Review

How to recognize and resolve reagent-dependent reactivity: a review

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Reagent-dependent reactivity can be described as agglutination of red blood cells (RBCs) in serologic testing that is not related to the interaction of RBC antigens and antibodies that the test system is intended to detect. In other words, reagent-dependent reactivity results in false-positive agglutination reactions in serologic testing. These false-positive reactions can cause confusion in antigen typing and RBC antibody detection and identification procedures, and may result in delays in patient transfusion. It is imperative that reagent-dependent reactivity is recognized and resolved during the investigation of ABO discrepancies, positive RBC antibody screens and antibody identification panels, and crossmatch reactivity. ***Immunohematology* 2016;32:96–99.**

Key Words: reagent-dependent reactivity, solid-phase red blood cell adherence, gel, LISS, antibody identification, false-positive reactions, panreactivity

Chapter 43 of the fourth edition of *Applied Blood Group Serology*[1] is titled "Miscellaneous Conditions That May Affect Results in the Blood Transfusion Laboratory." In the introduction to this chapter, authors P.D. Issitt and D.J. Anstee write, "For those who watch *Seinfeld,* this is a chapter about nothing." In the same vein, this article is about nothing: seemingly positive serologic results that generally can be proven to be nothing upon further investigation.

The AABB Standards for Immunohematology Reference Laboratories mandate that "the laboratory shall recognize and have a process to investigate reagent-dependent reactivity."[2] The need for this standard is a result of several testing platforms, both manual and automated, that are used throughout the United States for red blood cell (RBC) antibody detection and identification, and for the performance of ABO and other antigen testing of RBCs. Commercially prepared antisera, RBCs, and enhancement media have all been implicated in cases of reagent-dependent reactivity. Our immunohematology reference laboratory (IRL) regularly reports various causes of reagent-dependent reactivity to our client hospital transfusion services. The purpose of this article is to review multiple causes of reagent-dependent reactivity and how to recognize and resolve them.

Dyes Used in ABO Typing Reagents

Anti-A reagents contain a blue dye (such as FD&C Blue No. 1 and Patent Blue), and anti-B reagents contain a yellow dye (such as acriflavin, FD&C Yellow No. 5, and Naphthol Yellow);[3,4] antibodies to these dyes have been reported in the literature.[1,5] Antibodies in the patient's plasma can combine with the dye in the reagent to form antigen–antibody complexes; in the presence of these complexes, RBCs may agglutinate. Alternately, the dye may bind to the RBCs, which are then agglutinated by an antibody directed towards the dye. It has also been postulated that the dye might somehow modify the RBC membrane so that spontaneous agglutination of the RBCs occurs.[1] If unexpected reactions are observed in the ABO front (forward) type and an antibody to a dye is suspected, the patient's RBCs should be washed one to two times to remove all traces of plasma, and the testing should be repeated with the washed cells.[1]

Additives to ABO and Other Antisera

Most commercial antisera also contain bacteriostatic or other preservative agents. Patient plasma may contain antibodies to these agents, or immune complexes may be formed in the presence of these reagents.[1,5,6] These agents include chloramphenicol, gentamicin, neomycin, vancomycin, paraben, thimerosal, sodium azide, tetracycline, hydrocortisone, and other corticosteroids.[1,5–7] Reactions caused by these additives are an in vitro phenomenon and have no clinical significance in transfusion therapy, other than causing laboratory problems that delay transfusions.[7] If unexpected reactions are observed with any antisera, the patient's RBCs should be washed one to two times to remove all traces of plasma, and testing should be repeated with the washed cells.

