**Introduction**

Identification of human erythrocyte antigens (HEA) is a significant aspect of current immunohematology and transfusion practice. Serologic typing can be compromised by recent transfusion or a positive direct antiglobulin test. DNA-based analysis is an attractive alternative for determination of red cell antigen status. We evaluated a commercial multiplex “beadchip” array for assessing the polymorphic genes predictive of HEA status.

Early validation testing for Human Erythrocyte Antigen Testing (HEA) with HEA Beadchip™ kit, version 1.0 (BioArray Solutions, Ltd. Warren, NJ), showed a high proportion of inconclusive (IC) results for C antigen. The manufacturer, aware of the deficiency, implemented changes to the reagents and software to correct the problem. Repeat testing was performed on the same samples to investigate whether the improvements resolved the high proportion of IC results.

**Methods**

**Samples Tested**

Genomic DNA was isolated from 22 normal donors, 5 DAT+ patients and 5 patients transfused with >10 units of Red Blood Cells. All subjects had serological phenotypes available for Rh, Kell, Kidd, Duffy, and MNS. Serologic results were obtained by tube testing with commercially available antisera. Initial molecular testing was performed using the eMAP HEA Beadchip™ System, v.1.0. Repeat testing used the Web-based Human Erythrocyte Antigen DNA Typing (wHEA™ v.1.1 Beta) kit. Serologic results were compared to the molecular data.

**Description of Method**

DNA was obtained from whole blood using the Qiagen EZ1 BioRobot and Qiagen EZ1 DNA Blood kit. The extracted DNA was diluted 1:10 and 8ul used in a PCR reaction with 16ul of HEA eMAP PCR (BioArrays Solutions) and 1ul HotStar Taq Polymerase (Qiagen) per reaction. The PCR product is then subjected to ExoSAP-IT digestion to remove unconsumed dNTPs and primers. Lambda exonuclease is then used to preferentially degrade the 5’ phosphorylated strand of the double-stranded PCR product. The remaining single-stranded DNA is then mixed with eMAP Elongation Mix containing DNA polymerase, dNTPs, and fluorescently-labeled dCTP. This solution is incubated on the BeadChip™, allowing the oligonucleotides to anneal with the corresponding probes. The subsequent elongation reaction extends and incorporates the fluorescently-labeled dCTP only on those probes where the 3’ end exactly matches the hybridized oligonucleotide. The BeadChip is then imaged using a fluorescence microscope and the raw data sent via the internet to the BioArrays server for decoding of the array. Analysis of this image reveals which beads showed dCTP incorporation, and therefore which blood group antigen alleles are present in the DNA sample. Final results are produced in .pdf form and are retrieved via password through the BioArray BASIS site.

**Results**

In tests with v.1.0 on samples from normal donors, 17 results were IC for C, 3 for N, 1 for M and 2 for Jk+. On repeat testing with v. 1.1, results were IC for C on 1 sample; none were IC for N, M or Jk+. When DAT+ samples were tested using v.1.0, 4 gave IC results for C, 3 for N, 1 for Jk+. With v 1.1 no IC results were obtained with these DAT+ samples. In multi-transfused patients, there were 4 IC results for C, 1 for N and 2 for Jka with v.1.0. No IC results were seen in these same patients with v.1.1. Pre- and posttransfusion samples gave concordant results with both v.1.0 and v.1.1. Serological and molecular test results were in agreement for all samples except one IC result for C with v.1.1, and a discrepancy in C antigen status of one African American donor. The latter is likely due to expression of C from an RHD background (r’ hybrid gene), and has been noted in previous studies.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Inconclusive Results (IC)</th>
<th>n=35</th>
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</thead>
<tbody>
<tr>
<td>C</td>
<td>25 (71%)</td>
<td>1</td>
</tr>
<tr>
<td>M</td>
<td>1 (2.8%)</td>
<td>0</td>
</tr>
<tr>
<td>N</td>
<td>7 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>Jka</td>
<td>5 (14%)</td>
<td>0</td>
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**Conclusions**

Our data demonstrate that the problems inherent to v.1.0 were substantially resolved by improvements instituted by the manufacturer in v.1.1.

**References**


BioArray Solutions Training Manual, HEA BeadChip™ Kit