EDUCATIONAL COMMENTARY – ANTIBODY IDENTIFICATION SYSTEMS

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Learning Outcomes

On completion of this exercise, the participant should be able to

- define antibody identification and the principles involved;
- explain the current technologies used to identify antibodies and the advantages and disadvantages of each; and
- understand the different types of reactions caused by antibodies.

Introduction

Antibody identification is a principal tool used in transfusion medicine services to identify clinically significant antibodies in a patient or blood donor. A clinically significant antibody is defined as one that causes decreased survival of red blood cells (RBCs) that possess the target antigen. Antibody identification is performed after a positive antibody screen, when testing suggests a new antibody, or, if the facility requires, to confirm a previously identified antibody.

Antibodies to RBC antigens can be divided into two categories, alloantibodies and autoantibodies. Alloantibodies, also known as immune alloantibodies, are produced in response to an immune stimulus. In this case, the immune stimulus is blood given to the patient through transfusion, bone marrow transplant, or pregnancy. An autoantibody is an antibody directed toward the patient’s own RBCs. Usually autoantibodies are not clinically significant, but they can complicate the detection of underlying alloantibodies that are clinically significant and can cause decreased survival of transfused RBCs. Cold- and warm-reacting autoantibodies react with all cells when tested within their thermal amplitude, thus masking any underlying alloantibodies. Autoantibodies sometimes indicate an underlying disease state and can be clinically significant if they react at 37°C or in the antihuman globulin (AHG) phase.

The general process for antibody identification includes eight key steps: (1) review of the patient’s transfusion history; (2) performance of an antibody panel; (3) checking the autocontrol; (4) determining the panel pattern; (5) performing panel cross-outs for what is not there; (6) looking at what is there; (7) performing testing using special techniques if needed; and (8) performing a statistical significance check once the antibody or antibodies are identified. The platform for identifying antibodies changed greatly with the introduction of solid-phase testing in the 1980s and column-agglutination technology in the early 1990s in the United States. These platforms have paved the way for automation in blood banking, but at what cost? Each platform for antibody identification has advantages and disadvantages. This article
discusses antibody identification using each of the testing platforms, describes the pros and cons of each, and provides an overview of antibody-mediated transfusion reactions.

Hemagglutination (Tube)

The hemagglutination, or tube, method of antibody identification is the gold standard of blood bank testing. This method uses the indirect antiglobulin test (IAT) technique to determine if an antibody is present in serum; the positive endpoint is hemagglutination. The IAT technique consists of RBC reagents, enhancement reagents, AHG reagents, and patient serum. Two drops of patient serum are added to one drop of antigen-identified reagent RBCs and the enhancement media of choice. It is then incubated at 37°C for a prescribed length of time, centrifuged, and read for hemolysis and agglutination. The length of time for incubation depends on the enhancement media used. The tubes are then washed with 0.9% saline a minimum of three times to remove all unbound antibody, and AHG reagent is added. The tubes are again centrifuged and read macroscopically and/or microscopically, depending on the enhancement media used. If the result is negative, immunoglobulin G (IgG)–sensitized check cells are added to the test system and centrifuged to ensure that the system is able to yield a positive result. The most common reason for a failed test system is the presence of residual protein from inadequate washing.

The most commonly used enhancement media are low-ionic-strength saline (LISS), bovine serum albumin (BSA), and polyethylene glycol (PEG). LISS contains glycine in an albumin solution that reduces the ionic strength of the test system, thus increasing the uptake of antibody during the sensitization phase of the reaction. BSA contains bovine serum in a 22% or 30% solution that influences the second stage of agglutination (lattice formation) by reducing the zeta (ζ) potential, allowing the RBCs to move closer to each other. It may also change the degree of hydration of the RBC membrane, thus concentrating the test environment. The most sensitive enhancement media is PEG. It is made of polyethylene glycol prepared in a LISS solution. PEG removes water from the test system, thus concentrating any antibodies present and reducing the ionic strength.

Tube hemagglutination testing using the IAT method gives the technologist performing the test a wide variety of options for manipulation, which is helpful when trying to identify an antibody. The serum to cell ratio, the incubation times, and the potentiators can all be changed, or manipulated, to enhance the detection of the suspected antibody. It is most likely this ability to manipulate the test environment to optimize it for the suspected antibody that makes tube testing the gold standard of antibody testing.
Solid-Phase Testing

In 1978, Rosenfield and coworkers were the first to use solid-phase technology in blood bank testing. Solid-phase technology is divided into three categories: solid-phase red cell adherence, solid-phase protein A technology, and solid-phase enzyme-linked immunosorbent assay (ELISA).

Solid-phase red cell adherence technology is currently approved by the FDA for antibody screening, antibody identification, weak-D testing, IgG autologous control, and compatibility testing. This technology uses a plastic microwell plate that has screening or antigen-typed cells attached to the bottom surface. Plasma and LISS are added to the wells and incubated at 37°C. After incubation the plates are washed with isotonic saline, and anti-IgG–coated indicator cells are added to the plate. The microwells are centrifuged and read for positivity. A strongly positive sample will form a “carpet” across the entire well, whereas a negative sample forms a “cell button” in the middle of the well. Any result between the complete carpet and tight cell button is a varying strength of positive.