B(A) Phenomenon

A leading manufacturer of one source of anti-A includes the following message in the package insert for this product: "Anti-A may detect previously unrecognized A antigen in a

small number (0.1%) of Group B people. The agglutination is weak, mixed field, and easily dispersed."[3] This phenomenon is known as B(A). The package insert states that this problem can be resolved by testing with polyclonal anti-A or another monoclonal anti-A derived from a cell line other than MH04.[3]

RBC Preservative Problems

The package insert from one leading manufacturer of screening cells used in antibody detection procedures contains the following warning: "Infrequently, falsely positive results may occur in the presence of antibodies directed to components of the red blood cell diluent."[8] The chemicals in most of these commercial RBC diluents are adenine, chloramphenicol, glucose, inosine, neomycin sulfate, sodium chloride, and sodium citrate.[5] All manufacturers add antibiotics to their RBC suspension media to reduce or prevent bacterial contamination;[5] antibodies to any of the antibiotics could be present in a patient's plasma. Some manufacturers also add hydrocortisone to their RBC diluents, and numerous examples of IgM antibodies directed towards hydrocortisone have been described.[5] Finally, RBC diluents contain some source of sugar, and antibodies to one or more sugars in the diluent have been reported.[5]

An antibody to a commercial RBC diluent should be suspected if a laboratory reports panreactivity with screening or panel RBCs, but not with donor RBCs (which would lack the components of the commercial RBC diluent), at the same phase of testing. An IRL might suspect a RBC diluent problem if the referring laboratory uses a different source of commercial RBCs, and the IRL cannot duplicate the panreactivity seen in testing by the referring lab. Alternatively, the IRL might see panreactivity with one commercial source of RBCs and no reactivity with another commercial source of RBCs.

RBC diluent formulas are "proprietary" and vary from one manufacturer to the next. If an antibody to a component of the commercial RBC diluent is suspected, the commercial cells should be washed one to two times to remove the diluents, and the testing should be repeated. The components of the diluent may be washed away easily. If washing the cells is unsuccessful in resolving the problem, an alternate commercial source or fully phenotyped donor cells should be used in testing.

Antibodies to Chemicals in Commercial Antibody Potentiators

Albumin is rarely used as a potentiator in RBC antibody screening or identification procedures, but autoagglutinins reacting only in the presence of bovine albumin have been reported. In 1969, a group of researchers showed that the "albumin autoagglutinin phenomenon" was caused by antibodies in the patient's sera reacting with sodium caprylate, which was added as a stabilizer during the heating phase of the manufacturing of bovine albumin.[5] Other researchers point to patients with antibodies directed to the bovine albumin or contaminants in the bovine albumin.[5]

Low-ionic-strength saline (LISS) is a common potentiator used in tube testing as well as in gel testing and solid-phase RBC adherence (SPRCA) assays. LISS-dependent autoagglutinins are detected with some regularity in the IRL. A LISS panagglutinin is typically suspected when all reagent RBCs, including the autocontrol, react at 37°C and/or the antihuman globulin phase, but the direct antiglobulin test (DAT) is negative. Thimerosal and paraben are LISS additives that have been implicated in this phenomenon.[1,9–13] Antibody identification tests and the autocontrol should be repeated using a different potentiator such as polyethylene glycol (PEG). LISS-dependent reactivity should be reported when all cells including the autocontrol are reactive in the presence of one potentiator, but are nonreactive in the presence of others. The recommendation to the hospital is to transfuse RBCs that are crossmatch-compatible using a method that does not use LISS or another implicated potentiator.

Solid-Phase-Only Reactivity

SPRCA assays are generally automated and are well suited to antibody screening and antibody identification for transfusion services and blood bank laboratories with a high volume of testing and/or limited staff. Nonetheless, a small percentage of false-positive reactivity has been acknowledged by the U.S. manufacturer of this testing platform. The package insert reads under Specific Performance Characteristics: "Some patient and donor specimens were evaluated that reacted by Capture-R Ready-Screen, but were nonreactive by reference hemagglutination techniques. Most of these specimens were shown to contain solid-phase-only autoantibodies."[14]

Our laboratory began using a manual SPRCA assay (Capture-R; Immucor, Norcross, GA) as an adjunct to manual tube RBC antibody detection and identification methods approximately 10 years ago. We soon realized that the test seemed to be "too sensitive," as we could not detect antibody activity by any other method in many donor and patient samples. A call was made to the manufacturer's technical support department, and we were told that SPRCA assays can detect an antibody directed at the cryptantigens of the

