**Transfusion Medicine Practice in the Genomics Era**

**50th ANNUAL SPRING MEETING**
April 18 & 19, 2017

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**Objectives: DNA approach for antigen typing**

- **Why?**
  - strengths and limitations
- **How?**
  - differs from other tools and technology we have implemented
  - over 15 years experience - methods have evolved and are evolving
- **Who?**
  - will be doing the testing
  - where
  - for which patients
- **How change approach routine pre-transfusion testing?**
  - not just in IRL reference laboratories
  - integrated into daily practice

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**DNA-based antigen testing: Strengths**

- Type multiply transfused patients
  - avoid interference from circulating transfused donor RBCs
  - cell separations labor intensive and can be inaccurate
- Type RBCs coated with immunoglobulin (+DAT)
  - alternative – chemical treatment (AET, DTT)
  - labor intensive; destroy or weaken some antigens
- Type clinically significant blood groups for which there are no commercial reagents
  - Do(a/b), Hy, Jk(a), Je(a/b), Co(a), Yt(a), VVS, U, etc.
DNA-based antigen testing: **Strengths**

- Distinguish samples with weak antigens
  - FYX allele – 1-2% frequency in Caucasians
  - RBCs have weak expression of Fyb
  - Not detected with current monoclonal reagents
  - RBCs type as Fy(b-)

  Single largest number of discrepancies between serology typing and DNA typing of donors

- Distinguish between weak D and partial D
  - Typing of females and OB women
  - To determine RhIg candidates and transfusion therapy

DNA-based antigen testing: **Strengths**

- Do not need a RBC sample
  - Buccal swab
  - Fetal amniocytes

- Determine fetal risk for HDFN (antibodies to RBCs) NAIT (antibodies to platelet antigens)
  - Paternal testing to determine risk
  - Gene copy number (zygosity: RhD and HPA)

- Test for numerous minor antigens in a single assay
  - Improved accuracy
  - Antigen typing
  - Antibody ID
  - Find uncommon combinations of antigens in donor inventory
  - Provide higher level of care

**Power of Automation and Interpretation**

**Perspective**

Genotype is not always the phenotype!
Phenotype is not always the genotype!
Perspective – Kell System Example

Genotype is not always the phenotype!

38 year old pregnant female
Anti-K; titer 512
Test paternal sample to predict fetal risk
RBC phenotype: K–, k–
KEL genotype: Predict KEL*01/*02
K+k+, Kp(a–b+), Js(a–b+)
50% chance not at risk

Phenotype is not always the genotype!

48 year old female
plasma reactivity – all cells +
DTT treated – non-reactive
RBC phenotype: K–, k–, Kp(b–)
KEL null cell – non-reactive
Antibody to high in Kell system
Rare-Uncommon
KEL genotype: Predict
K–, k+ (Kp(a–b+), Js(a–b+) )

Kell null alleles (KEL*02N)

• Inability to distinguish silenced expression considered a limitation of genotyping (false positive)
• Ability to detect the presence of two alleles (K and k), even though one is silence in a paternal sample enables the accurate prediction of risk for HDFN
• K0 phenotype is very rare but chance of carrying one null (or mod) allele is higher
  – European studies: 3.5% - 7.5% of K+k– had one KEL*02N null silenced or mod allele

DNA-based testing: Limitations

• "prediction" of presence of antigen on RBCs
  silenced alleles (false positive)
  • only testing region of gene that encodes the antigen
  • a mutation can turn "off" expression in region you are not testing
  • cannot do routine ABO and RhD typing
    • Group O – is a silenced A or B gene
    • D negative – is a silenced RhD gene

• Laboratory testing environment and methods

• Test turnaround time
  – 5-8 hours

How to “balance” limitations
How??

approach and tools differ from other technologies we have embraced

DNA-based antigen testing: Limitations

• "prediction" of presence of antigen on RBCs
  - silenced alleles (false positive)
  - only testing region of gene that encodes the antigen
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• Laboratory testing environment and methods
  - Like HLA department

