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DNA-Based Methods in the Immunohematology Reference Laboratory

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Abstract

Although hemagglutination serves the immunohematology reference laboratory well, when used alone, it has limited capability to resolve complex problems. This overview discusses how molecular approaches can be used in the immunohematology reference laboratory. In order to apply molecular approaches to immunohematology, knowledge of genes, DNA-based methods, and the molecular bases of blood groups are required. When applied correctly, DNA-based methods can predict blood groups to resolve ABO/Rh discrepancies, identify variant alleles, and screen donors for antigen-negative units. DNA-based testing in immunohematology is a valuable tool used to resolve blood group incompatibilities and to support patients in their transfusion needs.

Keywords

Antibody identification; blood group antigens; molecular methods; DNA testing; blood groups; identification of blood groups

1. Introduction

Immunohematology reference laboratories arose from a need for a more thorough examination of the serological nature of a positive antibody screen, incompatible crossmatch, or unusual antigen reactivity. The discovery of blood group antigens has occurred on a relatively continual basis from the 1940s to the present day, with nearly 300 blood group antigens characterized to date [1]. Historically, resolution of problems in the immunohematology reference laboratory has depended on hemagglutination. While hemagglutination remains the gold standard method, DNA-based methods are now being applied to aid in solving these problems [2–5].

Blood group antigens are expressed on the outside surface of the red blood cell (RBC) membrane. They are inherited structural moieties located on proteins, glycoproteins, or glycolipids and are encoded by various alleles [6]. The consequence of the vast variation of blood groups is observed through an immune response that can be induced when RBCs expressing an antigen are introduced into a person whose RBCs lack the ‘foreign’ antigen.

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The antibodies that emerge from this immune response, can cause transfusion reactions, fetal and neonatal anemia, and in some instances autoimmune hemolytic anemia.

Hemagglutination, the original method to test for blood group antigens and antibodies, is simple, inexpensive, and when done correctly, serves the transfusion medicine well in the majority of clinical scenarios. However, hemagglutination is a subjective test and has limitations: it does not reliably predict a fetus at risk of hemolytic disease of the fetus and newborn (HDFN), it is difficult to phenotype RBCs from a recently transfused patient or when RBCs are coated with IgG, and has poor ability to predict zygosity in Rh-positive individuals. Technical drawbacks include the need for multiple methodologies for optimal reactivity by a vast array of antisera; limited automated high-throughput capability, and a paucity of potent reagents for all clinically relevant antibodies. In fact, over the last few years, source material has become expensive and its availability dwindling. The understanding of the molecular bases associated with blood group antigens and phenotypes has enabled the field to use this knowledge to screen donors for alleles that encode blood group antigens conserves expensive and rare antisera for confirmation by hemagglutination of predicted antigen-negativity. The purpose of this overview is to discuss how molecular approaches can be used in the immunohematology reference laboratory.

2. DNA analysis of blood group genes

The genes encoding the 30 blood group systems have been cloned and sequenced, and the molecular bases of most blood group antigens and phenotypes have been determined [7–9]. Analysis of DNA involves polymerase chain reaction (PCR) amplification of the target sequence, typically using primers targeting the intron regions that flank one or more exons, followed by analyses such as restriction fragment length polymorphism (RFLP) or direct dideoxy-nucleotide sequencing. In addition, PCR can be a sequence-specific (SS)-PCR, or performed in real-time using fluorescent probes. DNA array technology is currently semi-automated and has the potential to be fully automated. DNA array technology has the added advantage of computerized interpretation and documentation of results, and direct downloading to a database. Molecular testing using DNA arrays, makes it feasible to contemplate mass screening donors to increase inventories of antigen-negative RBC components, and precisely matching the antigen-negative status of a transfusion recipient to that of a donor [4;10–12]. DNA-based methods in the immunohematology reference laboratory have tremendous value; however, when performed alone, can have limitations (see later). Molecular immunohematology results should be evaluated in the context of available serological information.

