RBC Genotyping: Current and Emerging Applications

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Starting 7/12/2021:
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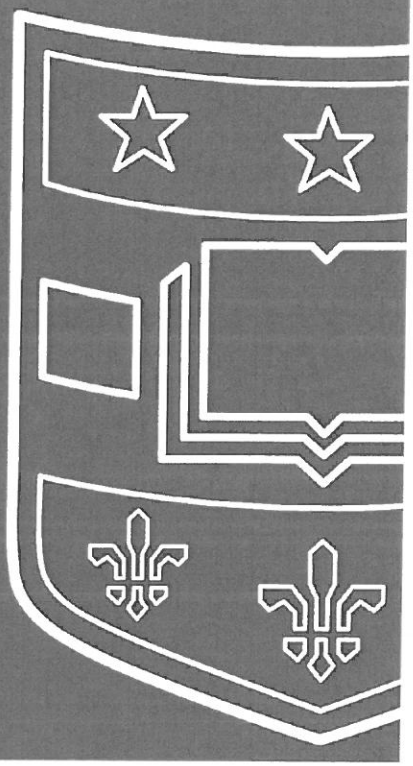
6/9/2021

Washington University in St. Louis



Financial Disclosures

- No Financial Disclosures

Learning Objectives

- Describe appropriate uses of red blood cell genotyping
- Demonstrate an understanding of different red blood cell genotyping techniques both commonly utilized as well as emerging methods
- Explain the why the complete assessment of RHD and RHCE by molecular techniques is difficult

Transfusion Therapy

- Risks
  - Infectious Disease Transmission
  - Iron Overload
  - Transfusion Reactions
  - Alloimmunization
- Phenotyping primary method for determining transfusion compatibility
- Genotyping supplementing

Red Blood Cell Genotyping Indications

Improvement on Phenotype

- Individuals with or at risk for alloimmunization (ie Hemoglobinopathies)
  1) Help antibody workup
  2) Obtain antigen matched units to prevent any or further immunization
- Donor Screening/Matching
- Paternal/Prenatal Testing
- RhIG candidate
- Transplantation

Unique Indications

- Discrepant Serologic Results
  - Ex:
    - Antibody against Antigen Positive By Phenotype
    - "Weak" D
- Significant serological interference
- Recent Transfusion

Pros/Cons of Phenotyping and Genotyping Methods

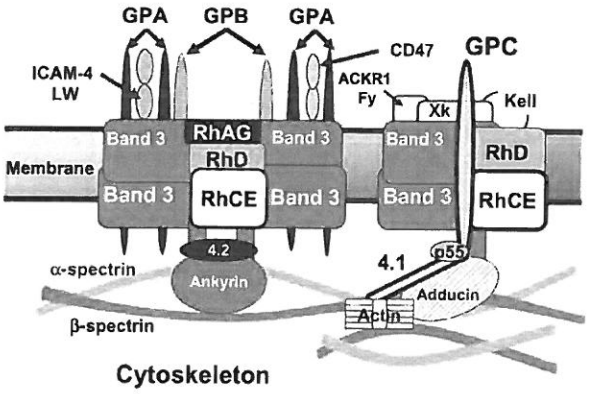
	Phenotyping (Serologic)	Genotyping (DNA)
Pros	- Actual Protein Being Tested - Fast Turn Around Time (TAT) - Cheap (common antigens)	- High Throughput/ Cheaper (rare antigens) - Not affected by recent transfusion - Help determine variant antigens - Resolve whether antibody Auto or Allo
Cons	-Sensitivity Issues Weak/Partial Antigen Difficulty - Low Throughput -Expensive (Rare antigens) -Interference (ie DAT positive) -Fresh RBCs needed	-Expensive (common Antigens) -Genotype ≠ Antigen Expression (Predicted) - Long TAT - Specialized Equipment - Limited FDA Approval

When Should We Think To Order Red Blood Cell Genotyping?

Discrepant Serologic Results

Red Blood Cell Antigens Overview

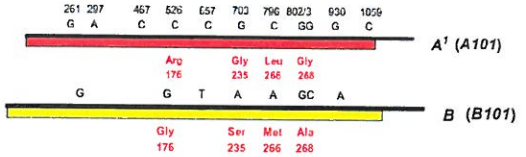
- 38 blood group recognized by ISBT
- At least 300 **serologically** defined antigens
- Non-ABO antigens commonly on most antigen panels:
  - RhD,RhCE, Kell, Kidd, Duffy, MNS, Lewis, Luth, P



Cohn CS, Delaney M, Johnson S, Katz L. Technical Manual 20th Edition. American Association of Blood Banks; 2020. 816 p

Most Red Cell Antigens Defined by Simple Polymorphisms

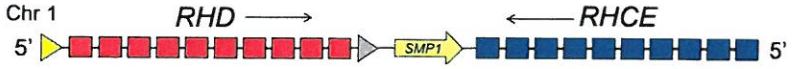
- Clinically relevant red cell antigens are defined by one or a few single nucleotide polymorphisms
- Yet > 2000 alleles defined (most rare)
- However, there are clinically relevant RhD and RhCE variants that are more complex