• Test turnaround time
  - 5-8 hours

How to “balance” or overcome limitations

Laboratory Environment for Testing

• 3 separate laboratory areas
  - Sample DNA extraction
  - Pre-PCR set-up ("clean")
  - Post-PCR analysis ("contaminated")

• Power of PCR to amplify contamination from environment
  - Sterile-like techniques
  - Hood with UV
  - or positive pressure room
  - Dedicated equipment and supplies
  - Gloves
  - Filter tips for pipetting

Methods and tools not routinely found in blood bank & repetitive precision pipetting
Evolution of Methods

Manual
RFLP/SSP
PCR
自动化
Real-time PCR
自动化
Luminex Progenika
线性探针在彩色珠子上的自动读出
自动解读扩展了使用

Semi-Automated
DNA probes on colored beads

Automated
DNA probes on miniature beads on silicone chip
BioArray/Immucor

Test Turn around Time

Manual DNA extraction of single sample = 45 min
PCR reaction: amplify 30-40 antigen targets/sample

Automated DNA extraction ~3-4 hours
Barcoded samples n=96

Not testing single samples
3,290 Ag typings ~ 6 hours

Power of DNA array = is the numbers

ID CORE-XT

Phenotype read-out
+ = positive
0 = negative
– 37 antigens
– single assay
Phenotype read-out

+ = positive
0 = negative

- 34 antigens
- one tube assay

How to Integrate Tool into Routine Practice?

- environment needed
- economy in scale (numbers) of tests
- turn around time
- frequency of testing

Integrating DNA-based Technology into Routine Patient Care
Why interest in more than ABO and D?

~3% transfused patients make antibodies (alloimmunized) to foreign red cell antigens
35% or more of chronic transfused patients
- increase costs of each subsequent transfusion
- delay in providing transfusion
- life-threatening in emergency

Is this level of complication acceptable medical practice today?
11.6 M transfusions in U.S./year
32,000 transfusions / day

Why interest in more than ABO and Rh?

65% of antibodies drop to undetectable levels in 6 months
- patient at risk for transfusion reaction
- can be life-threatening
  - 90% anti-Jk^a disappeared
  - all had disappeared by 10 years
  - only anti-D was very stable

The persistence and evanescence of blood group alloantibodies in men. Tormey CA, Stack G. Transfusion 2009, 49:555-12

What is the value of antibody screen and crossmatch for detecting compatibility?

FDA – U.S. Reported Fatalities 2010-14

Majority of HTR fatalities: failure to detect pre-existing antibodies or emergency transfusion
Laboratory Pre-transfusion Testing
Routine: ABO, RhD type and antibody screen

Approach has not changed in >60 years

2016: 36 Blood group systems (352 antigens)

Prevention of Alloimmunization
The most common antibody specificities: C, E, c, K

Higher Level of Patient Care
- Blood transfusions have declined significantly over the last five years
  - advances in surgical techniques
  - patient blood management (PBM) programs
- Lower hgb threshold for patients (7.0 gm/dl) and limited transfusion
  - Optimal RBC survival more important than ever
- Health Care Landscape
  - focus on outcomes – improved patient care
  - personalized medicine with Genomics
How to provide higher level of patient care?

- Cost effective
- Need more antigen typing information
  - Donors – on the labeling - scannable
  - Patients – in the medical record
- Operationally efficient
  - donor center
  - Hospital

“Operationalize” Process
- Vein to Vein

Operationalized at Donor Center

- Testing for extended antigens better done at the donor center
  - Can associate results with the donor
  - Saves $$$$$$$
  - Donor center typing on label – no need for hospital to repeat
    - Information is not “lost” to the system
    - ~75% donors are repeat donors
    - AABB standard – only need to repeat 2X
    - Increased accuracy (compare typing)
    - Automation and higher throughput
    - Electronic checks and balances
- Donor Center provide patient testing service
  - Saves $$$$$$$
  - Provide to hospital customers to make part of patient record

