When Should Transfusion Services Request Blood Group DNA Testing?

Illinois Association of Blood Banks, Spring 2016

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RBC Blood Group Genotyping in Transfusion-Service Patients

- Sickle cell disease—broad phenotyping; Rh C status
- Complex antibody problems
  - Multiple and/or unidentified antibodies
  - High-frequency-antigen antibodies
  - Autoantibodies
- Antibody-antigen discrepancies
- Obstetrical problems
  - Maternal weak D and RhIG candidacy
  - Fetal typing; paternal D zygoticy
- Allogeneic stem cell transplant antibody problems

Disclosure

- Fetal RhD Genotyping
  - Spouse’s sister: pathologist
    - Former medical director of LabCorp
  - On board of directors of Sequenom, Inc.
    - Vendor for fetal D typing from maternal blood

DNA Analysis Methods - Overview

Polymerase Chain Reaction Amplification

Analytic Test Limitations

- Other nucleotide variants not tested for may affect:
  - Serological phenotype; e.g.:
    - Null variants elsewhere in exons, splice sites, promoter regions
    - Interactions with other blood groups or genes
    - Typing sera reactivity
    - DNA genotype results
  - Specificity sites of primers, probes, restriction enzymes
  - May cause false-negatives (allele dropout)
    - Or misinterpretations by analysis software

- RBC phenotypes are PREDICTED in genotyping
RBC Blood Group DNA Testing In/For Chicagoland (alphabetical order)

- American Red Cross, National Molecular Lab, Philadelphia, PA
  - BioArray HEA, RHD, RHCE, and lab-developed tests

- Heartland -> Blood Center of Wisconsin, Milwaukee, WI
  - Lab-developed tests: www.bcw.edu

- LifeSource -> Virginia Blood Services, Richmond, VA
  - Progenika ID Core XT ->
  - Grifols Immunohematology Center, San Marco, TX
    - Lab-developed tests

- Northwestern Memorial Hospital, Chicago, IL
  - BioArray HEA, and (spring 2016) RHD for weak D

Beads: BioArray and Progenika

- Hybridization of amplified patient DNA with selected probe sequences identifying polymorphisms

- Bead-based microarrays
  - BioArray HEA—multiple blood groups, including RHCE
  - BioArray RHD and RHCE variants

- Liquid bead suspension
  - Progenika ID CORE XT-multiple blood groups, including RHCE

Immucor BioArray BeadChip™

Test DNA: Extracted Amplified

BeadChip Readout

- Beads with amplified DNA fluoresce

  - Fluorescent image obtained of each patient’s chip
  - Image transmitted to BioArray to match up with bead map of that chip
  - Analysis returned from BioArray

BeadChip HEA Genotyping Signal Report

- 24 sets of probes, mostly single-nucleotide-polymorphism (SNP) pairs (blue/green)

Progenika BLOODchips™: Europe, Canada

- DNA processing
  - Labeling
  - Hybridization
  - Scan, analyze signals

- Avent ND, Br J Haematol 144:3, 2008
- Canadian Blood Services, Jan 2014
Progenika BLOODchips™
Grifols brochure, Spain

- ABO, H (Bombay)
- RHD zygosities
- RHD variants
- RHCE variants
- Kell, Kidd, Duffy, MNS
- Diego, Dombrock, Colton, Cartwright, Lutheran
- HPA platelet antigens

Progenika ID Core XT System

Grifols Progenika ID Core XT
Immucor BioArray PreciseType HEA

Phenotypes Predicted in Both Assays (high-frequency)