3. Databases for genes encoding RBC blood groups

The Human Genome Project resulted in the generation of an enormous amount of genetic data. To use this genetic information effectively in the immunohematology reference laboratory, an understanding of the genes and alleles that encode blood group antigens is desirable. In some instances, the reference laboratory may need to develop a DNA-based assay to resolve a unique serological problem or to characterize an allele encoding a new antigen. The clinical laboratory scientist should be familiar with and be able to peruse the repositories that house the genetic information for blood group antigens. The National Center for Biotechnology Information (NCBI) is an excellent resource containing collections of databases and computational tools for public use. NCBI provides access to GenBank, the National Institute of Health (NIH) database that houses the collection of nucleic acid sequences [13]. NCBI is part of a consortium that includes the DNA DataBank of Japan (DDBJ) and the European Molecular Biology Laboratory (EMBL). These institutions work together to maintain and exchange all public DNA information submitted

world-wide. Entrez Gene is the NCBI online tool used to search the GenBank database and also serves as a link to chromosome maps, citation, and expression data among other more high-level data and tools [14]. The best single reference for information on all blood group antigens is the Blood Group Antigen Gene Mutation Database (dbRBC), whose custodian is NCBI [15]. Other useful electronic databases include: RefSeq, which contains a collection of reference sequences, with RefSeqGene entries representing the consensus sequences of genes, transcripts and proteins. The Online Medelian Inheritance in Man (OMIM) database provides an overview of genes encoding blood group systems, and the Single Nucleotide Polymorphism database (dbSNP) provides a catalog DNA variations of genes including those nucleotide changes not associated necessarily with blood group antigens. In addition, clinical scientists should be familiar with restriction enzyme databases, and be able to use electronic tools to align similar sequences, devise PCR amplimers, and develop and validate in-house assays. Alternatively, available DNA array platforms can be used to analyze nucleotides that the manufacturer determined are of relevance. Table 1 summarizes genes for some of the clinically relevant blood group system antigens, accession numbers, selected blood group antigens, and the corresponding nucleotide polymorphisms.

4. Applications of DNA-Based Assays in the Reference Laboratory

The immunohematology reference laboratory that relies on serology alone is limited in its capability to resolve complex transfusion associated incompatibilities. Applied to both the blood donor and the transfusion recipient, DNA-based analyses provide important information that serology alone cannot provide. Aside from the inheritance of an altered or non-functional (silenced) gene, DNA analyses are a valuable adjunct to hemagglutination and can increase the safety of blood transfusion. The clinical applications of molecular analyses in immunohematology are summarized in Table 2.

To apply molecular approaches to clinical situations, several areas of knowledge are needed. Namely, a knowledge of molecular biology, gene structure and the cellular processes from gene to gene product, the molecular bases of blood groups, hemagglutination techniques, and factors that may affect interpretation of a genotype (e.g., a natural chimera). Laboratory supervisors will be required to understand regulatory compliance (cGLP, IRB, FDA), as well as an ability to correlate DNA and serological results to the clinical problems being addressed.

4.1. Fetal Blood Group DNA Testing

Serological analyses, including titers, have limited sensitivity to predict severity of HDFN, especially for anti-K [16;17]. Fetal DNA blood group typing to predict an expressed antigen can be of value in assessing risk early in pregnancy and to identify the fetus who is *not* at risk of HDFN (i.e., predicted to be antigen-negative). In the latter case, the mother need not be aggressively monitored and potentially can return to her primary care physician rather than be followed at a high risk clinic. Sources of fetal DNA include amniocytes (in amniotic fluid or cultured) and maternal plasma [18;19].

DNA-based typing should be considered when a mother's serum contains an IgG alloantibody that has been associated with HDFN, and when the father's antigen status for the corresponding antigen is heterozygous, indeterminable, or he is not available for testing. The decision to perform amniocentesis should balance the potential for disease and the risks associated with this invasive procedure. If it were not for certain patient restrictions, the detection of fetal DNA in maternal plasma for all antigens implicated in HDFN could replace amniocentesis for fetal blood group testing [20].

In the prenatal setting, the approach to DNA typing should err on the side of caution. Thus, the strategy should be to detect a gene even if the product is not expressed on the RBC membrane, rather than fail to detect a gene, and should include the detection of common silenced alleles. Results should be evaluated for possible contamination by maternal DNA. For example, when testing for fetal DNA in maternal plasma, an underlying maternal *RHD* (weak or partial D) can be suspected by the strong intensity (amplification) of the PCR amplicons. In addition, DNA obtained from maternal plasma can be evaluated for a gene absent in the mother (e.g., Y chromosome), or for non-maternal short tandem repeat segments to establish that fetal DNA is present especially in light of a negative result [21].

