Incidence of weak D in blood donors typed as D positive by the Olympus PK 7200

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The incidence of weak D has been reported to be between 0.23 and 0.5 percent in Europe and 3.0 percent in the United States. All studies were performed before the introduction of monoclonal anti-D reagents. Using current commercial reagents, this study evaluated D+ samples for the presence of weak D. D+ donors, typed by the Olympus PK 7200, using diluted monoclonal blend anti-D and diluted polyclonal anti-D, were selected by sampling batches of 100 to 200 samples from the previous day's collection. Anti-D reagents used on the Olympus PK 7200 are required to detect RBCs with the weak D phenotype which do not agglutinate at immediate spin (IS) when tested with polyclonal anti-D by manual tube methods. More than 95 percent of donors tested were Caucasian. Using tube tests with two different monoclonal blend anti-D reagents and one polyclonal anti-D typing reagent, the presence or absence of the D antigen was evaluated after the IS reading. Donors found negative or weakly positive (< 2+) at IS were further typed for weak D by the IAT. The weak D samples were RHD genotyped by allele-specific PCR. Of 1005 donors tested, 4 (0.4%) were classified as weak D by one or more anti-D reagents. Polyclonal anti-D reagent demonstrated weaker reactions when compared with the monoclonal blends. All weak D samples were found positive for exon 4, intron 4, and exon 10, a finding consistent with most D+ samples. The incidence of weak D found in this study is not significantly different from that found in earlier studies using polyclonal anti-D reagents. Immunohematology 2005;21:152–4.

Key Words: incidence of weak D, anti-D typing reagents

The D antigen is carried on an integral protein inserted into the RBC membrane. It is proposed that point mutations that cause amino acid changes in the intracellular or transmembrane portions of the protein result in less protein being inserted into the membrane. This leads to a quantitative difference in reactivity with anti-D reagents when testing D+ and weak D RBCs. Depending on the anti-D reagent, RBCs with a weak D phenotype may not react or may react weakly (< 2+) in direct agglutination tests, but are reactive by the IAT.

Point mutations in extracellular loop regions or genetic mutations, such as recombination or frameshift mutations leading to absence of portions of the Rh protein, can result in partial D antigens. Some anti-D reagents can react with partial D RBCs by direct agglutination while others require IATs.

The incidence of serologically defined weak D has been reported to be between 0.23 and 0.5 percent in Europe and 3.0 percent in the United States. In 1974, Garretta reported an incidence of 0.56 percent with 203,240 samples typed with a Groupamatic using polyclonal anti-D. In 1989, Contreras reported 49 out of 16,484 donors (0.3%) typed as weak D by the Kontron Groupamatic G2000. Of these 49 donors, 27 were initially typed as weak D by the Kontron. In addition, 14 of 87 Groupamatic-typed D–, C+, E– donors and 8 of 90 D–, C–, E+ Groupamatic-typed donors were confirmed weak D. All 49 donors were confirmed weak D by a manual IAT.

An incidence of 0.23 percent (32 of 13,500) weak D donors was found in the Netherlands when testing with various anti-D reagents, including four polyclonal anti-D with enhancers, four monoclonal anti-D available in the Netherlands, two modified IgG anti-D, and bromelin anti-D. In this study, weak D was identified as samples typing D– with at least two of these anti-D reagents.

In the United States, Stroup Walters reported an incidence of 3.0 percent in typing 23,000 donors in 1988 with at least two reagents. This study was performed prior to the introduction of monoclonal anti-D reagents in the United States.

Materials and Methods

Sample selection

D+ blood donors, typed by the Olympus PK 7200 (Olympus America, Inc., Melville, NY) using diluted monoclonal blend anti-D and diluted polyclonal anti-D, were selected by sampling batches of 100 to 200
samples from the previous day’s collection. A total of 1005 D+ donor samples were tested. All samples were collected in EDTA-containing tubes (Becton Dickinson and Co., Franklin Lakes, NJ). More than 95 percent of samples were from Caucasian donors.

Anti-D reagents used on the PK 7200 included anti-D monoclonal blend Gamma-clone (ImmucorGamma, Houston, TX) diluted 1 to 12 with physiologic saline and polyclonal anti-D (ImmucorGamma) diluted 1 to 8 with 6% dextran (Mol.wt. 68800). The optimal working dilution was determined for each lot of anti-D and Rh control reagent. Following the Olympus PK 7200 Operator’s Manual, a series of dilutions were made for each reagent and tested with 16 D+, 50 D–, and 8 weak D RBC samples. All donor RBCs were treated with 0.8% bromelin. All batches typed on the PK 7200 included D–, D+, and weak D controls.