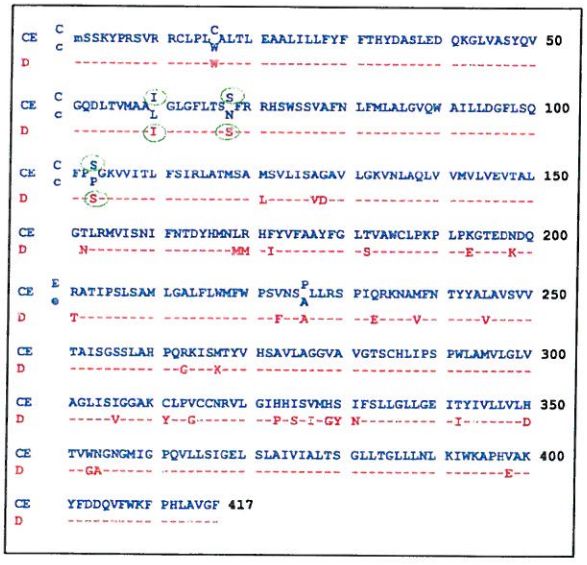


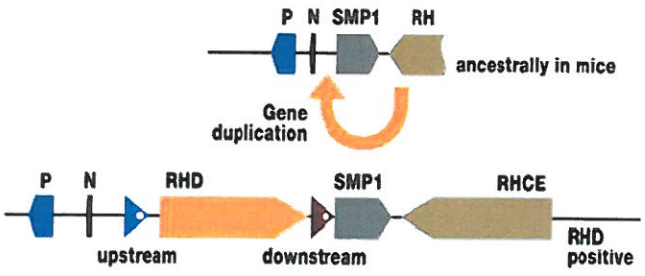
Blood Group System	Analyte	Polymorphism
Rh	c/C	307 C>T
		109 Ins
	e/E	676 G>C
	V5	733 C>G, 1006 G>T
	V	
Kell	K/k	698 T>C
	Js^a/Js^b	1910 C>T
	Kp^b/Kp^c	961 T>C
Duffy	Fy^a/Fy^b	125 G>A
	GATA (Silencing *FY*)	-67 T>C''
	Fy[Fy(b+^{w})]	265 C>T
Kidd	Jk^a/Jk^b	838 G>A
MNS	M/N	50 C>T
	S/s	143 T>C
	Silencing S (Uvar, Uneg)	230C>T
		In5 g>t
Lutheran	Lu^a/Lu^b	230 A>G
Dombrock	Do^a/Do^b	793 A>G
	Hy+/Hy	323 G>T
	Jo(a+)/Jo(a)	350 C>T
Landsteiner-Wiener	LW^a/LW^b	308 A>G
Diego	Di^a/Di^b	2561 C>T
Colton	Co^a/Co^b	134 C>T
Scianna	Sc1/Sc2	169 G>A

PreciseType HEA Molecular BeadChip Test Package Insert

RHD and RHCE Sequence is Very Similar



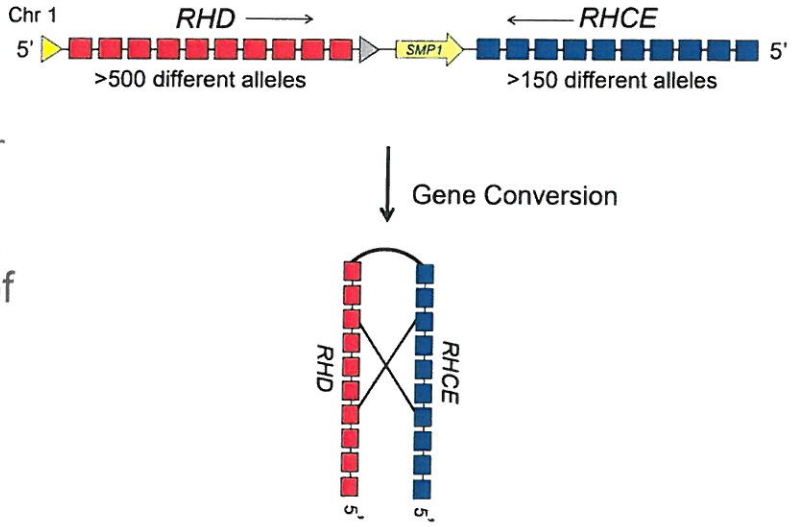




Flegel WA. The genetics of the Rhesus blood group system. *Blood transfusion. 2007;5(2):50.*
Rh and RHAG Blood Group Systems. In: *Human Blood Groups.2013:182-258.*

RHD and RHCE Genetics are Complex

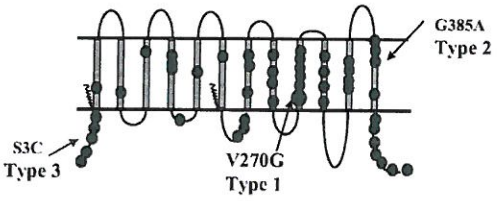
- Many different well characterized variants
  - Variants may cause loss of high prevalent antigens and/or generation of novel antigens
- In addition, gene conversion may occur resulting in loss of epitopes and new antigens
- Up to 85% of AA individuals may have at least one RH variant

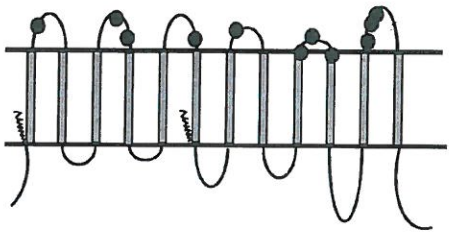


Rh and RHAG Blood Group Systems. In: *Human Blood Groups.2013:182-258.*

Genotyping Necessary to Distinguish RHD Variants

- "Weak"
  - 145 different weak D alleles
  - **weak expression** ,surface D-epitope same
  - Do not make anti-D (RhoGam not necessary)
  - Important for identification in donors
- "Partial"
  - ~130 alleles
  - Type D+ or even "weak"
  - May make anti-D
  - Important for identification in recipients

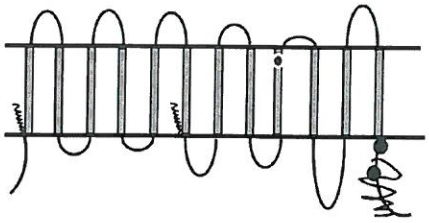




Adapted from Westhoff CM, The structure and function of the Rh antigen complex. *Seminars in hematology*, vol. 44, no. 1, pp. 42-50..