Costs – reagents for serologic testing

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Reagent Cost / Test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.95 - 1.15</td>
</tr>
<tr>
<td>E</td>
<td>0.95 - 1.15</td>
</tr>
<tr>
<td>c</td>
<td>0.95 - 1.15</td>
</tr>
<tr>
<td>e</td>
<td>2.20 - 3.68</td>
</tr>
<tr>
<td>M</td>
<td>3.56 - 4.63</td>
</tr>
<tr>
<td>N</td>
<td>3.74 - 4.91</td>
</tr>
<tr>
<td>S</td>
<td>7.18 - 18.79</td>
</tr>
<tr>
<td>s</td>
<td>3.18 - 8.63</td>
</tr>
<tr>
<td>K</td>
<td>1.07 - 1.55</td>
</tr>
<tr>
<td>Fya</td>
<td>2.58 - 7.11</td>
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<tr>
<td>Fyb</td>
<td>9.18 - 11.43</td>
</tr>
<tr>
<td>Jka</td>
<td>4.63 - 12.31</td>
</tr>
<tr>
<td>Jkb</td>
<td>4.98 - 13.21</td>
</tr>
<tr>
<td>Dia</td>
<td>2.68</td>
</tr>
<tr>
<td>Kpa</td>
<td>2.24</td>
</tr>
<tr>
<td>Kpb</td>
<td>2.24</td>
</tr>
<tr>
<td>Lub</td>
<td>3.20</td>
</tr>
<tr>
<td>K</td>
<td>5.13</td>
</tr>
</tbody>
</table>

*Average range from a number of facilities and manufacturers 2010

- ~ $150 reagents /sample
- + labor
- + manual data entry
- + pos & neg controls
- + supplies

* > $250 - $280
Currently

• **CHALLENGE** - providing extended antigen typed units
  • Labor intensive
  • Manual activity
  • Performed in IRL reference laboratory – highly trained staff
  • Testing repeated each time
• Inability to scale up
• No serologic reagents for some clinically significant antigens
• Consequently at many donor centers
  • extended antigen typing primarily only minority donors
• **Ultimate Goal** - for patient care and inventory management
  • antigen information on all units

How would more information (antigen profile on patient) change approach *routine* pre-transfusion testing?

Pre-transfusion Testing

- Every sample is “Black Box”
- Potentially has any of 300+ antibody specificities
- How would we design testing if
  - know what patient is at risk to make (antigen-negative)
  - reduces number of possible specificities by 50%
  - know what patient was exposed to
  - all part of electronic medical record
  - change importance of antibody screen?
Challenges for Current technology

• Limited number of markers
  - Cannot cover all antigens of potential interest
    - only common polymorphisms
    - cannot detect all silenced (null) alleles
    - accurate ABO & D typing will require sampling entire gene
  - Cannot determine "phasing" or "linkage"
    - which alleles are changes carried on?
• Population Diversity/Admixture
  - new variants
• Tests "hard-wired" if taken to FDA licensure
  - no pathway to add addition markers to existing design

346 + 6 (2016-ISBT) = 352 red cell antigens

Next Generation Sequencing – “Next Gen”

• Approaches
  - Whole exome (WES) – coding regions of genes only (~2%)
  - Whole genome (WGS) – coding and non-coding regions
  - Targeted - capture/amplify gene(s) of interest only - HLA

Massive parallel sequencing
Next Generation Sequencing Platforms

<table>
<thead>
<tr>
<th>Platform</th>
<th>Accuracy (bp)</th>
<th>Sequencing Method</th>
<th>Detection</th>
<th>Post-sequencing Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche 454</td>
<td>100x</td>
<td>454-pyrosequencing</td>
<td>Light-capture</td>
<td>454-pyrosequencing</td>
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<tr>
<td>Life Technologies</td>
<td>100x</td>
<td>Illumina HiSeq 2000</td>
<td>150bp</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Pacific Biosciences RS</td>
<td>100x</td>
<td>HiSeq 2000</td>
<td>150bp</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Illumina HiSeq</td>
<td>100x</td>
<td>HiSeq 2500</td>
<td>Real-time PCR</td>
<td>100% accuracy</td>
</tr>
</tbody>
</table>

Genomics Revolution

“Genomic data will soon become a commodity; the next challenge — linking human genetic variation with physiology and disease will be as great as the one genomists faced a decade ago.”