Solid-phase protein A technology works similarly to tube testing except that any antibodies attached to RBCs at the end of incubation are captured by protein A at the bottom of the microwells, forming a visible carpet that is read as a positive result.

The ELISA testing technique is currently used to screen and identify platelet antibodies. The ELISA technique is similar to the solid-phase red cell adherence method, except that the AHG reagent used has an alkaline phosphatase label. After washing, phase p-nitrophenyl phosphate is added, causing a gradient color change that correlates with the concentration of antibody present. The more antibody is bound, the darker the color.

The advantages of solid-phase technology include well-defined endpoints that provide for consistency across multiple technologists, the ability to test hemolyzed, lipemic, and icteric samples, and enhanced sensitivity that can detect weak alloantibodies. The main disadvantages of solid-phase technology are the need for specialized equipment, and enhanced sensitivity that allows for the detection of clinically insignificant weak autoantibodies.

Column Agglutination (Gel)

Column agglutination, or gel, testing technology was developed by Dr. Yves Lapierre of France in 1988. The system was licensed and approved by the US Food and Drug Administration (FDA) for use in the United States in 1994, and distribution rights were sold to Ortho Diagnostic Systems in January 1995. The principal process of gel testing technology is controlled centrifugation of predetermined reagents and
RBCs through a dextran-acrylamide gel. The gel uses a process known as *molecular sieving* to separate the RBCs based on agglutinated particle size, with larger agglutinated RBCs located at the top of the gel and nonagglutinated cells forming a pellet at the bottom. The antibody screening and panel cells made specifically for gel testing contain an antigen-typed cell diluted into a LISS solution. The pre-made gel card contains polyacrylamide gel covered in an AHG solution. The advantages of gel technology are the elimination of the washing phase and need for check cells in tube testing. Gel technology also provides standardization and automation to blood bank testing. Disadvantages of gel testing include the inability to perform testing on hemolyzed, icteric, or lipemic samples because color interference can lead to false-positive results.

**Methodology Comparison**

A study conducted by Haywood and colleagues compared 254 antibody positive samples received in a 1-year period. They found the tube method to be the most consistent, identifying all but six clinically significant antibodies. Gel and solid-phase technologies failed to identify 59 and 56 clinically significant antibodies, respectively. The solid-phase testing platform was weak at identifying anti-K antibodies, missing 12 examples during the study. Another study performed by Schmidt and colleagues consisting of more than 80,000 samples also found that solid-phase technology missed a statistically significant number of anti-K antibodies, at 27%. In contrast to the Haywood study, the Schmidt study also found that solid-phase testing identified almost twice the number of anti-Jk<sup>a</sup>, anti-s, anti-Fya<sup>a</sup>, and anti-Jk<sup>b</sup> antibodies that were missed using gel technology. In the Haywood study, the tube method significantly outperformed both of the automated platforms, identifying nearly all of the antibodies tested. These differences between the antibody detection methods can be attributed to the sensitivity and specificity of the assays.

**Overview of Antibodies**

Clinically significant antibodies are designated as such because they either cause hemolytic transfusion reactions, delayed hemolytic transfusion reactions, or hemolytic disease of the fetus and newborn. Antibodies known to cause hemolytic transfusion reactions are the IgM ABO antibodies anti-A and anti-B, which cause intravascular hemolysis, and non-ABO IgG antibodies such as Rh, Kell, or Duffy, which cause extravascular hemolysis. Most non-ABO IgG antibodies cause a delayed hemolytic transfusion reaction. The most well-known antibodies that cause a delayed reaction are those belonging to the Kidd system. These antibodies cause visible signs and symptoms of a transfusion reaction from 2 to 14 days after the initial transfusion. There are reports of patients experiencing symptoms as long as 4 weeks after the transfusion. After a delayed hemolytic transfusion reaction, the blood work is expected to show elevated concentrations of lactate dehydrogenase and bilirubin, a low serum haptoglobin concentration,
EDUCATIONAL COMMENTARY – ANTIBODY IDENTIFICATION SYSTEMS (cont.)

free hemoglobin in urine, and possibly elevated D-dimer level, prothrombin time, and partial thromboplastin time.¹

Summary

Antibody identification is a principal tool used in transfusion medicine services to identify clinically significant antibodies in a patient or blood donor. In the United States, there are currently three major platforms for antibody identification testing: the hemagglutination tube method, the solid-phase testing method, and the column agglutination gel method. Although both the gel and solid-phase platforms provide automation for blood banking, the hemagglutination tube method remains the gold standard for identifying clinically significant antibodies. Clinically significant antibodies are those that recognize blood cell antigens and cause either an immediate or a delayed hemolytic transfusion reaction. Overall, the main purpose of antibody identification is to provide the patient with the safest blood product possible.

References


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