Testing for D antigen

D antigen testing by conventional tube method was performed using the following anti-D reagents: polyclonal anti-D (ImmucorGamma, Houston, TX), anti-D monoclonal blend, Gamma-clone (ImmucorGamma, Houston, TX), and anti-D monoclonal blend, Series 4 (ImmucorGamma, Norcross, GA). RBCs found to be D– or weakly D+ (< 2+) were further tested for weak D by the IAT. A positive result in the IAT defined weak D in this study.

RHD genotyping

All weak D samples were RHD genotyped by allele-specific PCR. DNA was prepared from blood samples using one of the following methods: QIAamp Blood Kit (Qiagen, Venlo, the Netherlands), PUREGENE (Gentra Systems, Minneapolis, MN), or MagNA Pure LC DNA Isolation Kit I (Roche, Indianapolis, IN). RHD genotyping was performed in two multiplex PCR reactions. The first reaction detected intron 4 and exon 10. The second reaction detected exon 4, including the position of the 37-bp insert found in the RHD pseudogene. PCR products were analyzed by agarose gel electrophoresis in the presence of ethidium bromide followed by documentation by UV illumination.

Results

Four donors (0.4%) were classified as weak D by one or more anti-D reagents (Table 1). Polyclonal anti-D reagent demonstrated weaker reactions when compared with the monoclonal blends. Gamma-clonal monoclonal blend anti-D showed the strongest reactions on direct agglutination.

All weak D RBC samples were found positive for exon 4, intron 4, and exon 10, a finding consistent with most D+ RBC samples.

Discussion

When testing 1005 D+ donors, as defined by testing with the Olympus PK 7200, we found the incidence of weak D to be 0.4 percent. This is not significantly different from the results of earlier studies using polyclonal reagents, where the incidence of weak D was reported to be between 0.23 and 3.0 percent. Monoclonal reagents showed similar results to those seen with polyclonal reagents when using less than 2+ agglutination as the defining limit to determine the presence or absence of D antigen by direct agglutination.

It is possible that the incidence of weak D is higher than that found in our study because only donors who typed D+ on the Olympus PK 7200 were studied. Donors with very weakly expressed D antigens not detected by the Olympus methodology may have been missed. Gassner and colleagues performed molecular analysis on 1700 serologically typed D–, C+, or E+ RBC samples from Central European blood donors. Eighty-nine had various forms of RHD alleles when screened for RHD-specific DNA sequences. Five of these donors would have presumably been detected by serologic weak D testing. Wagner et al. investigated 1068 donors who serologically typed D–. Of these donors, 48 carried the RHD gene and all were C+ or E+ antigen positive. In addition, 7574 RBC samples determined to be D–, C–, E–, c+, e+ were tested and 2 RHD positive donors were found. Five donors in all were subsequently typed as weak D. The frequency of weak D in individuals typing as D– is low, on the basis of these two studies.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polyclonal Anti-D</th>
<th>Monoclonal blend Gamma-clone</th>
<th>Monoclonal blend Immucor (Series 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+/-</td>
<td>w+</td>
<td>w+</td>
</tr>
<tr>
<td>2</td>
<td>2+</td>
<td>3+</td>
<td>w+</td>
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<tr>
<td>3</td>
<td>0</td>
<td>2+</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>+/-</td>
<td>3+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

* +/– = weak granularity in the RBC suspension. A few macroscopic agglutinates in a turbid red background.
† w+ = tiny agglutinates in a turbid red background (free RBCs)
In our study all weak D samples were genotyped to confirm the presence of the \textit{RHD} gene and to look for unusual PCR results, in an attempt to determine whether the weak D status was due to quantitative or qualitative differences. All four weak D samples tested as having “normal” \textit{RHD} genes. It is unlikely that these weak D samples are any of the common partial D types. DVI, the most common partial D among Caucasians, reacts only in the IAT using a test tube method, while \textit{R}_0^{\text{Har}} reacts with Gamma-clone anti-D upon immediate spin (IS) and does not react with Ortho BioClone anti-D reagent. Partial D categories, including DII, DIII, DIV, and DVa, are detected by monoclonal anti-D reagents upon IS.\textsuperscript{10} The donors in our study all reacted weakly by direct agglutination with both examples of monoclonal anti-D used but reactivity was significantly enhanced in the IAT used to detect weak D. In addition, our PCR method would have detected the presence of DVI and \textit{R}_0^{\text{Har}}, as DVI would be negative for exon 4 and intron 4 and \textit{R}_0^{\text{Har}} would have tested as D–. As most weak D phenotypes are due to point mutations\textsuperscript{11} that would not be detected by our PCR assay, our results are consistent with those samples having either a \textit{RHD} allele encoding a weak D phenotype, or having \textit{RHC} in trans to D, causing weakened D antigen expression.

While only serologically determined D+ donors were tested in this study and the number tested was relatively small, the incidence of 0.4 percent compares to that previously found by serologic methods. Further molecular studies on the U.S. donor population, like those reported by Gassner and Wagner, may provide further information on the incidence and significance of weak D antigen.

References


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