Transfusion Medicine Practice in the Genomics Era

50th ANNUAL SPRING MEETING
April 18 & 19, 2017

Connie M. Westhoff, SBB, PhD
Executive Scientific Director
Immunohematology and Genomics
National Center for Blood Group Genomics

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

DNA-based antigen testing: Strengths

- **Type multiply transfused patients**
  - avoid interference from circulating transfused donor RBCs
  - cell separations labor intensive and can be inacurrate
- **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, Jo(a), Js(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 Fy^b**
    - 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

New York Blood Center

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

New York Blood Center

Perspective

Genotype is not always the phenotype!

Phenotype is not always the genotype!

New York Blood Center

Perspective – Kell System Example

Genotype is not always the phenotype!

48 year old female
plasma reactivity – all cells +
DTT treated – non-reactive

RBC phenotype: K– **k–** Kp(**b–**)
K_0 null cell – non-reactive
Antibody to high in Kell System
Rare-Uncommon

KEL genotype: Predict
K– **k+**, Kp(a–**b+**), Js(a–**b+**)

Phenotype is not always the genotype!

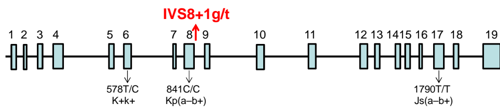
38 year old pregnant female
Anti-K; titer 512
Test paternal sample to predict fetal risk

RBC phenotype: K+k–
All children will be K+ and at risk

KEL genotype: Predict
*KEL*01/*02* K+k+
50% chance not at risk

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
- K_0 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
    - a mutation ican turn "off" expression in region you are not testing
- **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 pipets

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

Evolution of Methods

Manual
RFLP/SSP

PCR

Gel Electrophoresis

Semi-Automated

Real-time PCR

automated readout

Automated

DNA probes on colored beads
Luminex
Progenika

8 samples

96 samples

DNA probes on minature beads on silicone chip
BioArray/Immucor

automated interpretation expands use

New York Blood Center

Test Turn around Time

Manual DNA extraction of single sample = 45 min

Not testing single samples

Automated DNA extraction ~3-4 hours

Barcoded samples n=96

PCR reaction
amplify 30-40 antigen targets/sample

2 hours

3,290 Ag typings
~ 6 hours

Power of DNA array = is the numbers

New York Blood Center

ID CORE-XT

Phenotype read-out

+ = positive
0 = negative

- **37 antigens**
- **single assay**

New York Blood Center

Human Erythrocyte Antigen (HEA) Phenotyping by DNA Analysis Report

Blood Group	Antigen	Result	Notes
Rh	c	+	
	C	+	
	e	+	
	E	0	
Kell	K	0	
	k	+	
	Kpa	0	
	Kpb	+	
	Jsa	0	
	Jsb	+	
Kidd	Jka	+	
	Jkb	0	
Duffy	Fya	0	
	Fyb	+	
MNS	M	0	
	N	+	
	S	0	
	s	+	
Lutheran	Lua	0	
	Lub	+	
Diego	Dia	0	
	Dib	+	
Colton	Coa	+	
	Cob	0	
Dombrock	Doa	+	
	Dob	+	
	Joa	+	
	Hy	+	
Landsteiner-Wiener	LWa	+	
	LWb	0	
Scianna	Sc1	+	
	Sc2	0	
Hemoglobin S	HbS	0	

Detail Notes: V/VS

Phenotype read-out

+ = positive

0 = negative

- 34 antigens
- one tube assay

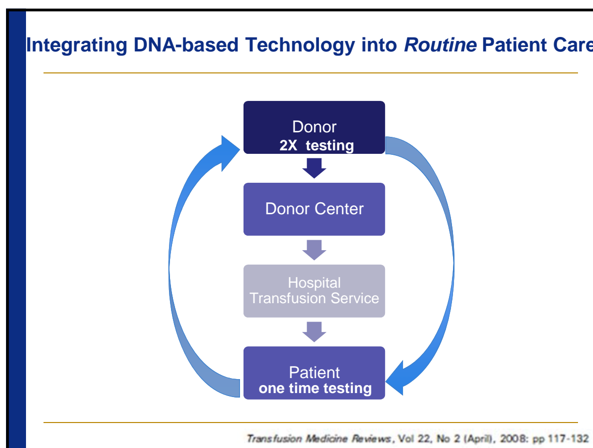
New York Blood Center

How to Integrate Tool into Routine Practice?

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

New York Blood Center

Integrating DNA-based Technology into *Routine* Patient Care



Transfusion Medicine Reviews, Vol 22, No 2 (April), 2008: pp 117-132

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 ?

