A CASE STUDY: AN ABO DISCREPANCY DUE TO AN ANTIBODY TO EDTA

Monica Tobar

Introduction

Additives in commercial reagent red blood cells (RBCs) may cause discrepancies in the ABO typing of patients who have developed an antibody against any of the added substances. Neomycin and chloramphenicol are bacteriostatic agents added to the suspending medium of reagent RBCs. Hysell et al reported an antibody against neomycin, and Beattie et al reported three cases of chloramphenicol antibody. Gillund et al and Reid et al have reported antibodies to EDTA-suspended RBCs. This paper describes an antibody directed against EDTA-suspended RBCs which was discovered when an ABO discrepancy was observed using EDTA-suspended, commercially prepared A and B reagent red cells. EDTA is added to some commercial reverse grouping cells to prevent lysis by anti-A or anti-B.5

Because of an ABO discrepancy in typing the blood of a thirty-six-year-old woman admitted to a local hospital for minor surgery, a sample of blood was sent to our reference laboratory. At the hospital, RBCs were typed as group A, but the serum agglutinated both group A and B reagent RBCs (American Dade, Miami, FL).

Methods and Materials

Standard serologic methods were used throughout. Group A and B reagent RBCs were obtained from four different commercial suppliers: American Dade (Miami, FL), Gamma (Houston, TX), Ortho (Raritan, NJ), and Amtec Diagnostics (Conroe, TX). The concentration of EDTA that produced the strongest reactions of the patient's antibody was ascertained as follows. Four sets of two-fold dilutions of the patient's serum were made. Four concentrations of EDTA ranging from 2-16 g/L in 0.85 percent saline were also prepared. To 0.1 mL of each serum dilution in each set was added 0.1 mL of one of the EDTA solutions. To each tube of serum-EDTA, 0.05 mL of a 4 percent suspension of group O washed red cells was added. The contents were mixed and immediately spun at 3400 rpm for 30 seconds; the cells were examined for agglutinates and the results recorded.

Results of Serologic Testing

No atypical antibodies were detected in tests of the patient's serum at room temperature, in LISS at 37°C, or in the indirect antiglobulin test. The direct antiglobulin test on the patient's red cells was negative. Her serum was tested against unwashed group A red cells from four manufacturers. Results are shown in Table 1. Two of the four manufacturer's reagent A1 RBCs were agglutinated. When those cells were washed, reactivity was abolished.

The supernatant fluids from reagent RBCs from the first and second manufacturers were removed and added to group O cells. The strength of agglutination of these modified group O RBCs, when tested with the patient's serum, was equal to that observed originally in the reverse grouping with group A1 RBCs.

Information supplied by the manufacturers established that EDTA was a constituent of reagents from the first and second manufacturers but not of reagents supplied by the third and fourth manufacturers. Titration of the patient's serum vs group O cells showed the optimum concentration of EDTA to be between 4 and 8 g/L (Table 2).

Antibody reactivity was destroyed by DTT-treatment of the serum, indicating that the antibody was an IgM immunoglobulin.

Discussion

Commercial food processors add EDTA to many food products to prevent oxidation, rancidity, and texture changes. Individuals who ingest EDTA and other chemicals may develop antibodies to these substances. EDTA added to the medium of reverse grouping cells chelates calcium, and this inactivates complement in the serum that is being tested. The purpose of inactivation is to prevent hemolysis and thereby avoid misinterpretation of the reverse group. While only two manufacturers are known to add EDTA to their reverse grouping cells, four manufacturers are known to add chloramphenicol and neomycin to prolong the viability of the reagent red cells. All of these drugs or chemicals may result in reverse grouping discrepancies and should be considered when serologic problems are resolved by washing the reagent red cells.1-4

References


Table 1. Reactions of patient's serum with group A1 red cells supplied by four manufacturers

<table>
<thead>
<tr>
<th>Source of cells</th>
<th>Agglutination of RBCs</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Unwashed</td>
</tr>
<tr>
<td>#1</td>
<td>3+</td>
</tr>
<tr>
<td>#2</td>
<td>4+</td>
</tr>
<tr>
<td>#3</td>
<td>neg</td>
</tr>
<tr>
<td>#4</td>
<td>neg</td>
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</table>

Table 2. Titration of patient's serum vs group O cells with varying concentrations of EDTA

<table>
<thead>
<tr>
<th>EDTA concentration</th>
<th>Serum dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>2 g/L</td>
<td>2+</td>
</tr>
<tr>
<td>4 g/L</td>
<td>4+</td>
</tr>
<tr>
<td>8 g/L</td>
<td>4+</td>
</tr>
<tr>
<td>16 g/L</td>
<td>4+</td>
</tr>
</tbody>
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antibody interfering with ABO grouping and antibody screening. Transfusion 1975;14:16-22.

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TECH HINTS

MICROFILTRATION FOR CAPILLARY TUBE USE

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Capillary tube testing requires reagents free of particulate matter and excessive lipids. It is difficult to filter, by the usual methods, the small amounts that are suitable for this technique. A microfilter that can be assembled rapidly (2 min) and has proved useful for this purpose, is described. A TampaxR tampon (Tam Brands, Inc., Lake Success, NY) should be pulled apart and enough fibers torn off to pack down to a 0.6 cm (1/4 inch) amount in a small 4.7 cm pipette filter tip (Electra Tips 620/650, Medical Laboratory Automation, Inc., MT. Vernon, NY) from which the last 0.6 cm has been cut off to enhance the flow. Approximately 1-2 cm of Seitz fibers should be tamped down on top of the TampaxR fibers. These fibers can be taken from the leftover Seitz material surrounding punched out pads in commercial filtration kits (11 or 22 mm filter discs made for the 2 or 25 mL sized Boerner Centrifugal Filters distributed by Arthur H. Thomas Co.). The microfilter is then inserted into a 400 μL polyethylene microcentrifuge tube (MC-1 from Analytical Lab Accessories), and two or more drops of serum are put into the microfilter pipette tip. This whole assembly is put into a 1 mL Fisher plastic tube for centrifugation. A hard spin, 7000 rpm for 5 min, is necessary for passage of reagents through the combination of fibers, therefore a Fisher type centrifuge is helpful. For virtually total recovery of reagents, two drops of saline or 6 percent bovine albumin may first be spun through the microfilter and then completely removed from the microcentrifuge tube before centrifugation of the serum.

This procedure is depicted in Figure 1.

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COMMUNICATIONS

To the Editor:

Hooray for John Judd! By condemning the description of exceptionally powerful examples of anti-N as anti-'N', he has struck a blow for clarity in communication between scientists.

If we are to succeed in understanding each other, it is important that we do not allow ourselves to lapse into jargon, or adopt loose terminology that departs from established conventions. The antibodies described in the reports by both Guizzo and Meadows2 and Kosanke and Behzad3 were plainly examples of anti-N possessing a sufficient reserve of potency to give detectable agglutination with cells lacking N but possessing 'N'. To call these antibodies anti-'N' is surely similar to calling anti-A, anti-A2 because it agglutinates A2 (as well as A1) cells, or anti-D, anti-D0 because it is capable of detecting D0 (as well as D). Are we entitled to call anti-A,B anti-A?, How about anti-i for an example of anti-I powerful enough that it requires dilution to show no agglutination of umbilical cord cells? Can we think of anti-Fy by as anti-Fy by if it detects the weak Fy by supposedly produced by Fy by?