<table>
<thead>
<tr>
<th>ISBT Blood Group</th>
<th>Antigens/Phenotypes (nucleotide markers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>002 MNS</td>
<td>M, N, S, U S-s-U- S-s-U+&lt;sup&gt;mar&lt;/sup&gt;</td>
</tr>
<tr>
<td>004 RhCE</td>
<td>C, c, E, e VS, V (733G, 1006T)</td>
</tr>
<tr>
<td>005 Lutheran</td>
<td>Lu&lt;sup&gt;a&lt;/sup&gt;, Lu&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>006 Kell</td>
<td>K&lt;sup&gt;a&lt;/sup&gt;, Kp&lt;sup&gt;a&lt;/sup&gt;, Kp&lt;sup&gt;b&lt;/sup&gt;, Jk&lt;sup&gt;a&lt;/sup&gt;, Jk&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>008 Duffy</td>
<td>Fy&lt;sup&gt;a&lt;/sup&gt;, Fy&lt;sup&gt;b&lt;/sup&gt; Fy(a-b-) (GATA-67C) Fy(b+)&lt;sup&gt;sh&lt;/sup&gt;</td>
</tr>
<tr>
<td>009 Kidd</td>
<td>Jk&lt;sup&gt;a&lt;/sup&gt;, Jk&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>010 Diego</td>
<td>D&lt;sup&gt;a&lt;/sup&gt;, D&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>014 Dombrock</td>
<td>Do&lt;sup&gt;a&lt;/sup&gt;, Do&lt;sup&gt;b&lt;/sup&gt;, Hy, Jo&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>015 Colton</td>
<td>Co&lt;sup&gt;a&lt;/sup&gt;, Co&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Blood Center of Wisconsin
Common and RhCE Variant Panels

PCR-hybridization probes

<table>
<thead>
<tr>
<th>ISBT Blood Group</th>
<th>Antigens/Phenotypes (high-frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>002 MNS</td>
<td>M, N, S, U S-s-U- S-s-U+&lt;sup&gt;mar&lt;/sup&gt;</td>
</tr>
<tr>
<td>004 RhCE</td>
<td>C, c, E, e VS, V, VS, Crawford (Rh43)</td>
</tr>
<tr>
<td>005 Lutheran</td>
<td>Lu&lt;sup&gt;a&lt;/sup&gt;, Lu&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Do&lt;sup&gt;a&lt;/sup&gt;, Do&lt;sup&gt;b&lt;/sup&gt;</td>
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www.bcw.edu, RBC genotyping, patient panels
Genotyping in Sickle Cell Disease: 
Blood Phenotyping Partial C Status

RBC Antibodies in Sickle Cell Disease
• 319 adult SCD patients, Duke Univ.
  - 27%, alloantibodies—most frequent:
    • E, C, S, Fy\(\alpha/Fy\beta, K, Jk^{b}, M, D\)
    • Most, 2 or more antibodies
  - Warm autoantibodies in 25% of alloimmunized, vs <1% when no alloAb
    • Mechanisms in common?
    • [More DATs in workups?]
  - Worse survival in alloimmunized

Blood Bank Support in Sickle Cell Disease
• Chronic or frequent episodic RBC transfusions in some
• Extended phenotype matching
  • D, C, E, K
  • Some programs extend further -- Fy\(a/-\)Jk\(a/-\)
• Full phenotype is recommended for future antibody information
  • Fy\(a/-\)Jk\(a/-\), M, N, S, s
• SCD patient’s RBCs can be obtained even after transfusion
  • Hypotonic lysis, 0.3% NaCl: does not lyse sickle cells

RBC Phenotyping in Sickle Cell Patients
• Serological: licensed antisera
  • Specificities limited
• DNA phenotyping
  • More antigens
  • Several high-frequency antigens
  • Cost-effective compared to antisera

Notable African-American RBC Phenotype Frequencies

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<th>ISBT Blood Group</th>
<th>Antigens/Phenotypes</th>
<th>African-American Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>002 MNS</td>
<td>S-s-U-, S-s-U+(^{+})</td>
<td>1-2% U-; 0.2-0.4% U+(^{+}) (1)</td>
</tr>
<tr>
<td>004 RhCE</td>
<td>V+, V+</td>
<td>32% and 30% V+ VS usu. on partial-e allele</td>
</tr>
<tr>
<td></td>
<td>Partial C (r(^{+}))</td>
<td>5-6% (2,3)</td>
</tr>
<tr>
<td>006 Kell</td>
<td>Js(b-)</td>
<td>1%</td>
</tr>
<tr>
<td>008 Duffy</td>
<td>Fy(a-b-)</td>
<td>67% [RBC FY*B GATA promoter]</td>
</tr>
<tr>
<td>014 Dombrock</td>
<td>H+y, Jo(a-)</td>
<td>&lt;1% and &lt;1% (“0%”, Cauc.)</td>
</tr>
</tbody>
</table>