Since the occurrence of alleles varies substantially in different populations, it can be helpful to know the ethnicity of the parents. An important part of DNA testing to predict the blood group of a fetus is to obtain an appropriate history to ascertain whether the mother has undergone medical procedures such as artificial insemination, *in vitro* fertilization, or whether she is a surrogate mother. A serological work-up and testing maternal DNA should be performed to appropriately evaluate the fetal blood group DNA test result.

The *RHD* type is a prime target because anti-D is often clinically significant (reviewed in Avent and Reid [22]). DNA analysis for the prediction of a paternally-derived fetal D antigen is based on detecting the presence or absence of portions of *RHD*. In Europeans, the molecular basis of the D-negative phenotype is usually associated with deletion of the entire *RHD*, but several other molecular bases have been described [23;24]. One tenth of D-negative Asians have an intact but inactive *RHD* and as many as 30% of Asians have the D_{el} phenotype [25–30]. About a quarter of D-negative African Americans have an *RHD* pseudogene (*RHD*Ψ) [31] and many others have a hybrid *RHD-CE-D* gene (e.g., the r'^{S} phenotype) [32], neither of which encode the D antigen. To predict the RhD antigen type by DNA analysis requires probing for at least two regions *RHD* [33].

The use of sequence-specific (SS)-PCR requires special consideration when working with DNA obtained directly from amniotic fluid, because these assays include an internal control to ensure that DNA was added to the reagent mix. Because amniotic fluid is mainly dead cells, the DNA is degraded and therefore, the amplified product should be relatively short and the internal control product should be larger than that of the test allele. This rule ensures that a negative test result is truly negative. If the internal control is amplified, then the blood group allele of interest would have been amplified if it was present.

When performing fetal blood group genotyping in the prenatal setting, it is advisable to determine the *RHD* status of the fetus, in addition to the blood group allele of interest. In so doing, the *RHD* positive fetus does not require Rh-negative blood for intrauterine transfusions. This is especially true if the mother has anti-c and fetal DNA is being typed for *RHCE*c* as R_1R_1 blood is far more common than r'r' blood.

4.2. Value of DNA Typing of Blood Group Antigens for Patients

When a patient receives transfusions, the presence of donor RBCs in the patient's peripheral blood makes RBC phenotyping unreliable and presents a major problem to the reference laboratory. The historical practice of "best guessing" a patient's antigen type based on the strength of hemagglutination, the number of RBC components transfused, the length of time since transfusion, the estimated blood volume of the patient, and the prevalence of the antigen in question is more often inaccurate. To overcome this problem, PCR-based assays using DNA isolated from WBCs, buccal smear, or even urine sediment can be used to predict the antigen type of a patient [34–36]. Thus, reference laboratories can abandon tedious and largely inaccurate methods of separating recipient RBCs from a post-transfusion

blood sample and use DNA to predict the patient's phenotype. In patients with SCD, the techniques of hypertonic wash technique is a useful tool [37].

DNA methods are valuable to predict the type of a patient with warm autoimmune hemolytic anemia (WAIHA) when direct agglutinating antibodies, or murine monoclonal antibodies are not available, or if the antigen is sensitive to chemical treatment (acid treatment to remove the IgG inactivates Kell blood group system antigens [38]). DNA-based assays are also useful as tools to distinguish alloantibodies from autoantibodies and to identify the molecular basis of unusual serological results.

However, care must be taken when using molecular methods in antibody investigations. One must consider that the serological problem may involve the inheritance of a null allele, an autoantibody with a particular specificity masking an autologous antigen [39], or a new variant. Knowing the patient's ethnicity, their parental and sibling phenotypes, and having a good understanding of which blood group systems express variants, can provide clues to the interpretation of molecular results or suggest further investigation like gene sequencing. As genomic DNA contains intervening sequences of nucleotides, changes in which can affect splicing, consideration should be given as to the value of preparing cDNA from mRNA in addition to genomic DNA.

In summary, when recommendations for clinical practice are based on DNA-based analyses, it is important to remember that, in rare situations, a genotype determination will not correlate with antigen expression on the RBC [40]. When feasible, the appropriate assays to detect nucleotide changes that alter the predicted phenotype should be included in algorithms for DNA-based testing; e.g., *FY* GATA box and the Fy^x associated polymorphism at nt265 with *FY* testing [41;42], the presence of non-functional African and Asian *RHD* genes with *RHD* typing [29;31], exon 5 analysis with $GYPB^S$ typing [43], and silencing alleles in the Kidd system [44].