DEL RHD variant be troublesome for blood centers

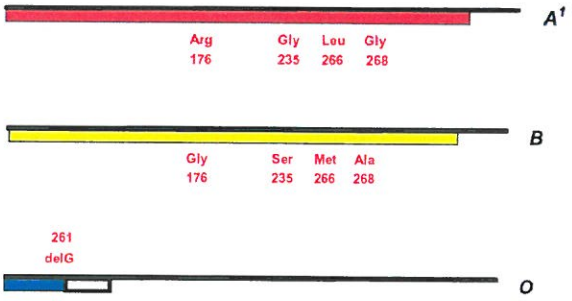
- 45 different DEL alleles
- **Very weak expression**, type D negative (even with Coombs reagent)
- Case reports of stimulating anti-D in D- patients
  - Immunogenicity may be less though
- As high as 30% prevalence in east Asian D- population



Westhoff CM, The structure and function of the Rh antigen complex. *Seminars in hematology*, vol. 44, no. 1, pp. 42-50.
Kwon DH, Sandler S, Flegel WA. DEL phenotype. Immunohematology. 2017;33(3):125.

ABO Genetics

- Chromosome: 9q34.1–q34.2
- Organization 7 exons distributed over 19.5 kbp of gDNA
- Variants may cause extra reactivity in reverse type, weak forward ,or mixed field forward (A_3,B_3)
- Per ISBT
  - 83 A variants
  - 49 B variants
  - 12 cis AB variants
  - 60 O variants
    - all same phenotype –>null
- Confirming type important for donor retention



Adapted From: Daniels G. Human Blood Groups. 3rd edition. Wiley-Blackwell; 2013. Chapter 2: ABO, H, and Lewis Systems

Names for ABO (ISBT 001) blood group alleles v1.1 171023 Available from: https://www.isbtweb.org/fileadmin/user_upload/Working_parties/WP_on_Red_Cell_Immunogenetics_and/001_ABO_Alleles_v1.2.pdf

All red cell variants may cause discrepancy

- Instances of RhD and ABO better characterized and well known
- However, all red cell antigens in theory may have discrepancy

- Phenotype not as strong as one suspect
  - 1) Decreased cell surface expression
  - 2) Change in cell surface epitope in which *monocolonal* reagent does not bind as well
- Antigen positive with apparent antibody (negative auto-control)
  - Any variant changing presentation at cell surface may cause

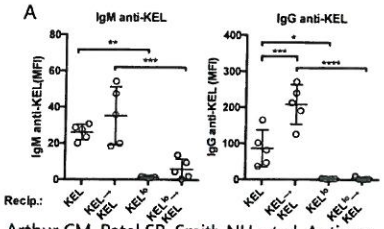
When Should We Think To Order Red Blood Cell Genotyping?

Individuals with or at risk for alloimmunization

Donor Screening

Red Blood Cell Antigens Have Different Immunogenicity

- Type
  - Not all antigens equal
  - Rh most immunogenic followed by Kell
- Density
  - KEL^{lo} and weak RhD low immunogenicity



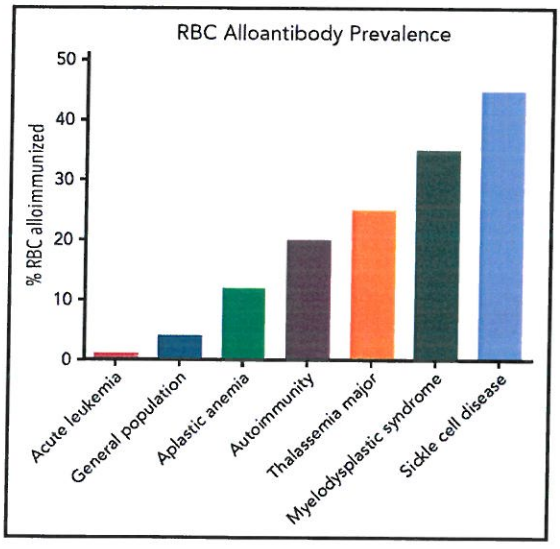
Arthur CM, Patel SR, Smith NH, et al. Antigen density dictates immune responsiveness following red blood cell transfusion. *The Journal of Immunology. 2017;198(7):2671-2680.*

Table 3–7 Relative Immunogenicity of Different Blood Group Antigens

Blood Group Antigen	Blood Group System	Immunogenicity (%)*
D (Rh_0)	Rh	50
K	Kell	5
c (hr')	Rh	2.05
E (rh'')	Rh	1.69
k	Kell	1.50
e (hr'')	Rh	0.56
Fy^a	Duffy	0.23
C (rh')	Rh	0.11
Jk^a	Kidd	0.07
S	MNSs	0.04
Jk^b	Kidd	0.03
s	MNSs	0.03

MT DMHP. Modern Blood Banking & Transfusion Practices. 7th Edition. Philadelphia: F.A. Davis Company; 2018. 672 p.

Disease Status Impacts RBC Alloimmunization



Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. *Blood, The Journal of the American Society of Hematology. 2019;133(17):1821-1830.*

Can Alloimmunization Risk be Reduced?