Technical Manual, AABB, 2014
1) Blood Group Antgens FactBook
2) Moulds JM, Transfusion 2013:53(2S):169A
3) Casas J, Transfusion 2015;55:1388
• 494 SCD patients—extended information in genotyping
  • FY: Fy(a-) 87.7%, at risk for anti-Fy\(a\)
    • FY(b-) 82.6%, of which 98.5% had GATA RBC promoter mutation
    • [although FY(a-b-) persons can occasionally make anti-Fy3]
  • Negatives for high-frequency antigens:
    • MNS: 5 (1%) U-neg, 3 (0.6%) U+var
    • Dombrock: 3 (0.6%) Jo(a-), 1 (0.2%) Hy-

Buccal-Mucosa RBC Genotyping in SCD Children
• Rampersand A, J Pediatr 2014;165:1003, Indiana Blood Center
• 92 children, ages 6 days–2.8 yr, identified in state SCD screening
• IN State Health Dept pilot project: buccal swabs, BioArray
  • 4% Js(b-), 2% Hy- Jo(a-), 1% Jo(a-), 8% likely carrying r'S
• 15 children had serologic typing for comparison
  • 3 had genotype-serotype discrepancies:
    • Genotyping correct in 2, sample contamination in 1
    • [BioArray HEA not FDA-approved for buccal mucosa]
• Reference Lab cost for HEA, 33 antigens:
  • 24% less than cost of serotyping for 12 antigens

RHD and RHCE genes—Chromosome 1: Partial-C and Other Hybrid Variants
• D+ or D-neg Rh gene in haplotype with RHCE gene, carrying Ce, cE, ce, or CE
  • D and CE genes transcribed in opposite directions
  • European D-negative gene shown here: deletion
  • Hybrid Rh box: marker for D-negative gene
  • Absence of D protein: anti-D readily made to D+ RBCs

Partial C Antigens in African-Americans
• (C)ce\(^6\), or r'S
  • D-CE(4-7)-D hybrid RHD gene
  • D-negative Rh protein carrying variant C+ antigen
  • The RHCE gene in cis is RHce: C-negative
  • When no normal C is present on the other RHCE gene, these persons can make anti-C
  • Figure: Reid ME, in Moulds JM, BeadChip Molec Immunohematol, 2011:101
**Partial C (r^5) and Anti-C in Sickle Cell Patients**

<table>
<thead>
<tr>
<th></th>
<th>Paris</th>
<th>Shreveport</th>
<th>Philadelphia</th>
</tr>
</thead>
<tbody>
<tr>
<td>C^+ serotype</td>
<td>22.5%</td>
<td>112/494, 22.6%</td>
<td></td>
</tr>
<tr>
<td>Possible partial C</td>
<td>36/416</td>
<td>30/416</td>
<td>30/494</td>
</tr>
<tr>
<td>(BioArray)</td>
<td>8.4% of SCD</td>
<td>6.1% of SCD</td>
<td></td>
</tr>
<tr>
<td>Confirmed r^5</td>
<td>36/177 C^+</td>
<td>23/416</td>
<td>23/494</td>
</tr>
<tr>
<td></td>
<td>4.6% of all SCD</td>
<td>5.5% of all SCD</td>
<td>4.7% of all SCD</td>
</tr>
<tr>
<td>Anti-C</td>
<td>10/36</td>
<td>7/23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28% of partial C</td>
<td>30% of partial C</td>
<td></td>
</tr>
</tbody>
</table>

* Paris: Tournamille C, Transfusion 2010;50:13
* Shreveport: Moulds JM, Transfusion 2013;53(2S):169A
* Philadelphia: Casas J, Transfusion 2015;55:1388

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**Genotype Identification of r^5**

- BioArray HEA, then serology:
  - 1) VS^+, V^- no normal C (no bp109 insert) in BioArray
  - Possible r^5
  - 2) Serotype with anti-C MS24 clone (Immucor, BioRad)
    - Positive in 75%—all r^5
    - Negative in 25%—none r^5
  - Progenika ID Core XT:
    - Probe for r^5 type 1—introns 3 breakpoint IVS3+3100G
    - 1% of r^5 are type 2, different breakpoint
  - BCW: allele-specific PCR, RHCE variant panel

- Moulds JM, Transfusion 2015;55:1418
- [www.bcw.edu](http://www.bcw.edu)

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**Sickle Cell Disease Summary**

- Complete phenotype improved by genotyping
- More information than conventional antisera
- Cost-effective
- Patients serotyped as C^+:
  - 20% have partial C antigen
  - Options: 1) give C-negative RBCs anyway
  - 2) Resolve by genotyping