4.3. Value of DNA Typing for Patients with Sickle Cell Disease (SCD)

It is clear from the Stroke Prevention Trial II (STOP II) that to prevent strokes, SCD patients benefit from continuous transfusions [45;46]. When this study was aborted prematurely, the NHLBI issued an alert to advise physicians who treat children with SCD that interruption of transfusions for primary stroke prevention is not recommended. However, with increased transfusion comes the increased incidence of blood group alloimmunization: incidence rates in SCD are approximately 20% or above, compared to 5% in other transfusion-dependent patient populations [47–51]. Patients with SCD often produce multiple blood group alloantibodies, which makes the provision of antigen-negative blood difficult. Because screening a large number of donors for minor antigens by hemagglutination is labor-intensive for testing and data entry, and the supply of typing grade reagents may be limited or unavailable, we have been limited in ability to supply antigen-negative blood. To provide appropriate transfusion support, there is a need to increase inventories of antigen-negative components to reduce the risk of transfusion reactions and prevent alloimmunization, especially in patients with SCD.

In order to provide antigen-negative blood products to patients with SCD of the type commonly needed [C–, E–, K–, Fy(a–), Jk(b–)], blood components from African Americans are screened because this phenotype is more prevalent than among Caucasians. However, RBCs of up to 20% of African Americans express immunogenic antigens (VS, V, Go^a, DAK, Js^a), which are not present on RBCs from Caucasians and this has resulted in a high proportion of patients who have antibodies to these antigens. Thus, antigen-negative blood orders now often require RBC components that are VS–, V–, Go(a–), DAK–, or Js(a–) Providing RBC products for these patients is particularly difficult because these antigens are

not on antibody screening RBCs, the corresponding antibodies are not available to screen donors, and the crossmatch is not always reliable for their detection. DNA-based assays provide a tool to mass screen donors, thereby increasing the antigen-negative inventory for a number of blood group systems, thereby improving patient care.

4.4. Screening Donors for Blood Group Antigens

DNA-based methods, especially DNA arrays, can be used to predict the phenotype of donors to increase inventories of antigen-negative blood components and of donor blood used for antibody identification reagent panels [52]. This is particularly useful when antibodies are not available (e.g., anti-VS/V, -Do^a, -Do^b, -Js^a) or are weakly reactive (e.g., Fy^b in Fy^x, and Kn^a/Kn^b to aid in antibody identification).

With donor typing, the presence of a grossly normal gene whose product is not expressed on the RBC surface would lead to the donor being falsely thought to be antigen-positive, and although this would mean loss of an antigen-negative donor, it would not jeopardize the safety of blood transfusion. As automated procedures accommodate larger numbers of alleles with faster throughput at lower cost, typing of blood donors by DNA-based methods is becoming more widespread. Several molecular typing platforms have been published for use as a screening tool to identify antigen-negative donors [53–56]. Antigens predicted to be absent should be confirmed by hemagglutination, using the appropriate antibody or crossmatch. In this manner, precious antibodies and expensive antisera are conserved for the confirmation of DNA typing interpretations.

DNA array technology simultaneously performs multiple assays on one sample; thereby providing the ability to predict antigen types for large number of donor samples with very rapid throughput. Results are analyzed and interpreted by computer, and the data can be used to create a useful repository of possible antigen-negative blood. Coupled with confirmation of the lack of antigen expression with available antisera, phenotypic discrepancies and human error should be reduced. The cost of DNA arrays is less than that for comparable phenotyping methods and will depend on market influences and whether manufacturers can develop FDA-approved kits. Laboratories that chose to apply DNA array technology to blood group antigens should be aware of the added expense of investigating any discrepancies. However, the biggest advantage is that high-throughput DNA arrays have the potential to dramatically increase inventories of antigen-negative blood.