- General Transfused Individual Risk – 2-5%
- Studies have shown reduction in prevalence of alloimmunization by C/c,E/e,K matching for sickle cell patients[1]
  - 18-66%(1.7-3.9 antibodies/100 units transfused) for ABO/RhD only
  - 5-25% (0.26-0.50 antibodies/100 units transfusion) additional C/c E/e K matching
  - 0-7% (<=0.10 antibodies/100 units transfusion) + Fya/Fyb,S,Jka/Jkb (less evidence)
- Lower alloimmunization in ethnically homogenous countries -> ethnically matched donors

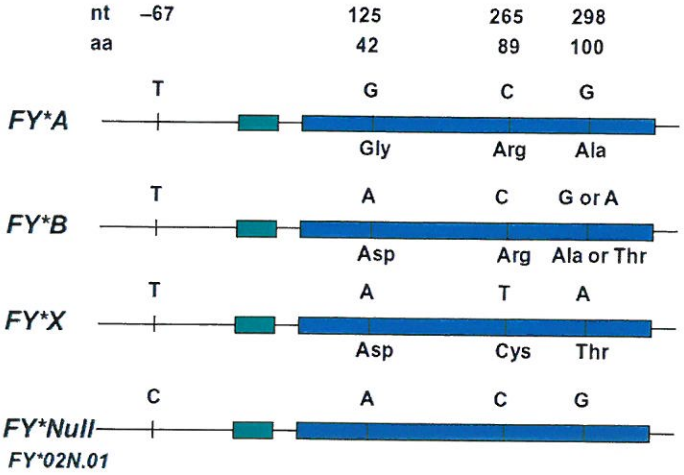
Fasano RM, Sullivan HC, Bray RA, et al. Genotyping applications for transplantation and transfusion management: the Emory experience. *Archives of pathology & laboratory medicine. 2017;141(3):329-340.*

ASH 2020 Guidelines for Sickle Cell Transfusion Support

- *Suggests* an extended red cell antigen profile by genotype ***or serology*** over only ABO/RhD typing for all patients with SCD (all genotypes)
  - Genotyping is **preferred** over serologic phenotyping
    - GATA mutation in Fy, Partial Rh Alleles
- Recommends prophylactic red cell antigen matching for Rh (C, E or C/c, E/e) and K antigens over only ABO/RhD matching for patients with SCD
  - Extended red cell antigen matching (Jk^a/Jk^b,Fy^a/Fy^b,S/s) may provide further protection from alloimmunization

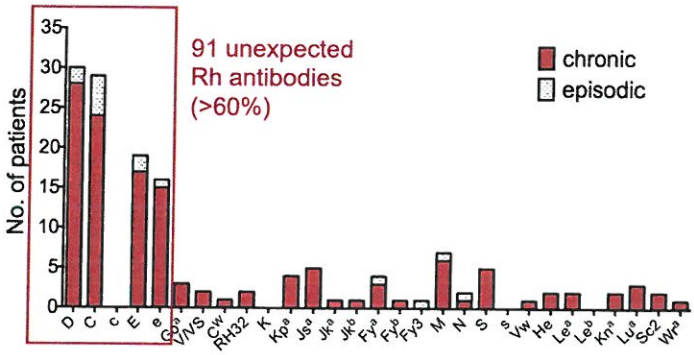
Genotyping Is Important to Identify Patients with GATA Mutation

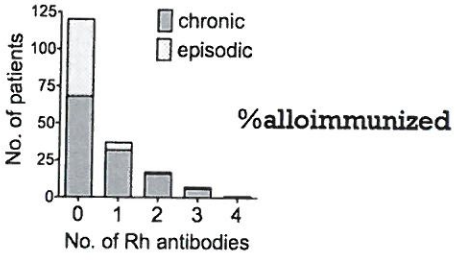
- Receptor for P. Vivax
- High number of African Americans have GATA mutation where Duffy is not expressed **on RBCs**
- Still present on tissues = no risk of alloimmunization

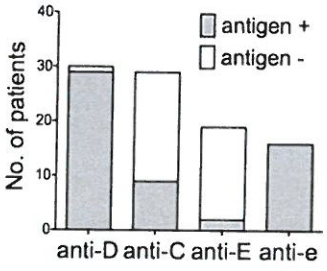


Duffy Blood Group Systems. In: *Human Blood Groups.2013:182-258.*

Phenotype Matching not be Enough in Sickle Cell







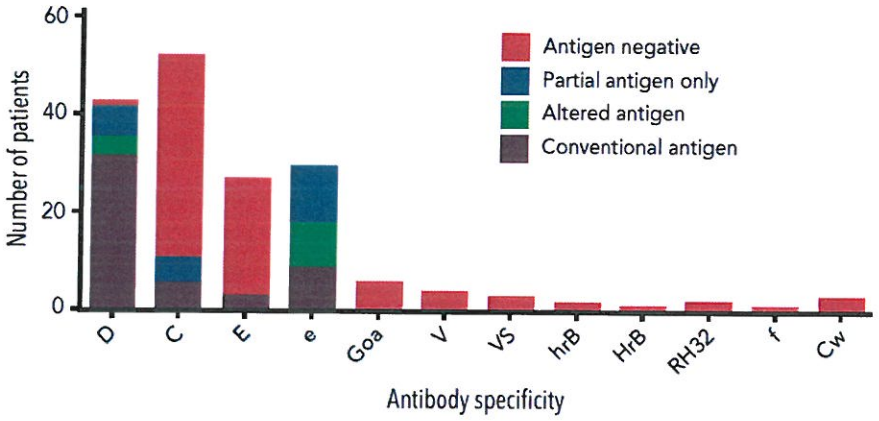
Chou ST, Jackson T, Vege S, et al. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. *Blood, The Journal of the American Society of Hematology. 2013;122(6):1062-1071.*