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**RHD-CE Haplotypes in 110 Africans**

- Nonpygmoid (n = 270)
  - D^w^D with partial e: 20%
  - D^w^-neg with partial e: some of 21%
  - Yellow wedges: Partial D 34%
  - Partial e 52% of haplotypes

Granier T, Transfusion 53:3009, 2013, ISBT variant categories added

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**Genotyping in Complex Antibody Cases**

Morton Arboretum / GR
Complex Antibody Patients

- Warm autoantibodies
- Multiple antibodies
  - And/or nonspecific reactivity
  - Antibody to high-frequency antigen
- With or without recent RBC transfusions
- Identifying or ruling out new alloantibodies can be challenging

Autoantibodies and Alloantibodies: Often a Team

- Autoantibodies are often accompanied by alloantibodies
  - 12-40% of patients with warm autoantibodies had RBC alloantibodies (1978-1999 data, manual testing)
  - Automated testing often more sensitive for autoantibodies than tube testing
- Sickle cell patients: 25% of alloantibody patients had warm autoantibodies, vs <1% when no alloantibodies
  - Telen MJ, Transfusion 2015;55:1378
- Autoantibodies can appear when new alloantibodies develop
  - Numerous reports reviewed: Garratty G, Transfusion 2004;44:5
  - After D+ RBCs experimentally injected to D- persons
  - Sickle cell patients

Autoantibodies Mimicking Alloantibodies?

- Alloantibodies develop to transfused RBCs...
  - Then persist on patient's RBCs up to 300 days after transfusion
  - Salama A, Transfusion 1984;24:188
  - Ness PM, Transfusion 1990;30:688
- Alloantibodies that develop autoantibody reactivity?
  - Garratty G, Transfusion 2004;44:5

Autoantibodies Mimicking Alloantibodies? II

- Autoantibodies with RBC antigen specificity and
  - Loss of the antigen from the patient's own RBCs
  - Negative DAT
- Return of antigen after autoantibody resolves
- They resemble alloantibodies
- 2009 review, blood groups involved (cases reported):
  - Kell (6), Rh (3), Kidd (3), Cromer (3)
  - LW (2), Gerlich, AnW
- Possible mechanisms
  - Altered antigen or altered glycosylation
  - Loss of the entire protein
  - RBCs altered during erythropoiesis

Alloantibody to A High-Frequency Antigen, Mimicking An Autoantibody

- Consider a delayed hemolytic/serologic reaction to a high-frequency antigen (e.g., Hr, k, Kp, Dp, etc.)
- Broad plasma reactivity
- Positive direct antiglobulin test
- Eluate “pan”-reactive
- Alloabsorption at reference lab removes plasma activity
- This could look a lot like a warm autoantibody!

Potential Benefits of Genotyping with Autoantibodies/Complex Alloantibodies

- Recurrent transfusion need:
  - Delayed hemolysis?
  - Identify which alloantibodies patient could form
- “Alloantibody” may be autoantibody
  - Still need antigen-negative RBCs after antibody resolves?
- “Autoantibody” may be alloantibody
  - Antibody to high-frequency/multiple antigens, and recent transfusion?
Streamlining Future Workups In Patients With Complex Antibody Reactions

- RBC genotyping to determine antigens patient has/doesn’t have
- Focus antibody ‘rule-out’ testing:
  - Antigens for which patient is negative
- Some patients with multiple antibodies are running out of antigens to make more antibody to
  - Genotyping: which antigens are left on their ‘list’?

RBC Genotyping and Future Workload: NMH Pilot Study—ILABB Case Studies 2016

- 58 patients in study period
- 10 (17%) known to have been recently transfused
- Examined no. of screen/panel RBCs needed in future workups after genotyping available

<table>
<thead>
<tr>
<th>Followup Workups</th>
<th>Before DNA Typing</th>
<th>After DNA Typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>9 (16%)</td>
<td>20 (34%)</td>
</tr>
<tr>
<td>Workups: Total</td>
<td>13</td>
<td>55</td>
</tr>
<tr>
<td>Mean (Range)</td>
<td>1.4 (1-3)</td>
<td>2.75 (1-11)</td>
</tr>
<tr>
<td>No. Antibodies:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>31% of workups</td>
<td>55% of workups</td>
</tr>
<tr>
<td>2</td>
<td>54%</td>
<td>29%</td>
</tr>
<tr>
<td>3</td>
<td>15%</td>
<td>16%</td>
</tr>
</tbody>
</table>

Antibody-Antigen Discrepancies

- Patient has antibody to “X”, but his/her RBCs type positive for “X”
  - Review antibody and typing workup
    - Were antibody ID and typing correct?

