABO or Rh alleles. So serology will still be required to type for these systems. Molecular genotyping will help to type donors for a wider spectrum of minor blood group antigens and also genotype blood group antigens of multiply transfused patients such as sickle cell anaemia or β-thalassemia or patients having positive direct antiglobulin test. Providing a donor’s blood to the patient after studying extended antigen profile will help in preventing alloimmunization. Alloimmunized transfusion recipients will also be benefited if the donor’s blood is electronically cross matched using the extended array of SNPs. This technology can be used to screen for uncommon or rare antigens or to look for the absence of high-frequency antigens or to detect the antigens where specific antisera are not available. Molecular genotyping will also play an important role in non-invasive prenatal RhD typing of foetus of RhD-negative pregnant woman. In short, molecular genotyping will make transfusion medicine more personalized and patient-oriented.

**Conflicts of Interest:** None.

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Reprint requests: Dr Ajit Gorakshakar, ICMR-National Institute of Immunohaematology, 13th Floor, New Multistoreyed Building, K. E. M. Hospital Campus, Parel, Mumbai 400 012, Maharashtra, India e-mail: ajit5678@yahoo.com