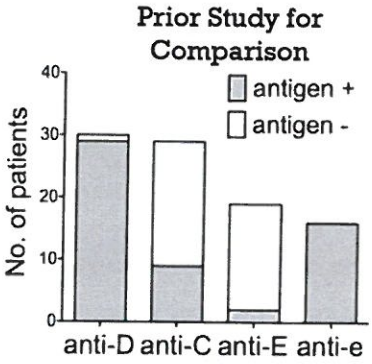
RHD/RHCE Genotyping Matching May Be Better

- 857 patients with SCD and 587 AA donors
- Genotyped utilizing RHD and RHCE BeadChip Arrays + custom PCR assays + sanger sequencing
- Created a Donor Patient Matching Virtual Simulation
- Goal to assess:
  1) Antigen Variation in SCD and AA donors
  2) Antigen Variation in SCD with Antibodies to RHD/RHCE
  3) Feasibility of a RH Genotyping Matching for SCD

Chou ST, Evans P, Vege S, et al. RH genotype matching for transfusion support in sickle cell disease. *Blood, The Journal of the American Society of Hematology. 2018;132(11):1198-1207.*

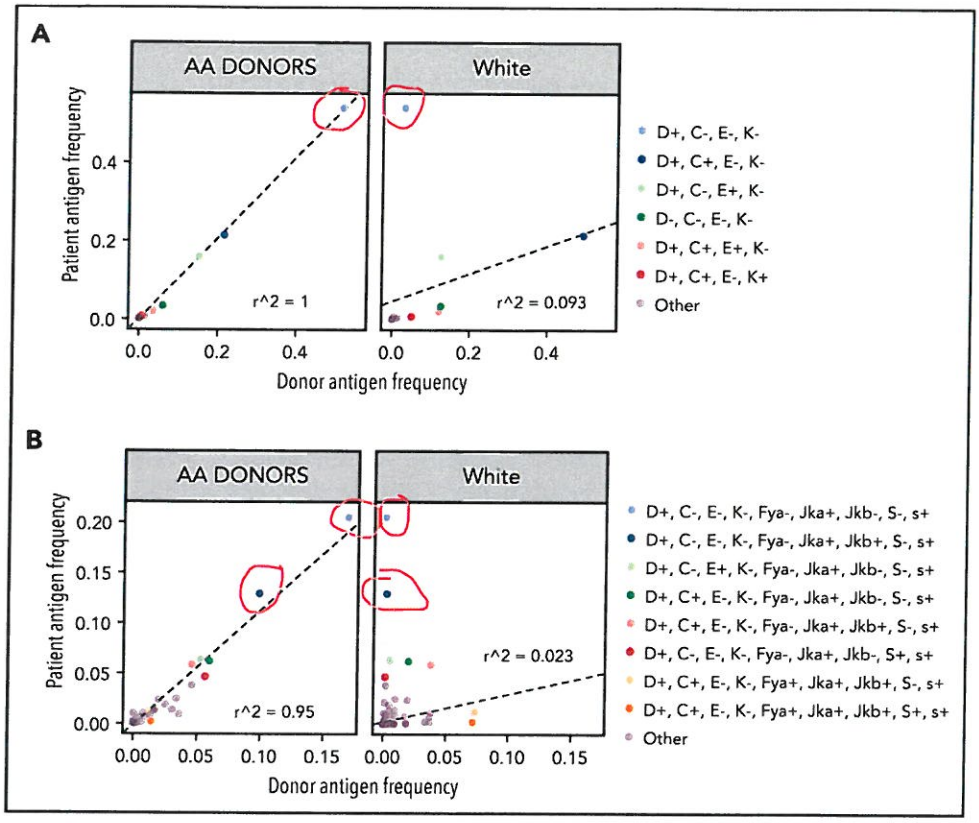
Rh Antigen Status in SCD with Rh Antibodies





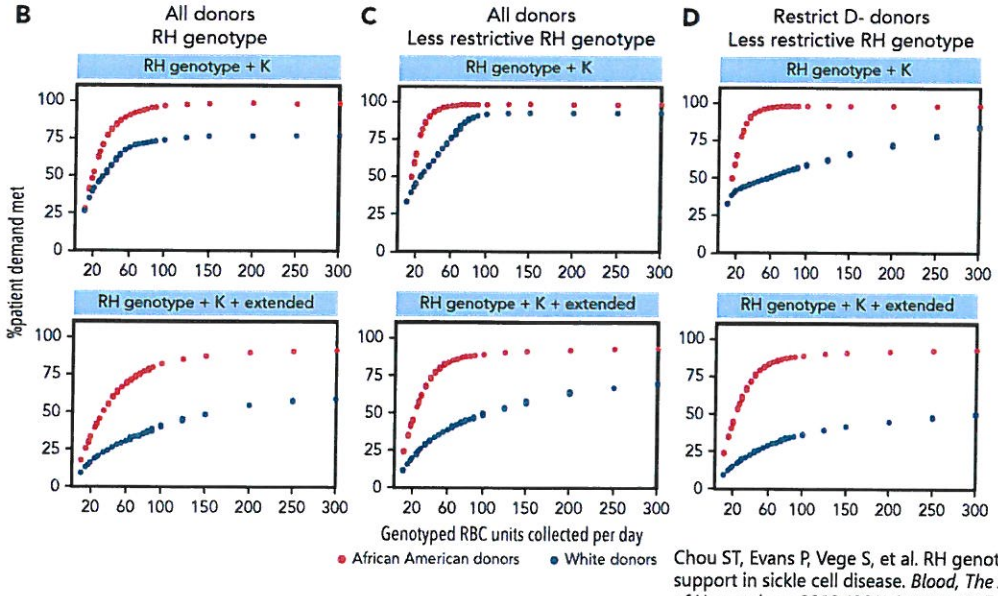
30% variants due Ag+ with at least one conventional allele (64% Anti-D) – Likely Due to Donor