If antigen-negative inventories were large enough to meet demand, the following uses of antigen-matched blood could be contemplated:

- To match the antigen profiles of chronically transfused patients with SCD, especially those who have made an antibody to at least one blood group antigen
- To match unusual Rh phenotypes especially in African American recipients (e variants, e.g., $hr^S–$, $hr^B–$, and D variants, e.g., DIIIa, Del)
- To match patients with multiple antibodies or with antibodies for which there is no antiserum (e.g., V/VS, Go^a, DAK, Js^a, Do^a, Do^b)
- To transfuse patients with antibodies to high prevalence antigens
- To transfuse antigen-matched blood to patients with WAIHA to eliminate periodic labor-intensive procedures that are required to ensure that there are no underlying clinically significant antibodies

4.5. Testing for Do Antigens

RBC typing for Do^a, Do^b, Hy and Jo^a antigens of the Dombrock blood group system is notoriously difficult because the corresponding antibodies, although clinically significant, are often weakly reactive, available only in small volume, and present in sera containing other alloantibodies. DNA-based assays to type patients and donors for *DO*A*, *DO*B*, *DO*B.HY* and *DO*A.JO* are now frequently used and provide the reference laboratory with a larger inventory of Do(a–), Do(b–), Hy– and Jo(a–) donors both for transfusion and for red cell reagents. Due to the dearth of appropriate antiserum, testing for polymorphisms in the Dombrock blood group system by DNA-based methods surpasses hemagglutination for antigen typing [57].

4.6. DNA Analyses for ABO and RH Discrepancies

In the reference laboratory, DNA analyses can be used to resolve ABO discrepancies and to distinguish an acquired phenotype from an inherited one without having to perform laborious family studies. With well over 100 alleles encoding A and B antigens, DNA-based analysis is complex; however, molecular methods can be useful to resolve ABO forward and back typing discrepancies. The DNA analysis can identify donors with weakly expressed ABO variants, thus resolving what was considered in the past an FDA reportable error.

DNA analyses can be valuable for the prediction of Rh antigens when suitable panels of monoclonal antibodies are not readily available, or the antibodies are not available in the needed strength or volume. DNA assays also may be useful to define variants, predict the expression of antigens (e.g., V and VS) and to precisely match the D and e antigen status of a donor to a recipient, especially those with SCD. Moreover, DNA-based methods are useful in solving D discrepancies and predicting D antigen epitopes expressed on the Rhce polypeptide [58–60].

4.7. DNA Typing for High Prevalence Antigens

Testing DNA to obtain Lu(b–), Yt(a–), Sc1–, LW–, and Co(a–) is the desirable alternative to hemagglutination because antibodies to these high prevalent antigens are inconsistently available. However, the availability of anti-k, -Kp^b, -Js^a, -Fy3, and -Jk3 often makes hemagglutination the method of choice to type for these antigens. As addition of appropriate nucleotide changes to high-throughput a DNA array adds little incremental cost, all of the above antigens could be screened at the DNA level using this platform, with confirmation using hemagglutination on the few donors with the nucleotide change associated with high prevalence antigen-negativity. Detection of Vel–, Lan–, At(a–), or Jr(a–) donors is restricted to hemagglutination because the molecular bases of these antigen are unknown. Detection of null phenotypes such as Rh_{null}, K_0, Gy(a–), Ge–, or McLeod is complex due to the diverse molecular bases associated with these phenotypes [15].

4.8. Other Applications of DNA-Based Testing

Other applications of molecular testing in the immunohematology reference laboratory include expression of antigens in heterologous systems by using mRNA in transfection studies to potentially detect and identify blood group antibodies in a single, objective, automated assay [61], to explore expression of new alleles or biochemical pathways required for expression [62;63], to produce soluble recombinant forms of an antigen for use in inhibiting an antibody [64], and to use as immunogens for the production of monoclonal antibodies [65;66].

5. Limitations of DNA analyses

Although DNA-based testing for the prediction of blood groups has great value there are several limitations that preclude this methodology from being the sole method by which clinical decisions should be made. There are several reasons for this and some of the limitations of DNA testing are listed in Table 3.

Also the DNA-based analyses have technical, medical, and genetic pitfalls. Medical pitfalls include recent transfusions, stem cell transplantation, and natural chimerism [36;67;68]. In these scenarios, results of testing DNA may not agree with hemagglutination results. Stem cell transplantation and natural chimerism cause confounding results especially in methods using allele discrimination and because results of testing DNA from somatic cells can differ from results of testing DNA from WBCs. Thus, when using DNA testing, it is important to obtain an accurate medical history. There are many genetic events that cause apparent discrepant results between hemagglutination and DNA test results; the genotype is not the phenotype. These limitations should be documented in the appropriate section of the laboratory's quality plan and/or standard operating procedures, and to follow defined algorithms for the use of these procedures.

