EDUCATIONAL COMMENTARY – UPDATE ON GEL AND AUTOMATED TESTING METHODS AND A REVIEW OF ABO DISCREPANCIES

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

• Characterize the gel testing system.
• List the advantages of the gel testing system and of automated systems for pre-transfusion testing.
• Describe the test adjustments to resolve ABO discrepancies.

The gel test was developed by Dr. Yves Lapierre of Lyon, France in 1985.¹ He designed gel testing to standardize antiglobulin tests as well as to improve sensitivity and specificity. The gel testing system consists of plastic cards with 6 microtubule dextran-acrylamide gel columns with testing chambers. Each microtube consists of a reaction chamber above a gel matrix. Agglutination complexes are visibly detectable at various levels in the microtube. A negative reaction is characterized by a pellet of RBCs in the bottom of the microtube. A positive reaction is characterized by trapped sensitized RBC agglutinates in the gel matrix. Reactions are graded according to the distribution of trapped RBC agglutinates in the column. Sensitized RBCs are trapped in the gel, and unsensitized RBCs form a pellet in the base of the microtube.

There are 3 types of gel media: specific, neutral, and anti-IgG. The specific gel media has incorporated in the gel antisera specific for phenotyping cells. An example is Rh phenotyping: the gel card consists of specific Rh antisera in the microtubule. The neutral gel media consists of dextran-acrylamide gel, and applications include reverse ABO grouping and an antibody panel with enzyme-treated RBCs. The antiglobulin test consists of an anti-IgG gel matrix used for compatibility testing, and antibody screening and identification.

An advantage of gel testing is fewer procedural steps. The centrifuge time and speed allow RBCs to enter the gel column but leave serum or plasma in the reaction chamber, eliminating the need for saline washing or antiglobulin control cells. The stability of the final reaction phase is another advantage of gel testing; the test can be read by more than one individual, or it can be photocopied. The gel technique provides well-defined endpoints. Gel test results are read as 4+, 3+, 2+, 1+, MF (mixed field), or negative based on agglutination reactions.

Automated Systems
Automated testing platforms have been developed that include all steps in the testing process. Automated testing platforms are available for solid phase, gel column, and microtiter plate technology.
The testing platforms optimize efficiencies and error reduction. Tube testing, manual gel testing, and automated gel platforms have been compared to evaluate the number of defect opportunities and number of steps requiring operator intervention. The automated platform reduced defect opportunities and number of process steps. Potential analytical and post-analytical errors are reduced by decreasing manual processes and interfacing with the laboratory information system.

The ABO System Discrepancies

The ABO system antigens and antibodies remain the most significant for blood transfusions. The ABO system is the only blood group system with predictable non-RBC-stimulated antibodies present in the sera. ABO discrepancies between the RBC and serum tests require resolution to interpret the forward and reverse testing to determine the ABO group. ABO discrepancies can be classified into 1 of 4 categories:

1. Weak or missing antibody reactions in reverse typing due to missing or weak antibody reactivity.
2. Weak or missing reactions in forward typing due to missing or weak antigens.
3. Unexpected antibody reactivity in reverse typing due to cold allo- or auto-antibodies.
4. Unexpected antigen reactivity in forward typing due to acquired antigen or plasma proteins.

Resolving Discrepancies

Preliminary steps to resolve ABO discrepancies are:

1. Repeat testing after washing the RBCs.
2. Obtain patient-related information to include:
   a. Diagnosis.
   b. Historical blood group.
   c. History of transfusion.
   d. Medication history.
3. Review reactivity of sera against O RBCs (screening cells) and patient’s RBCs (auto-control).
4. Collect a new blood sample for testing to rule out contamination.

To help resolve discrepancies for each category, additional testing or investigation is necessary. For the first category—weak or missing antibody reactions in reverse typing—checking the patient’s age and also checking for the presence of hypogammaglobulinemia may help in the investigation. Newborns and elderly patients may not have detectable antibodies. Incubation at room temperature for 15 to 30 minutes and/or incubation at 4°C may enhance antibody reactivity.

The second category—weak or missing reactions in forward typing—may indicate the presence of an ABO subgroup. Anti-A and anti-B may be incubated with washed RBCs at room temperature for 15 to 30 minutes to enhance reactions. If a subgroup of A is suspected, testing with anti-A₁ lectin and testing serum against A₁, A₂, and O RBCs may help classify the subgroup. Many small, community hospital
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blood banks do not have the reagents on hand to define subgroups of A and rely on their reference blood bank to help resolve ABO discrepancies due to a subgroup. Leukemia and other malignancies may weaken antigen reactions. The presence of an acquired B antigen associated with cancer of the colon or other diseases of the digestive tract is included in this category and usually demonstrates a reaction of $<4+$.\(^1\)

The third category—unexpected reactions in reverse typing—may be due to rouleaux or elevated fibrinogen levels. Saline washing and/or saline replacement technique may help differentiate rouleaux from true agglutination.\(^2\)

The fourth category includes reactivity in forward and reverse reactions due to cold reactive autoantibodies and unexpected non-ABO alloantibodies. The patient’s RBCs can be incubated at 37°C and washed with warm saline to elute the cold antibody off of the patient’s RBCs so that the RBCs do not spontaneously agglutinate in forward typing. Alloantibodies reacting with antigens present on the reverse reagent RBCs other than the A and B antigens may cause a discrepancy. The antibody specificity can be determined with an antibody panel.

Technical errors that may cause an ABO discrepancy include specimen mix-up, clerical errors, failure to add reagents, missed observation of hemolysis, and RBC suspension that is too heavy or light.

**Conclusion**

The advantages of the gel testing system and automated platforms for pre-transfusion testing include less hands-on time, standardization of testing, and error reduction. The automation platforms expedite routine testing and optimize efficiencies. Pre-transfusion services are still reliant on the laboratory professional’s cognitive skills and technical troubleshooting abilities to thoughtfully and thoroughly investigate pre-transfusion problems and ABO discrepancies to provide safe transfusions for recipients.

**References**