Chou ST, Evans P, Vege S, et al. RH genotype matching for transfusion support in sickle cell disease. *Blood, The Journal of the American Society of Hematology. 2018;132(11):1198-1207.*
Chou ST, Jackson T, Vege S, et al. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. *Blood, The Journal of the American Society of Hematology. 2013;122(6):1062-1071.*



Chou ST, Evans P, Vege S, et al. RH genotype matching for transfusion support in sickle cell disease. *Blood, The Journal of the American Society of Hematology. 2018;132(11):1198-1207.*

Genotype Matching Percent Demand Met with Decent African American Donor Pool

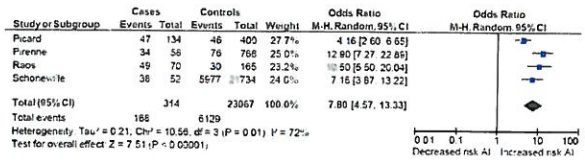


Chou ST, Evans P, Vege S, et al. RH genotype matching for transfusion support in sickle cell disease. *Blood, The Journal of the American Society of Hematology. 2018;132(11):1198-1207.*

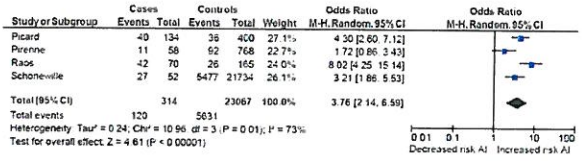
Future Thought
Should we sequence/genotype to find risk factors for alloimmunization?

Anti-Fy^a

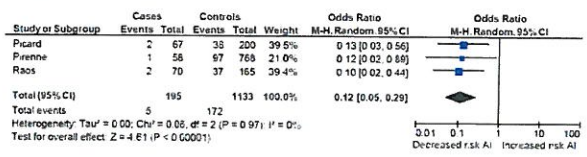
HLA-DRB1*04



HLA-DRB1*15

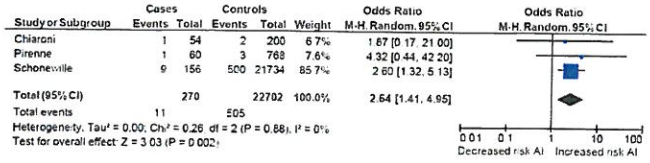


HLA-DRB1*03

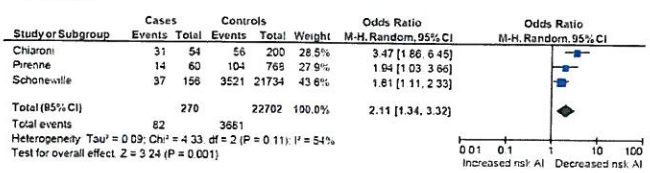


Anti-K

HLA-DRB1*10



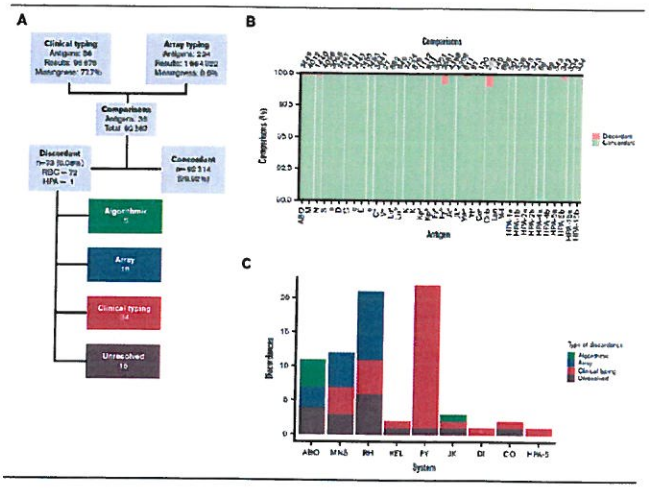
HLA-DRB1*11



Gerritsma J, Oomen I, Meinderts S, et al. Back to base pairs: What is the genetic risk for red bloodcell alloimmunization? *Blood Reviews. 2021:100794.*

Development and validation of a universal blood donor genotyping platform: a multinational prospective study

- Published by the Blood Transfusion Genomics Consortium
- Developed array that can be run at ~$40 a sample
- 99.92% concordance between serologic typing and array
- 34 discordances
  - Included DEL variants



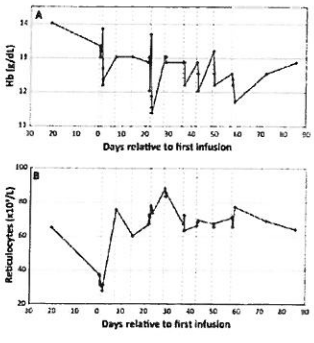
Gleadall NS, Veldhuisen B, Gollub J, et al. Development and validation of a universal blood donor genotyping platform: a multinational prospective study. *Blood advances. 2020;4(15):3495-3506.*

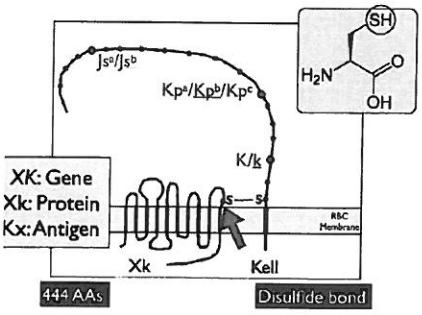
When Should We Think To Order Red Blood Cell Genotyping?