RBC membrane; these are antigens that can be exposed when RBC stroma is present, but not usually seen with intact RBCs. Therefore, we launched a joint project with the American Red Cross IRL in Columbus, Ohio, to investigate this so-called "SPRCA-only reactivity" in normal, healthy blood donors. A total of 283,971 donor antibody screens were performed in an 8-month period, and 694 donors were identified as having a SPRCA-only antibody; this translated to a rate of 0.24 percent.[15] The criteria used to categorize an antibody as SPRCA-only included the following: panreactivity in SPRCA, negative antibody screen in gel, negative antibody screen in tube using PEG, and a negative DAT.

Our laboratory regularly receives samples referred from client hospitals that use automated solid-phase platforms for antibody screens and antibody identification. Anecdotally, it seems that automated platforms detect more of these so-called SPRCA-only antibodies than does the manual SPRCA assay used by our laboratory; it also leads to the speculation that these antibodies might also be detected more frequently in a patient population than in a healthy, donor population. In one study characterizing the performance of the 2003 walk-away analyzer (Galileo, Immucor), the authors cited a false-positive rate of 1.4 percent.[16] A false positive in their study was defined as a positive screen with a negative panel.[16]

A SPRCA-only antibody should be suspected if panreactivity is seen in an antibody identification procedure using a solid-phase platform and the DAT is negative. Although an antibody to a high-prevalence antigen or the presence of multiple alloantibodies would act in the same manner, it may be prudent to initially repeat an antibody screen on the sample in question with a tube method using either LISS or PEG, or test in a gel platform if available. If the antibody screen is negative with a method other than solid-phase, it is most likely that the solid-phase results are false positives. Obviously, if the antibody screen is positive by a second test method, the presence of antibodies must be further explored.

Practical Applications: Why Recognizing Reagent-Dependent Reactivity Is Important

The overview of reagent-dependent reactivity in this article is not meant to be all-inclusive, and the information given is largely based on the kinds of problems referred to our IRL. As such, the main reason for this article is to educate readers to simple solutions for the resolution of these problems.

Recognizing and subsequently resolving reagent problems is important because it prevents the time and expense of referring samples to an outside laboratory. Unfortunately, many transfusion services have only one way of performing a test or approaching a problem and are reluctant to try anything else, even if suggested to them. The reflex decision is to send the sample to an IRL, which delays patient treatment and adds expense.

Many transfusion services have come to rely on automation—and rightfully so—for high-throughput testing and the ability to cross-train employees. Unfortunately, the cost of automation often means that the transfusion service has limited back-up resources for problem-solving. For example, when a client hospital reports panreactivity in a sample using either gel or solid-phase technology, an IRL will ask (after confirming that the DAT is negative), "Did you repeat the testing in tube?" Common responses include, "We don't do that" or "We don't do tube testing." When tested by tube method by the IRL, no atypical antibodies are detected. In contrast, some hospitals request that the IRL attempt to duplicate their results using a solid-phase method even when tube testing is nonreactive. These unnecessary delays in patient treatment and charges could be prevented if the referring transfusion service would simply take the time to perform a tube antibody screen. It is a simple, low-cost test that may solve the problem in less than 30 minutes—yet many transfusion services are unwilling or unable to do this follow-up testing.

For those readers who are blood bank educators as well as technical specialists, perhaps it should be your role to teach students and employees about reagent-dependent reactivity. Blood bank curricula contain lectures about potentiators and how they work, but may not cover LISS-dependent autoagglutinins. The principle of SPRCA is covered and students learn how to operate the instrument, but the phenomenon of SPRCA-only antibodies may not be addressed. Discussion of common reagent-dependent problems and their resolution should perhaps be as important as discussing how to identify a true RBC antibody. After all, a "false positive" can only be called that after you prove it is actually a "Seinfeld" nothing.

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