“Sequence Once; Read Often”

• Whole genome sequence data will be available on our patients
• Especially for patients with chronic disease
• We will only need to “read” the information

Comprehensive red cell and platelet antigen prediction from whole genome sequencing: proof of principal

Transfusion. 2016, 56(3):743-54
Lane WJ, Westhoff CM, Uy JM, Aguilera M, Rafferty BA, Redman RL, Green RC, Silberstein LE.

Illumina HiSeq - 30X coverage
45 RBC genes
-346 antigens
6 platelet genes
-33 antigens
Comparison of WES for RH genotyping

- Whole exome sequencing (WES) with DNA BeadChip Array (n=54 patients)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>L62F</td>
<td>9.3%</td>
<td>98.1%</td>
</tr>
<tr>
<td>A137V</td>
<td>8.3%</td>
<td>100%</td>
</tr>
<tr>
<td>N152T</td>
<td>100%</td>
<td>98.1%</td>
</tr>
<tr>
<td>N152T</td>
<td>1.3%</td>
<td>ND*</td>
</tr>
<tr>
<td>T201R</td>
<td>7.5%</td>
<td>100%</td>
</tr>
<tr>
<td>T221V</td>
<td>8.3%</td>
<td>98.1%</td>
</tr>
<tr>
<td>R233G</td>
<td>0.9%</td>
<td>98.1%</td>
</tr>
<tr>
<td>R233G</td>
<td>98.1%</td>
<td></td>
</tr>
<tr>
<td>T236X</td>
<td>2.6%</td>
<td>100%</td>
</tr>
<tr>
<td>T236X</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>L349V</td>
<td>0.9%</td>
<td>100%</td>
</tr>
<tr>
<td>L349V</td>
<td>35.2%</td>
<td>98.1%</td>
</tr>
<tr>
<td>I342T</td>
<td>9.3%</td>
<td>100%</td>
</tr>
<tr>
<td>I342T</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>T379M</td>
<td>15.7%</td>
<td>ND*</td>
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<tr>
<td>T379M</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>T379M</td>
<td>100%</td>
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</tr>
</tbody>
</table>

RHCE

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>W16C</td>
<td>49.1%</td>
<td>92.0%</td>
</tr>
<tr>
<td>A85G</td>
<td>3.7%</td>
<td>100%</td>
</tr>
<tr>
<td>109Ins</td>
<td>9.3%</td>
<td>ND*</td>
</tr>
<tr>
<td>R114W</td>
<td>0.9%</td>
<td>100%</td>
</tr>
<tr>
<td>A226P</td>
<td>0.9%</td>
<td>100%</td>
</tr>
<tr>
<td>G233R</td>
<td>6.9%</td>
<td>100%</td>
</tr>
<tr>
<td>G233R</td>
<td>0.9%</td>
<td>100%</td>
</tr>
<tr>
<td>L149V</td>
<td>0.9%</td>
<td>100%</td>
</tr>
<tr>
<td>D385Y</td>
<td>0.9%</td>
<td>94.4%</td>
</tr>
<tr>
<td>D385Y</td>
<td>8.3%</td>
<td>100%</td>
</tr>
<tr>
<td>T340D</td>
<td>0.9%</td>
<td>100%</td>
</tr>
</tbody>
</table>

How to continue to integrate new Genomic Technology Into practice of Transfusion Medicine

Centralized Testing Model

**Goals**

1. Drive down testing cost
2. Provide “precise matched” blood products
3. Changing testing paradigm(s)
4. Training the next generation of professionals
5. Exploring next generation of testing
Thank You!!

New York Blood Center
NYBC

Community Blood Center of Kansas City
CBC