  - Could this be an autoantibody?
    - DAT+? Associated warm autoantibody?
    - (Note: polyclonal typing sera may be invalid)
    - Elute antibody from patient’s RBCs?

  - ‘Partial’ antigen variant, with alloantibody to normal antigen?
    - Is patient heterozygous or homozygous for the antigen?
    - Heterozygous—more likely to be a variant
      - Homozygous—would need 2 variant genes or ?null
Weak or Partial Antigen Variants

- Rh: D, C, c, E and e all may have partial variants
- Anti-e like antibodies associated with VS+ alleles

- Kidd: many variants reported in recent years in genotyping
  - Weak Jk^a or Jk^b antigens
  - Can make alloantibody to normal antigen

- Lurie Childrens’ thalassemic: delayed hemolysis, anti-Jk^a
  - Had Jk nt130G>A variant associated with weak Jk^a
  - Ramsey G et al, Transfusion 2012;52(5):143A

RhD Typing and Normal RhIG Algorithm

Prenatal Type and Screen

- RhD+: 15%
  - Each pregnancy:
    - 26-28 weeks
    - Antibody screen
    - One RhIG dose
    - Delivery
    - RhIG evaluation:
      - Antibody screen
      - Type baby:
        - D-neg: stop (40%)
        - D+: One dose RhIG
      - Screen for excess fetal bleed
      - Screen+: quantify fetal Hgb
      - Give more RhIG if indicated (0.3%)

- RhD-negative: 15%
  - Europe: fetal D genotype,
    - 12wk maternal blood;
    - D-neg (40%) -> stop
  - Ambiguous D:
    - Weak D typing
    - Discrepant D typings
    - 0.4%
    - 0.08%
    - 0.32%
    - 0.08%
    - Resolve with one-time RHD genotyping

Reasons for Ambiguous/Discrepant RhD Typings

- Variable D typing methods and reagents
- Manual or automated testing
- Multiple vendors, multiple anti-D monoclonal reagents
- Variable RHD genetics
  - Dozens of RHD genetic variants in several categories
    - Weak D: weakly reactive with IgM or only with IgG
    - Partial D: missing part of D antigen, can make anti-D
    - Weak partial D
    - Same variant may type differently depending on method
    - In same lab or across different labs
- Variable laboratory reporting of weak-D results
  - Positive, Negative, or Weak-D Positive
- Many opportunities for confusion—patients and obstetricians
**COMMENTARY**

It’s time to phase in RHD genotyping for patients with a serologic weak D phenotype

S. Cedric Sandler, MD, MPH; Andrew Bridges; Tracey Miller-Fiske; and Elizabeth H. Lopas

Transfusion 2015;55:680-9

AABB (American Association of Blood Banks)

College of American Pathologists

ACOG: John T. Queenan, MD, Georgetown University

Weak D typing & RhIG lab practices: Sandler SG, Arch Pathol Lab Med 2014;138:620-5


Cost-benefit analysis: Kacker S et al, Transfusion 2015;55:2095-103

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**Estimated US Frequencies of Weak D Variants**

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>X % of D-negative all who are weak-D</th>
<th>% of Weak D's who are:</th>
</tr>
</thead>
<tbody>
<tr>
<td>All US</td>
<td>14.6%</td>
<td>3.0% 0.43%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>17.3%</td>
<td>2.3% 0.40%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>7.3%</td>
<td>10.9% 0.80%</td>
</tr>
<tr>
<td>Al-Amer</td>
<td>7.1%</td>
<td>8.0% 0.57%</td>
</tr>
<tr>
<td>Asian</td>
<td>1.7%</td>
<td>0.6% 0.01%</td>
</tr>
</tbody>
</table>

Note: With broad US diversity, anti-D risk should not be based on ethnicity.


