As we cross train and utilize more generalists in the blood bank, it becomes essential to educate “casual” blood bankers on the rationale for certain practices and protocols that are new or might have changed over the years. One area of hot discussion currently is that of the use of molecular testing in immunohematology, particularly in the area of red cell genotyping (RCG).

Hemagglutination has traditionally been the basis of testing in immunohematology. According to Connie Westhoff, SBB, PhD, director, Immunohematology and Genomics of the New York Blood Center,¹ “Serologic testing involves using a specific antibody to detect a blood group antigen on the patient or blood donor red blood cells. If the antigen is present, the positive result is detected as agglutination (clumping) of the red blood cells.”

Serologic testing requires that a specific antibody be developed to target each antigen of interest and manufactured as reagent grade material, and extensively tested for specificity, which is an expensive process. The cost of commercial reagents that are FDA approved is escalating exponentially, but expense is only one of the limitations of serological testing.²

Addressing Limitations
Interpreting serological results is largely a subjective process. It can be unreliable if the cells being tested are sensitized. If the patient was recently transfused (possibility of mixed red blood cell population or a weak alloantibody), results can be inaccurate or difficult to interpret at best. Other limitations include variability in reactivity of commercially available antibodies. Weak reactivity of clinically significant antibodies like E, K, Jka and Jkb may lead to failure to detect, or positively identify, those antibodies. In such cases, a “negative screen” may erroneously allow an electronic crossmatch. One should bear in mind that even a serological crossmatch may not detect incompatibilities.

This is where molecular testing comes in—to address some of the limitations of serological testing. Since the genes encoding most of the RBC antigens have been sequenced, molecular testing can determine the genotype (versus the phenotype) of a particular antigen. Molecular testing is best described as DNA-based testing to predict the presence of an antigen on the patient or blood donor red blood cells, explained Westhoff. This approach has been made possible by the innovations in technology that came with the sequencing of the genomes of human and other organisms. Active testing continues to evolve and improve due to technology refinements developed with the 1000 genomes project.³

For example, current DNA-based testing technology is not able to perform blood group typing for ABO, the most important blood type, except to resolve discrepancies found by serology. However, new methods will eventually allow DNA-based testing for ABO as well.

In terms of cost, DNA assays utilize
reagents that are readily available because they are not dependent on human source material. Molecular testing can be utilized with great benefit in the areas of donor testing, patient testing and in the manufacture of reagent cells.

**Benefits to Donor, Patient Testing**

In donor testing, it allows the testing of more donors fairly easily because of automation and multiplex testing. It can also assist greatly in the management of rare donor units—from accurate identification to less wastage.

In patient testing, the immunohematologist or MLS might use molecular testing in patients where the cells have been sensitized with antibodies (e.g., those samples giving a positive DAT) and in patients with multiple RBC populations. It can be used to resolve unusual or confounding serological findings and to determine a more rational approach to transfusion practices involving multi-transfused or transfusion-dependent patients. Fetal genotyping aids in the prediction and management of hemolytic disease of the newborn (HDN).

Westhoff says that, in her laboratory, molecular testing is part of the workup protocol for every patient who presents with antibody sensitization to blood transfusion. She describes the advantages of molecular testing as, “The ability to test for numerous blood group antigens in a single assay. The ability to predict the blood group antigen type of patients who have been transfused because there is no interference from the transfused donor cells and to type the patient whose red blood cells are already coated with an antibody (auto antibody), making serologic testing difficult or impossible.”

For manufacturing, molecular testing can provide a probable genotype when antisera are not available to determine the phenotype of an antigen of interest. It may also assist in the selection of homozygous cells to increase the chance of detecting weak but clinically significant antibodies such as Jka and Jkb.

For several reasons, it is unrealistic to expect molecular testing to completely replace serological testing. For one, molecular testing does not detect or identify significant antibodies. Serology is sometimes necessary to resolve phenotype versus genotype discrepancies. There is also some reluctance to completely give up the serological crossmatch that has been around for a long time.

Westhoff gives a balanced view of the advantages and disadvantages of molecular testing based on the science as well as her experience. “The only limitation currently is the inability to detect the presence of a mutation in the gene that silences the expression of the antigen, which results in a false positive test. In our experience, this occurs

**Online**

- A tandem approach utilizing RCG offers new ways to solve complex problems in transfusion, providing patients with better and proactive care. Search “Utilization of Patient Red Cell Genotyping” at www.advanceweb.com/laboratory to learn how.
in 1/500-1/1,000 depending on the ethnic population and the blood group gene being tested.

“In very rare cases, approximately 1/5,000 in our experience, a false negative results due to ‘allele drop out,’ i.e., a gene mutation that results in failure of the target to amplify. These limitations will be overcome with new technologies that use whole genome sequencing. Human errors in manual serologic testing and recording of results far outnumber these limitations with DNA testing.”

**Tomorrow’s Testing**

So, what is the future for molecular testing in the typical immunohematology laboratory? Once again, I asked Westhoff for her analysis. The profession has had more than 10 years’ experience with DNA-based testing for determination of minor blood group antigens. This is the wave of the future, she says, although the technology is so very different, making it difficult for routine or smaller hospital blood banks/transfusion services to adopt and fully implement.

Unlike serologic testing for ABO and Rh, however, testing for minor blood group antigens does not have to be repeated. One-time testing will be sufficient and readily available through the electronic medical record. Such testing can be performed by central referral laboratories with the requisite staff, equipment and expertise, which will reduce cost by offering economies of scale.

Although molecular testing will not be adopted by every laboratory right this moment, most laboratories will increasingly utilize, and benefit from the advantages of, some degree of molecular testing in their blood bank.

_Glen McDaniel is a healthcare consultant, clinical lab scientist, speaker and freelance writer._

**References**


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