Transfusion services utilize hemagglutination as the gold standard method for antibody identification and antigen phenotyping. Although serological techniques have served the transfusion community well for the last century, grading hemagglutination is largely subjective and has limitations; antigen phenotyping can be unreliable when the direct antiglobulin test is positive and accuracy is in question when performed after recent transfusion. Molecular methods are effectively used as an alternative approach to determine blood group antigens rather than agglutination. Red blood cell genotyping (RCG) is a predictor of phenotype and the antigen status should be further investigated when a phenotype vs. genotype discrepancy is suspected.

Numerous blood group antigens are considered clinically significant in transfusion medicine (Table). Patients receiving allogeneic blood products are exposed to these non-self antigens expressed on donor red cells. This exposure can lead to alloimmunization. To reduce alloimmunization, precise matching of donor and recipient blood groups is beneficial before the transfusion regimen begins. Using molecular techniques to determine blood group alleles present is applicable to:

- determination of blood groups in recently transfused patients and direct antiglobulin test (DAT) positive patients
- aiding in future antibody identification problems (assist in antibody “rule outs”)  
- preventing alloimmunization of chronically transfused patients through antigen-matched blood
- prenatal diagnostics and paternal RHD zygosity

**Patient Genotyping**

Resolving antibody identification is challenging for patients requiring complex workups that test the limitations of serology. These include recent transfusions, inconsistent antigen expression on reagent red cells, reagent availability and/or cost, and positive DAT. Labor intensive practices are used to resolve these issues in many transfusion services. With these complex workups, RCG is performed to help aid in antibody identification. Patient RCG helps confirm the presence of an antibody by proving the patient is antigen-negative for the suspected antibody in their serum. This methodology can be performed regardless of transfusion history. By knowing the patient’s genotype, the next time the patient presents for transfusion, the
transfusion service will know what antibodies the patient can make, therefore enhancing their problem-solving capabilities.

In transfusion practice, patients are matched for ABO and Rh only. Extended typing is performed only when an antibody screen is positive. Following transfusion, 1-4% of patients can become alloimmunized.\textsuperscript{3} That frequency is significantly higher for chronically transfused patients (32-40%).\textsuperscript{3} Alloimmunization consequently leads to increased turnaround time for antibody investigations, provision of compatible red cell products, increased risk of transfusion reactions, a reduction in the pool of compatible blood if future transfusion is needed, and potentially increasing frequency of transfusion and subsequent iron overload.

While most transfusions may not require extensive matching, certain patient groups can be considered for red cell genotyping, such as:
- Patients facing chronic transfusion, e.g., sickle cell disease,\textsuperscript{5} myelodysplastic syndrome, aplastic anemia, etc.
  - C, E, and K negative (potentially including Kidd, and Duffy for responders)
- Patients with warm autoantibodies
  - Potential decrease or elimination of complex, 6-8 hour allo- and autoabsorptions
- Female children and women of child bearing age
  - Avoid anti-K and anti-c

The benefit of developing a high-throughput molecular system, in which patient results are electronically captured and documented on patient charts and/or donor databases, is the ability to more closely match patients to donors, decreasing the chance of alloimmunization.

Prenatal Diagnostics

HDFN results from the alloimmunization of the mother to paternal alloantigen inherited by a fetus. Maternal IgG antibody crosses the placenta and binds to fetal antigen positive RBCs, destruction of fetal RBCs results in anemia. In the majority of cases the Rh D antigen is responsible for HDFN. Antigen prediction by RCG can be of value to identify the fetus not at risk of HDFN, so that aggressive monitoring of the mother is not needed.\textsuperscript{1}

In addition to Rh D, other great clinical value can be found for prenatal diagnosis with RCG. Establishing the fetal Kell genotype to determine the risk for severe anemia is beneficial since maternal anti-K antibody titers do not correlate with the severity of infant anemia.\textsuperscript{5}

Limitations

Although being widely adopted, RCG has specific considerations. Currently, testing takes hours; similar to complex allo- and autoabsorption techniques, the turnaround time limits the value in stat testing. Also, in rare instances a genotype can yield a different phenotype depending on additional nucleotide changes that are ethnically linked.\textsuperscript{1} Furthermore, the molecular basis of several antigens is not known, including Vel, Lan, Jr\textsuperscript{a}, At\textsuperscript{a}, MAM, and AnWj.\textsuperscript{6} Antibodies to these antigens are rare but clinically significant because they can cause severe hemolytic transfusion reactions and HDFN.

References

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