1) South Asian <1%; East Asian <0.5% of which 10-20% are Del; Westrock C, Blood Transfus 12:3, 2014
3) Muller TH, Transfusion 41:45,2001
5) Chou ST, Blood 122:1062,2013 (sickle cell patients)

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**Weak or Discordant D: The AABB/CAP/ACOG Algorithm**

- **Weak D** (low-strength D) ≤2+ initial test
  - Types 1,2,3
  - No RhIG
- **Discordant D**
  - Reagent-variable
  - Give RhIG

**Partial D Variants**

All other weak D except 1, 2 or 3

Variants with uncertain anti-D risk

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**Benefits of Identifying Obstetrical Patients With Weak D Types 1, 2 or 3**

- RhIG management not needed in all future pregnancies
  - No antenatal 26-28-week RhIG needed
  - Often with prior repeat antibody screen
  - No postpartum neonatal RBC typing
  - No test for excess fetomaternal hemorrhage
  - No postpartum RhIG

- Transfusions can be D+ units


- Confusion resolution for patients and caregivers

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**RHD Weak Variant Genotyping**

Table: BioArray RHD BeadChip; BCW & Grifols below

<table>
<thead>
<tr>
<th>Weak D types</th>
<th>Allo-anti-D Seen?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3</td>
<td>No</td>
</tr>
<tr>
<td>4/DAR (multiple)</td>
<td>4.0, DAR</td>
</tr>
<tr>
<td>1, 15, 41</td>
<td>Yes</td>
</tr>
<tr>
<td>9, 14/40/51, 17, 29, 34, 47</td>
<td>Not yet [but RhIG candidates]</td>
</tr>
</tbody>
</table>

- Red: BCW RhD Discrepancy Analysis: allele-specific PCR (www.bcw.edu)
- Grifols Immunohematology Center: Week D 1,2,3 sequencing assay
- Rh variant identification: allele-specific probes and sequencing

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**Partial-D Variant Genotyping**

Table: BioArray RHD BeadChip; BCW and Grifols below

| ISBT RHD and RHCE Partial D categories: All considered at risk for anti-D |
|-----------------------------|------------------|-----------------|------------------|---------------------|
| 03                          | 04               | 05              | 06               | 09                  |
| Genotype                   | Genotype         | Genotype        | Genotype         | Genotype            |
| DII: a, b, c, 4, 6, 7      | DII: 1-4         | DII: 1,2,3 (DBS),4,6,8,9 | DII: 1-4         | DII: Weak D type 4 category |
| 14                          | 16               | 17              | 19               | 25                  |
| DIT: 1,2                    | DCS: 1,2         | DFE: 1-4        | DMM             | DNN                 |
| 37                          | 38               | 39              | 40               | 41                  |
| DAU: 1-5                    | DAU:             | DOL: 1-3        | DOL:             | DOL: 1,2            |
| 208                         | 314              | 21              | 22               |                    |

- BCW: Partial D Analysis (AS-PCR) includes red, plus DII-DIII
- Grifols: Rh variant identification: allele-specific probes, sequencing

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</thead>
<tbody>
<tr>
<td>All US</td>
<td>14.6%</td>
<td>3.0% 0.43%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>17.3%</td>
<td>2.3% 0.40%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>7.3%</td>
<td>10.9% 0.80%</td>
</tr>
<tr>
<td>Al-Amer</td>
<td>7.1%</td>
<td>8.0% 0.57%</td>
</tr>
<tr>
<td>Asian</td>
<td>1.7%</td>
<td>0.6% 0.01%</td>
</tr>
</tbody>
</table>

Note: With broad US diversity, anti-D risk should not be based on ethnicity.


1) South Asian <1%; East Asian <0.5% of which 10-20% are Del; Westrock C, Blood Transfus 12:3, 2014
3) Muller TH, Transfusion 41:45,2001
5) Chou ST, Blood 122:1062,2013 (sickle cell patients)
Paternal Genotyping

- Zygosity for D in partner with anti-D
- Genotyping for antigens with no commercial antisera
- Seek low-frequency antigen against which mom might have antibody
  - Baby has +DAT, mom’s screen negative
  - Mom’s crossmatch vs dad’s RBCs positive, if feasible

Fetal Genotyping

- Dad heterozygous, what is baby’s type?
  - Is pregnancy monitoring necessary?
- Amniotic fluid cells
  - American Red Cross
  - Heartland -> Blood Center of Wisconsin
- Maternal blood—cell-free fetal DNA in plasma, >10-12+ weeks
  - Widely used in Europe for D-negative moms
  - Anti-D moms, is baby D+?
  - Determine whether antenatal RhIG is needed
  - US rights held by Sequenom Labs, San Diego, CA
  - Offers fetal RhD typing on maternal blood