Significant Serologic Interference

Daratumumab & Isatuximab Interferes with RBC Antibody Testing

- CD38 coats RBC membrane
- Associated with only mild hemolysis
- Treatment with DTT destroy CD38 disulfide bonds but also destroys RBC antigens (Kell most important)
- Genotyping recommended because of interference



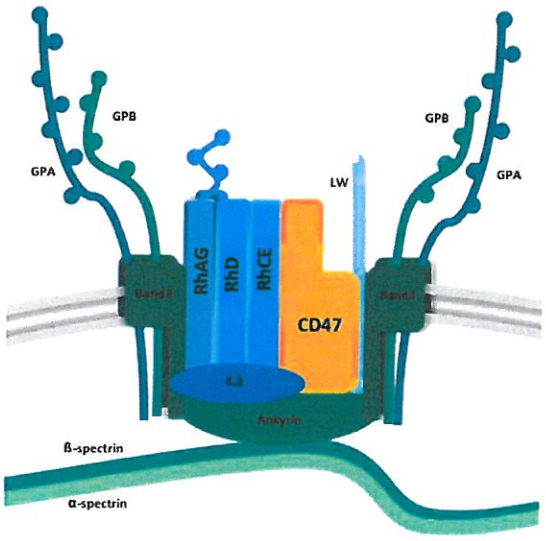


Oostendorp M, Lammerts van Bueren JJ, Doshi P, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. *Transfusion. 2015;55(6pt2):1555-1562.*

Cohn CS, Delaney M, Johnson S, Katz L. Technical Manual 20th Edition. American Association of Blood Banks; 2020. 816 p.

Hu5F9-G4 or anti-CD47 Interferes with RBC Genotyping

- CD47 coats RBC membranes
- An IgG4 antibody
- Ortho Reagents not Anti-IgG4
  - Immucor Reagents have Anti-IgG4 component
- No well establish techniques to get rid of
- Genotyping recommended



Velliquette RW, Aeschlimann J, Kirkegaard J, et al. Monoclonal anti-CD47 interference in red cell and platelet testing. *Transfusion. 2019;59(2):730-737.*

When Should We Think To Order Red Blood Cell Genotyping?

Transplantation

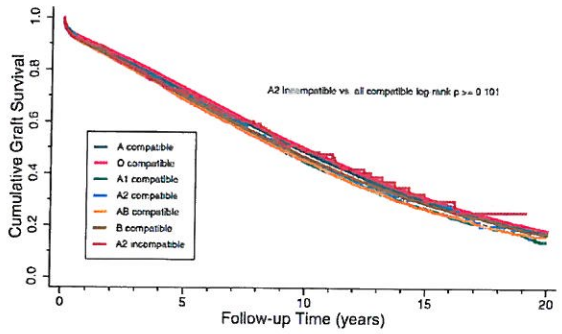
ABO genotyping may be helpful in transplantation

- A_2 subgroups can be utilized as O patients
- Some transplant registries collect buccal swabs routinely but not peripheral blood
- Deceased individuals with massive transfusion

Clin Transplant 2016: 30: 596–597 DOI: 10.1111/ctr.12724

Clinical Transplantation

A2 incompatible kidney transplantation does not adversely affect graft or patient survival



When Should We Think To Order Red Blood Cell Genotyping?

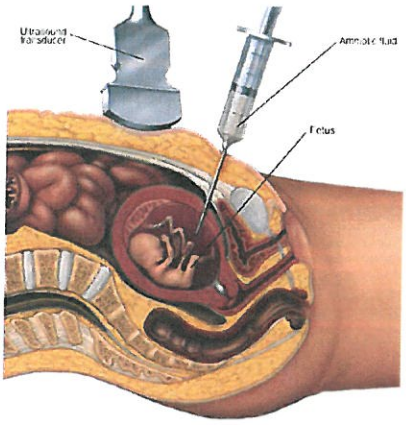
Prenatal Testing

Material Testing/RhIG candidate

Hemolytic Disease of the Newborn

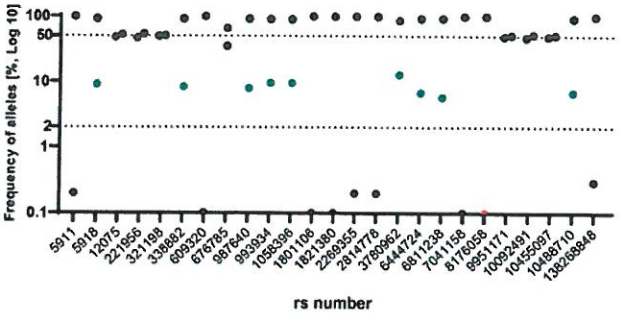
- Immune vs Non-Immune causes
- D antibody not as common cause due to RhIg
- ABO incompatibility (Type O mothers) most common cause now
  - Only IgG can cross placenta
  - Only Type O make IgG ABO antibodies
- Most severe D, c, and K "KELL kills"

- Amniocentesis most common source of fetal DNA for testing



Utilizing cell-free DNA to assess RBC genotype

- Cell Free DNA assessment reduces risk of amniocentesis
- Both maternal and fetal DNA sequenced at same time
- Timing of procedure tricky due to variance of fetal fraction
- Increased traction in Europe, less so in America