The majority of DNA-based assays target specific nucleotide(s) and will detect an apparent grossly normal allele, which can be silenced by a nucleotide elsewhere in the gene. Thus, antigens are not expressed on RBCs, and the interpretation can lead to a donor being falsely identified as antigen-positive. This would mean that a valuable antigen-negative (e.g., system null) donor would be lost to the inventory, but would not jeopardize the safety of a patient receiving blood transfusion. In a patient, a false positive interpretation has greater significance. For example, RBCs from a patient with a silenced KEL2 allele *in trans* to a KEL1 allele would be predicted to be K+k+ and she/he could make anti-k. Furthermore, homozygosity for a silenced allele will result in a blood group system null and, thus the patient could make antibody to the protein absent from his/her RBCs. Confirmation by hemagglutination of predicted antigen-negativity is recommended using a reagent antibody if applicable (ie. for donors), and/or by cross-matching using a method optimal for detection of antibody/antigen incompatibility.

In some blood group systems, a large number of alleles encode one phenotype (e.g., ABO, Rh, and the null phenotypes in many blood group systems), some alleles have large deletions (e.g., Ge–) or are hybrid genes (e.g., in the Rh and MNS systems) (see dbRBC database). Additionally, there is a high probability that not all alleles in all ethnic populations are known; this was recently illustrated in the Dombrock blood group system [69]. Thus, analyses of the molecular bases associated with many antigens have been restricted to a relatively small number of people with known antigen profiles. This information is being applied to DNA typing with the assumption that such analysis will correlate with RBC antigen typing in all populations. A much larger number of people from a variety of ethnic backgrounds need to be analyzed in order to establish more firmly the correlation between genotype and the blood group phenotype. Until such data is available, caution should be exercised when recommending clinical practice based on DNA typing for blood group antigens.

6. Consent for the Use of DNA

Molecular testing applied to immunohematology is generally not considered to be genotyping or genetic testing, but simply represents antigen typing using a different methodology. As the DNA testing is not used to identify or diagnose a genetic disease, and if results will not be published, informed consent is not required; however, it would be wise to check local laws in this regard. The interpretations do not differ from those that can be

presently accomplished by hemagglutination. Consideration of Institutional Review Board (IRB) depends on whether the samples are being tested for clinical purposes or research, whether the samples already exist or are collected specifically for a study, whether personal identification is unlinked or linked, and whether there is any risk to the human subject (in the context of the Helsinki declaration). No IRB approval is needed if a sample is tested only for patient care. However, informed consent from the patient should be obtained if results are to be published as an abstract or article.

7. Final remarks

Repositories of serologically-defined variants contributed to the rapid rate with which the genetic diversity of blood group genes was determined. Initially, the molecular information associated with a variant was obtained from only a small number of samples and the information was applied to molecular techniques in the clinical laboratory with the hope that the results would correlate with RBC antigen typing. As more information was obtained, it became apparent that multiple molecular changes result in genotype-phenotype discrepancies, and that more than one genetic event can give rise to the same phenotype. This is especially true for null phenotypes, e.g., Rh, Kell, Kidd, and Kx blood group systems and the p phenotype (see dbRBC database).

The analyses of multiple parts of a gene has made it possible to predict some antigen types, e.g., the *FY* GATA and *FY* nt265 with *FY*A/FY*B* typing, and the identification of additional nucleotide targets for all blood group systems will likely continue. The concept of whether results of DNA-based tests could be used without confirmation by hemagglutination is being debated. The use of DNA-based results alone should be considered only if such a practice is unlikely to harm the patient. If there was a simple, inexpensive way to positively identify a donor at subsequent donations, DNA typing could be performed only once. Electronic fingerprint identification at the time of donation is one possibility. In addition, to have a fully automated system of DNA preparation and analysis with positive sample identification from the beginning of the process to the end (i.e., from bar-coded tube of blood to downloading of interpreted results to a patient/donor database) would have great value.

In the immunohematology reference laboratory, hemagglutination is the gold standard test to detect reactions between antibody and antigens. DNA-based testing, used as an adjunct to hemagglutination, is a valuable tool that has radically changed the approach used to resolve blood group incompatibilities and to support patients in their transfusion needs.

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Table 1

List of blood group genes, transcripts, clinically relevant antigens, and nucleotide polymorphisms.

