Letter to the Editors

An unusual antibody reacting with pre-diluted 0.8% reagent RBCs and with 0.8% older (aged) RBCs prepared at the time of testing

A 58-year-old, nontransfused, group O male presented at hospital for pretransfusion testing prior to surgery. The preoperative screens and initial panel were performed using a gel test and reagent RBCs pre-diluted with LISS to 0.8% by the manufacturer (Ortho-Clinical Diagnostics, Inc., Raritan, NJ). All cells tested, except the autocontrol, were 2+ positive. The patient’s DAT was negative. The hospital had also performed tube LISS crossmatches and screens but observed no reactivity.

The sample was referred to our red cell reference laboratory for investigation of a potential antibody to a high-frequency antigen reacting only by gel test. As is our standard testing protocol, the patient’s serum sample was tested with a three-cell screen plus an autocontrol using the following: LISS-antiglobulin (ImmuAdd; Immucor Inc., Norcross, GA), ficin antiglobulin technique (GammaZyme-F; Gamma Biologicals Inc., Houston, TX), and a gel test using RBCs diluted at the time of testing with Diluent 2 (MicroTyping Systems Inc., Pompano Beach, FL).

No reactivity was observed with any of the test methods. This excluded clinically significant alloantibodies to common RBC antigens and antibodies to high-frequency antigens reacting by routine methods currently used by our laboratory.

The hospital had tested the sample using a gel method and pre-diluted RBC suspensions. Since our center was also using Ortho’s pooled pre-diluted 0.8% RBCs for high-volume donor screening, we tested this patient’s serum against these pooled RBCs in gel and also observed a 2+ reaction. Another lot number of the pooled pre-diluted cells was available and the 2+ reaction was reproduced. This latter example was washed (× 3) free of diluent with 0.9% normal saline and retested. There was no change in reactivity. This demonstrated that the reactivity was neither diluent-dependent nor caused by something that could be washed from the RBC surface.

Since this case was referred to us as a possible antibody to a high-frequency antigen, we prepared a selected panel of RBCs negative for high-frequency antigens from our liquid inventory. We prepared the RBCs as 0.8% suspensions in Diluent 2 and tested the RBCs with the patient’s serum immediately by the gel test. To this point, the only reactive RBCs had been those that had been pre-diluted by the manufacturer. We now had some 2+ reactions and some negative results. We then observed that the only reactive RBCs were greater than 3 months old at the time of testing.

We then tested older (aged) examples of previously nonreactive RBCs, side by side, in the gel cards and found that the older examples now gave us up to 2+ reactions. The DAT on these older RBCs was negative using gel IgG cards.

We then decided to “age” some reagent RBCs at 37°C overnight in a water bath and test them the next day. The so-called “aged” cells gave 2+ reactions and their “fresh” counterparts were nonreactive.

The patient’s serum and ten random male donor sera were tested against two 0.8% pre-diluted, DAT-negative screening cells by the gel technique. The patient’s serum reacted 2+ with both reagent RBCs and all ten of the donor sera were nonreactive. These same donor sera were then tested in parallel with the patient’s serum against DAT-negative RBCs from one donor. The single-source RBCs were tested “fresh,” i.e., 2 weeks from expiration, 3 months postexpiration, and 6 months postexpiration. The three RBC samples were diluted to 0.8% suspensions and tested immediately using the gel test. The ten donor sera were nonreactive with all cells tested but the patient’s serum reacted as expected. No reactivity was observed with the “fresh” RBCs, a 1+ reaction was observed with the 3-month aged RBCs, and a 2+ reaction with the 6-month aged RBCs.

The conclusion was that a constituent of the diluent used by Ortho to predilute their RBCs was...
producing an immediate effect on the RBC surface that was detected by this patient’s serum, an effect not apparent with other commercially prepared RBCs until they were aged during storage. This effect was demonstrable by gel test.

Although the unusual antibody observed in this case had no relevance to treatment of the patient, it is prudent to share abnormal findings with our colleagues. Sharing such results enables us to better understand the advantages and disadvantages of different antibody screening techniques.

Jeff Trimble, BSc, ART(CSMLS), CLS (NCA)
Reference Laboratory Supervisor
Michigan Community Blood Centers
1036 Fuller Ave., NE
PO Box 1704
Grand Rapids, MI 49504-1704

Attention SBB and BB Students: You are eligible for a free 1-year subscription to Immunohematology. Ask your education supervisor to submit the name and complete address for each student and the inclusive dates of the training period to Immunohematology, P.O. Box 40325, Philadelphia, PA 19106.