Post-SCT Auto- and Alloantibodies

- Warm AIHA after 533 allogeneic SCTs
- 19 cases (3.6%), 4-18 months (median 7) post-SCT
- >95% donor chimerism, peripheral blood, in 16/18 cases
- AIHA correlated only with unrelated or same-gender donors
- AIHA caused/contributed to death in 4 cases (0.8%)
  - Including one auto-anti-e (with allo-anti-E)
- 58% of AIHA patients developed plasma alloantibodies (Rh or Kell) vs. 4% of SCT patients with all-negative DATs (p<0.0001)
  - Presumed to be alloantibodies, due to high donor chimerisms, and not autoantibodies
- No genotyping was done

Allogeneic Stem Cell Transplant: Determining The Source of Alloantibody

- NMH AML patient developed anti-E after unrelated allogeneic SCT
- Peripheral blood chimerism ~50:50 donor/recipient
- Ongoing RBC transfusion need precluded serotyping
- Whose antibody was it? What if it disappeared?
  - If from donor, would need to keep on E - RBCs
  - If from recipient, and donor engrafted, E - RBCs not needed?
- Ramsey G, Zinni JG, Sumugod RD, Lindholm PF. Transfusion 2015;55(35):159A
**Allogeneic Stem Cell Transplant: Determining The Source of Alloantibody**

- To examine source of anti-E:
  - BioArray HEA testing:
    - SCT donor DNA from HLA Lab: E+
    - Patient DNA from buccal mucosa: E-negative
  - Anti-E was from recipient
  - (BioArray HEA not FDA-approved for buccal mucosa)
  - Ramsey G, Zinni JG, Sumugod RD, Lindholm PF. Transfusion 2015;55(3S):159A

**Evolving Roles of RBC Genotyping**

- “Basic-science” blood group genetics
- Rare-donor identification
- Esoteric studies of novel patients
- Frequent applications in transfusion and obstetrical care in every transfusion service

**RBC Blood Group DNA Testing In/For Chicagoland (alphabetical order)**

- American Red Cross, National Molecular Lab, Philadelphia, PA
  - BioArray HEA, RHD, RHCE, and lab-developed tests
- Heartland > Blood Center of Wisconsin, Milwaukee, WI
  - Lab-developed tests: www.bcw.edu
- LifeSource > Virginia Blood Services, Richmond, VA
  - Progenika ID Core XT :
  - Grifols Immunohematology Center, San Marcos, TX
    - Lab-developed tests
- Northwestern Memorial Hospital, Chicago, IL
  - BioArray HEA, and (spring 2016) RHD for weak D

**RBC Blood Group Genotyping in Transfusion-Service Patients**

- Sickle cell disease—broad phenotyping; Rh C status
- Complex antibody problems
  - Multiple and/or unidentified antibodies
  - High-frequency-antigen antibodies
  - Autoantibodies
- Antibody-antigen discrepancies
- Obstetrical problems
  - Maternal weak D and RhIG candidacy
  - Fetal typing; paternal D zygosity
- Allogeneic stem cell transplant antibody problems

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- BioArray: Nick ("I'll-buy-a-vowel!") Maioriello
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  - Mindy Goldman, MD, Canadian Red Cross, Ottawa, Canada
  - Chelsea Sheppard, MD, Virginia Blood Services, Richmond, VA
- Blood Center of Wisconsin: Greg Denomme, PhD, Sue Johnson
- Northwestern Medicine Team:
  - BioArray Blood Bank Technologists
  - Jules Zinni, Karyn Hartman, Ricardo Sumugod
  - Paul Lindholm, MD
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Apply Now!

Questions?
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Munich Treasury / GR

BioArray HEA PreciseType:
Billing for FDA-Approved In-Vitro Diagnostic Test

- Immucor USA web site
- Search for "81403"
- Web page has:
  - 1) CPT Code 81403
  - 2) Z-Code ZB04H
- Contact information at Immucor

- ICD-10 diagnosis codes being approved by Medicare carriers include various anemias such as sickle-cell, thalassemia, AIHA, renal disease, cancer, other anemias (not all listed here)