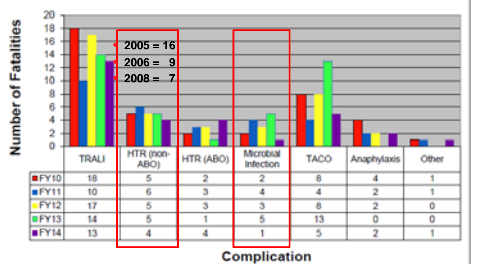
65% of antibodies drop to undetectable levels in 6 months

- patient at risk for transfusion reaction
- can be life-threatening
  - 90% anti-Jkª 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, 49:505-12

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

FDA – U.S. Reported Fatalities 2010-14

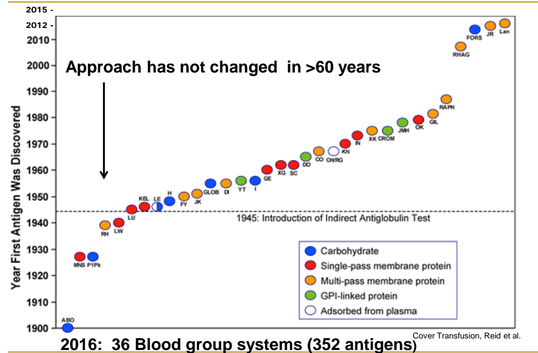


	TRALI	HTR (non-ABO)	HTR (ABO)	Microbial Infection	TACO	Anaphylaxis	Other
FY10	18	5	2	2	8	4	1
FY11	10	6	3	4	4	2	1
FY12	17	5	3	3	8	2	0
FY13	14	5	1	5	13	0	0
FY14	13	4	4	1	5	2	1

<u>Majority of HTR fatalities</u>: failure to detect pre-existing antibodies or emergency transfusion

Laboratory Pre-transfusion Testing

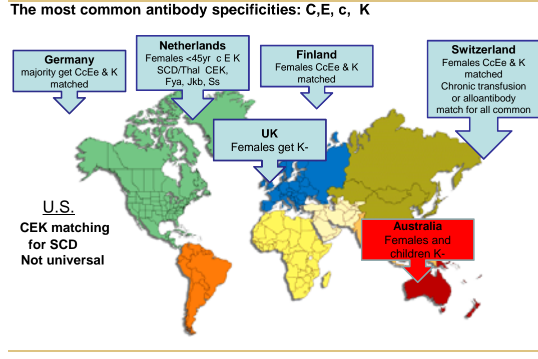
Routine: ABO, RhD type and antibody screen



New York Blood Center

Prevention of Alloimmunization

The most common antibody specificities: C,E, c, K



New York Blood Center

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

New York Blood Center

How to provide higher level of patient care?

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

"Operationalize" Process

- Vein to Vein

New York Blood Center

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

New York Blood Center

Costs – reagents for serologic testing

Antisera	Reagent Cost / Test*
C	0.95 -1.15
E	0.95 - 1.15
c	0.95 - 1.15
e	2.20 - 3.68
M	3.56 - 4.63
N	3.74 - 4.91
S	7.18 - 18.79
s	3.18 - 8.63
K	1.07 - 1.55
Fy^a	2.58 - 7.11
Fy^b	9.18 - 11.43
Jk^a	4.63 - 12.31
Jk^b	4.98 - 13.21
Dia	2.68
Kpa	2.24
Kpb	2.24
Lub	3.20
k	5.13

*average range from a number of facilities and manufacturers 2010

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

- > $250 - $280

New York Blood Center

Currently

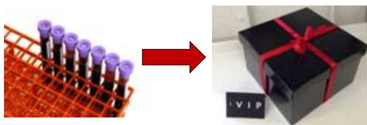
- **CHALLENGE - providing extended antigen typed units**
  - Labor intensive
  - Manual activity
  - Performed in IRL reference laboratory – highly trained staff
  - Testing repeated each time
- Inability to scale up
- No serologic reagents for some clinically significant antigens
- Consequently at many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

28 New York Blood Center

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

29 New York Blood Center

Pre-transfusion Testing



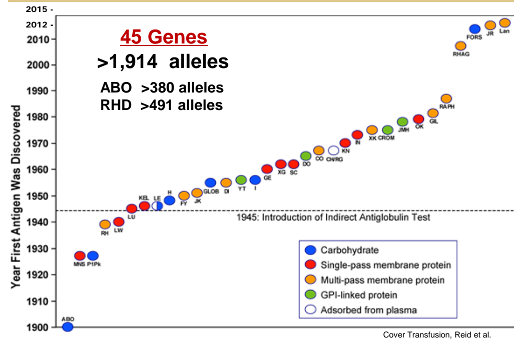
- Every sample is "Black Box"
- Potentially has any of 300+ antibody specificities
- How would we design testing if
  - know what patient is at risk to make (antigen-negative)
    - reduces number of possible specificities by 50%
  - know what patient was exposed to
    - all part of electronic medical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

