Resolution of discrepant typings observed in paternity testing

M. L. Guizzo, N. Lang

Abstract: Discrepant results in phenotyping the red blood cells (RBCs) of a child and his alleged parents were attributable to a contaminating antibody, anti-Bg\sup(b) (HLA B-17), in typing reagents (anti-C and -C\sup(w)). This case demonstrates the necessity for using reagents from at least two sources for paternity testing.

In this study, red blood cell (RBC) and white blood cell (WBC) typings were performed on samples from two alleged fathers, the presumed mother, and a four-month-old infant to determine the probability of paternity for each man. Results of the C and C\sup(w) typings on the child's RBCs could have been misinterpreted if testing had been done with one reagent.

Materials and Methods

Paternity testing at the Community Blood Center, Dayton, Ohio, is routinely done using a double-blind technique. Technologists, working independently and using two sources of commercial reagents, determine the RBC and WBC phenotypes of the parties involved. Double-blind testing is used to minimize the potential for errors and to assure highest quality in terms of accuracy.

Testing for RBC antigens was performed with commercially available reagents according to the manufacturers' directions. The red cells were tested with the following reagents: anti-A, -B, -A,B, -A\1; -D, -C, -c, -E, -e, -C\sup(w); -M, -N, -S, -s; -K, -k; -Fy\sup(a), -Fy\sup(b); -Jk\sup(a), and -Jk\sup(b).

WBC antigens (human lymphocyte antigens, HLA-A and -B) were determined using standard lymphocyte toxicity tests,\1 with antisera obtained from commercial sources.

Calculations were determined using a Comparison of Sperm Probability test\2 to obtain a paternity index and a relative chance of paternity (RCP). Gene frequencies used for RBC antigen calculations were derived from tables compiled in a paternity seminar manual published by the American Association of Blood Banks (AABB).\3 HLA haplotype frequencies were derived from tables compiled at the University of California–Los Angeles tissue typing laboratory.\4

Results

RBC phenotype

Red cell phenotypes of the two alleged fathers, mother, and child indicated that father #1 could be excluded as the father of the child with two second
class exclusions occurring within the MNSs system (Table 1). There were no exclusions in the ABO, Kell, Duffy, or Kidd blood group systems.

In the Rh system, a discrepancy was found in the results for C and C\textsuperscript{w} typing. A technologist using one source of anti-C and -C\textsuperscript{w} found the red cells of the child to be C\textsuperscript{−}, C\textsuperscript{w}\textsuperscript{−}. The second technologist, using different reagents, found the child's RBCs to be C\textsuperscript{+}, C\textsuperscript{w}\textsuperscript{+}. However, it was noted that the positive control results for C and C\textsuperscript{w} were perceptibly stronger with the same reagents (Table 2).

**Further Testing**

Adsorption/elution tests could not be performed due to the small RBC sample obtained from the infant. However, testing by the reagent manufacturer showed that the discrepant results were due to the presence of anti-Bg\textsuperscript{b} (-HLA B-17) in both the anti-C and the anti-C\textsuperscript{w} and to an exceptionally strong expression of the Bg\textsuperscript{b} (HLA B-17) antigen on the child's RBCs.

As a result of the manufacturer's testing, alleged father #2 could not be excluded as the biological father of the child, and the RCP of alleged father #2 was 98.7 percent when data from both RBC and HLA results were used for computation.

**Discussion**

A correlation between HLA B-17 and the Bg\textsuperscript{b} antigen on RBCs has been described.\textsuperscript{5} The mother and the child both typed as HLA B-17. Since no red cell discrepancies were encountered when the mother's RBCs were tested with the same anti-C and -C\textsuperscript{w} reagents, one might assume that the Bg\textsuperscript{b} (HLA B-17) antigen expression on her RBCs was not of sufficient

<table>
<thead>
<tr>
<th>Red cells</th>
<th>A</th>
<th>B</th>
<th>A\textsubscript{B}</th>
<th>A\textsubscript{1}</th>
<th>M</th>
<th>N</th>
<th>S</th>
<th>s</th>
<th>K</th>
<th>k</th>
<th>Fy\textsuperscript{a}</th>
<th>Fy\textsuperscript{b}</th>
<th>Jk\textsuperscript{a}</th>
<th>Jk\textsuperscript{b}</th>
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<tbody>
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<td>0</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
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<td>0</td>
<td>4+</td>
<td>0</td>
<td>2+</td>
<td>1+</td>
<td>4+</td>
</tr>
<tr>
<td>Alleged father #2</td>
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<td>4+</td>
<td>4+</td>
<td>0</td>
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<td>3+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td>Presumed mother</td>
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<td>4+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>4+</td>
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<td>2+</td>
<td>0</td>
<td>3+</td>
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<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Child</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>3+</td>
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<td>2+</td>
<td>2+</td>
<td>4+</td>
</tr>
</tbody>
</table>

**Table 2**

Results of Rh typing using reagents from two sources

<table>
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<tr>
<th>Red cells</th>
<th>-D</th>
<th>-C</th>
<th>-E</th>
<th>-c</th>
<th>-C\textsuperscript{w}</th>
<th>-D</th>
<th>-C\textsuperscript{t}</th>
<th>-E</th>
<th>-c</th>
<th>-C\textsuperscript{w}\textsuperscript{t}</th>
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</thead>
<tbody>
<tr>
<td>Alleged father #1</td>
<td>4+</td>
<td>0</td>
<td>3+</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>Alleged father #2</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>4+</td>
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<td>0</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>Presumed mother</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
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<td>1+</td>
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</tr>
<tr>
<td>Child</td>
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<td>4+</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

*Heterozygous expression
\textsuperscript{t}contains anti-Bg\textsuperscript{b}

**WBC data**

Based on the HLA phenotypes, alleged father #1 was also excluded in the HLA system. However, alleged father #2 could not be excluded, as he could have donated the child's A2, B51 phenotype (Table 3).

**Table 3**

HLA phenotypes

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleged father #1</td>
<td>A2, 25;</td>
<td>B18, 40</td>
</tr>
<tr>
<td>Alleged father #2</td>
<td>A1, 2;</td>
<td>B8, 51</td>
</tr>
<tr>
<td>Presumed mother</td>
<td>A1, 2;</td>
<td>B17, y</td>
</tr>
<tr>
<td>Child</td>
<td>A1, 2;</td>
<td>B17, 51</td>
</tr>
</tbody>
</table>
strength to cause a typing discrepancy. It has been shown that Bg\(^a\) (HLA B-7) and Bg\(^b\) (HLA B-17) antigens on RBCs are expressed to variable degrees over time and among family members.\(^6\) Therefore, hemagglutinating reactions of differing strengths can be expected when a serum, containing either antibody, is tested with red cells from individuals positive for the appropriate antigen.

This case confirms the importance of using two sources of reagents for red cell testing in cases of paternity dispute, as recommended by the AABB Committee on Parentage Testing. It further illustrates a need for careful interpretation of results in the event of a single exclusion when all other systems fail to provide additional evidence of exclusion.

**References**


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**LITERATURE REVIEWS**


**Antigens and antibodies**