ISBT Name (Symbol No.)	Reference Seq Gene (mRNA)	Common Antigens	Nucleotide polymorphism (dbSNP)
ABO (ABO 001)	NG_006669.1 (NM_020469.2)	A1>A2 A>B A>O	nt1061ΔC (rs56392308) nt526C>G (rs7853989) nt703G>A (rs8176743) nt796C>A (rs8176746) nt803G>C (rs8176747) nt261G/ΔG (rs8176719)
MNS (MNS 002)	NG_007470.2 (NM_002099.4) NG_007483.1 (NM_002100.3)	M>N S>s	nt59C>T;71G>A;72T>G (rs7682260;7687256;7658293) nt143T>C (rs7683365)
Rh (RH 004)	NG_007494.1 (NM_016124.3) NG_009208.1 (NM_020485.3)	D C>c E>e	(multiple targets) nt307T>C (rs676785) nt676C>G (rs609320)
Lutheran (LU 005)	NG_007480.1 (NM_005581.3)	$Lu^a > Lu^b$	nt230A>G (rs28399653)
Kell (KEL 006)	NG_007492.1 (NM_000420.2)	K>k $Kp^a>Kp^b$ $Js^a>Js^b$	nt578T>C (rs8176058) nt841T>C (rs8176059) nt1790C>T (rs8176038)
Duffy (FY 008)	NG_011626.1 (NM_002036.2)	$Fy^a>Fy^b$ $Fy^b>Fy^x$ Fy_{null}	nt125G>A (rs12075) nt265C>T (rs34599082) nt-67t>c (rs2814778)
Kidd (JK 009)	NG_011775.1 (NM_015865.4)	$Jk^a>Jk^b$	nt838G>A (rs1058396)
Diego (DI 010)	NG_007498.1 (NM_000342.3)	$Di^a>Di^b$	nt2561T>C (rs2285644)
Yt (YT 011)	NG_007474.1 (NM_015831.2)	$Yt^a>Yt^b$	nt1057C>A (rs1799805)
Scianna (SC 013)	NG_008749.1 (NM_018538.3)	Sc1>Sc2	nt169G>A (rs56025238)
Dombrock (DO 014)	NG_007477.1 (NM_021071.2)	$Do^a>Do^b$ Hy Jo	nt793A>G (rs11276) nt323G>T (rs28362797) nt350C>T (rs28362798)
Colton (CO 015)	NG_007475.1 (NM_198098.1)	$Co^a>Co^b$	nt134C>T (rs28362692)
Cromer (CR 021)	NG_007465.1 (NM_000574.3)	Cr(a+)>Cr(a−)	nt679G>C (rs60822373)
Knops (KN 022)	NG_007481.1 (NM_000573.3)	$Kn^a>Kn^b$ $McC^a>McC^b$ $Sl^a>Vil$	nt4681G>A (rs41274768) nt4768A>G (rs17047660) nt4801A>G (rs17047661)

Table 2

Clinical applications for molecular immunohematology.

- identify a fetus at risk for hemolytic disease of the newborn
- type a patient whose red cells are coated with immunoglobulin (DAT+)
- type a patient who has been recently transfused
- type patients and donors for antigens where antibodies are not available
- mass screen donors for antigen-negative blood types
- type donors for antibody identification panels
- resolve phenotype discrepancies (especially A, B D, and e)
- determine *RHD* zygosity
- identify null and novel alleles

Table 32

Limitations of DNA-based approaches for the predication of blood groups in clinical applications

- DNA-based assays will detect nucleotide of interest but allele contains silencing changes; the encoded antigen is not expressed on the red cell (interpretation error)
- DNA-based assays may not detect an allele with an alteration where probe/primer binds (e.g., when there are three of more alleles at a locus) or where a restriction enzyme cuts, but antigen is expressed
- Certain hybrid alleles (especially in MNS and RH systems) can give false positive or false negative results
- Preferential amplification of one allele in heterozygotes
- There may be many alleles per phenotype and it is impractical to assay for all of them (ABO, Rh, null system genes)
- Medical procedures such as insemination with non-spousal sperm
- In chimeras, the results may be difficult to interpret after transfusion, transplantation, or in a natural chimera
- Unlikely that all alleles in all populations are known
- Not all blood groups can be analyzed (Vel, Lan, Jr^a, At^a)