30 New York Blood Center

Challenges for Current technology

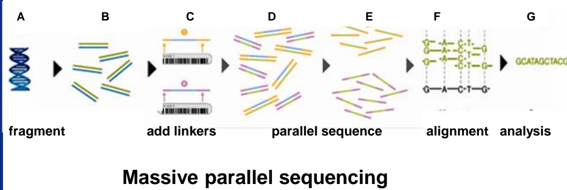
- Limited number of markers
  - Cannot cover all antigens of potential interest
    - common polymorphisms only
    - cannot detect all silenced (null) alleles
    - accurate ABO & D typing will require sampling entire gene
  - Cannot determine "phasing" or "linkage"
    - which alleles are changes carried 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** (W**E**S) – coding regions of genes only (~2%)
  - Whole **genome** (W**G**S) – coding and non-coding regions
  - **Targeted** - capture/amplify gene(s) of interest only - HLA



Next Generation Sequencing Platforms

Table 1 | Sequencing platform comparison

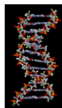
	Roche/454	Life Technologies SOLiD	Illumina Hi-Seq 2000	Pacific Biosciences RS
Library amplification method	emPCR* on bead surface	emPCR* on bead surface	Enzymatic amplification on glass surface	NA (single molecule detection)
Sequencing method	Polymerase-mediated incorporation of unlabelled nucleotides	Ligase-mediated addition of 2-base encoded fluorescent oligonucleotides	Polymerase- mediated incorporation of end-blocked fluorescent nucleotides	Polymerase-mediated incorporation of terminal phosphate labelled fluorescent nucleotides
Detection method	Light [illegible] secondary reactions initiated by release of PPi	Fluorescent emission from ligated dye-labelled oligonucleotides	Fluorescent emission from incorporated dye-labelled nucleotides	Real time detection of fluorescent dye in polymerase active site during incorporation
Post incorporation method	NA (unlabelled nucleotides are added in base-specific fashion, followed by detection)	Chemical cleavage removes fluorescent dye and 3′ end of oligonucleotide	Chemical cleavage of fluorescent dye and 3′ blocking group	NA (fluorescent dyes are removed as part of PPi release on nucleotide incorporation)
Error model	Substitution errors rare, insertion/ deletion errors at homopolymers	End of read substitution errors	End of read substitution errors	Random insertion/deletion errors
Read length (fragment/paired end)	400 bp/variable length mate pairs	75 bp/50+25 bp	150 bp/100+100 bp	>1,000 bp

Nature 470:198, 2011

Genomics Revolution

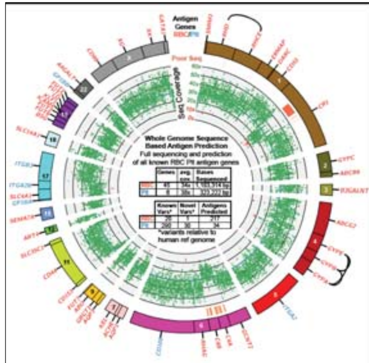
"Genomic data will soon become a commodity; the next challenge — linking human genetic variation with physiology and disease will be as great as the one genomicists faced a decade ago."

J. Craig Venter. Opinion *Nature* 464, 676-677 (1 April 2010)



- **"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.* 56(3):743-54, 2016 **Lane WJ, Westhoff CM, Uy JM, Aguad M, Smeland-Wagman R, Kaufman RM, Rehm HL, Green RC, Silberstein LE**



Ilumina 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)

RHD Mutation	Mutation Frequency	Concordance
L62F	9.3%	98.1%
A137V	9.3%	100%
N152T	9.3%	100%
Psi D*	1.9%	ND*
T201R	7.5%	98.1%
F223V	9.3%	96.3%
E233Q	0.0%	98.1%
Y269X	2.8%	100%
V279M	4.6%	100%
I342T	9.3%	100%
T379M*	15.7%	ND*

RHCE Mutation	Mutation Frequency	Concordance
W16C	49.1%	92.6%
A85G	3.7%	100%
109Ins*	9.3%	ND*
R114W	0.9%	100%
A226P	5.6%	100%
Q233E	0.9%	100%
M238V	0.9%	100%
L245V	35.2%	98.1%
I306V	0.9%	94.4%
G336C	8.3%	100%
T342I	0.9%	100%

Jonathan Flanagan, Baylor; Stella Chou, Philadelphia; Russell E. Ware, Cincinnati

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
4. Exploring next generation of testing

Thank You !!

New York Blood Center
NYBC

Community Blood Center of Kansas City
CBC

40