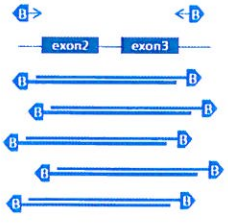
Wienzek-Lischka S, Bachmann S, Froehner V, Bein G. Potential of next-generation sequencing in noninvasive fetal molecular blood group genotyping. *Transfusion Medicine and Hemotherapy. 2020;47(1):14-22.*

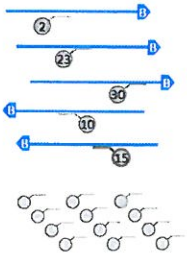
RBC Genotyping Options

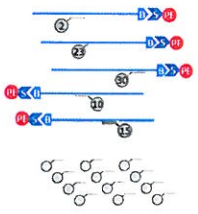
- Ready Made Panels/Arrays
  - FDA Approved
    - Grifols
      - ID Core XT
    - Immucor
      - PreciseType HEA
  - RUO
    - Thermofisher
      - RhD/CE + Red Cell Antigens, ABO
    - Grifols
      - RhD
    - Immucor
      - RhD, RhCE
- Custom
  - PCR Restriction Fragment Length Polymorphism (RFLP)
  - Melting Curve Analysis
  - Allele Specific PCR
  - Sanger Sequencing
  - NGS
    - Complete RBC genes and potentially alloimmunization risk genes may be available

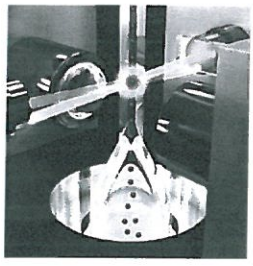
Grifols ID Core XT

- Biotin-labeled PCR amplicons hybridize to DNA probes attached to beads -> Fluorescent Streptavidin-phycoerythrin (SAPE) attached -> Beads quantified by Luminex
- Detects 29 polymorphisms determining 37 red blood cell antigens



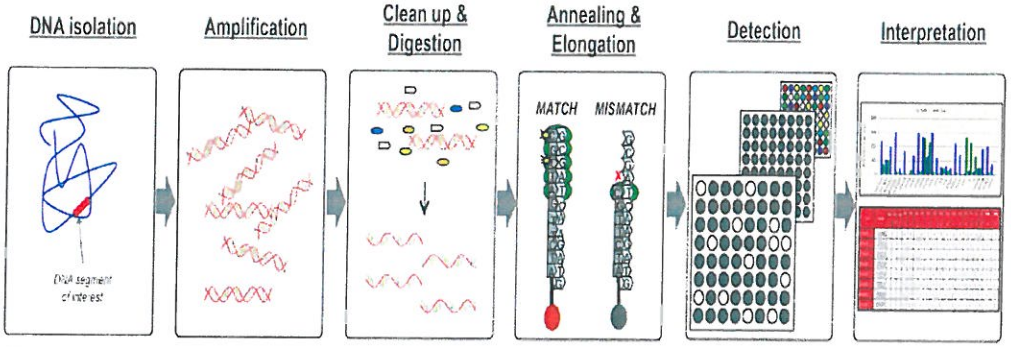


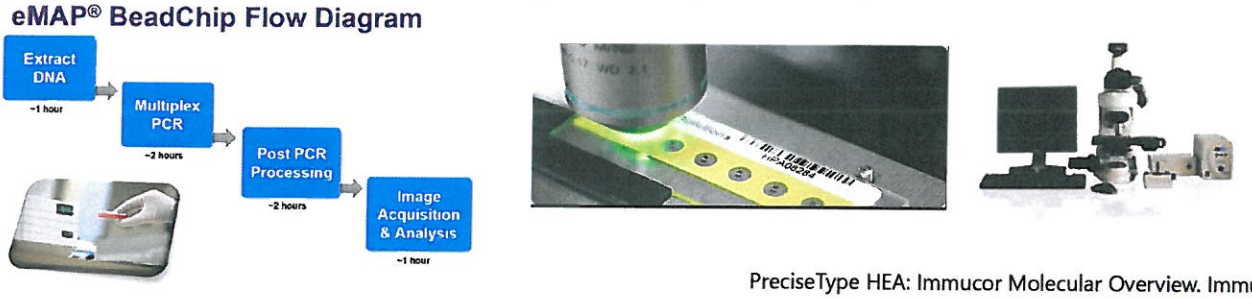




Goldman M, Núria N, Castilho LM. An overview of the Progenika ID CORE XT: an automated genotyping platform based on a fluidic microarray system. *Immunohematology. 2015;31(2):62-68.*

BeadChip Workflow





PreciseType HEA: Immucor Molecular Overview. Immucor.

HEA Precise Type

- First FDA approved test for molecular typing of red cell antigens
- Identifies 35 red blood cell antigens and 3 phenotypic variants from 11 blood groups
- > 99% accuracy for all antigens in FDA validation

Table 1: Genetic Markers for Red Blood Cell Antigens in the PreciseType HEA Test

Blood Group System	Analyte	Polymorphism	ISBT Phenotype	ISBT Genotype
Rh	c/C	307 C>T	RH4, RH2	*RHCE*4, RHCE*2*
		109 Ins		
	e/E	676 G>C	RH5, RH3	*RHCE*5, RHCE*3*
	VS	733 C>G, 1006 G>T	RH20	*RHCE*01.20.01, RHCE01.20.02, RHCE*01.20.04, RHCE*01.20.05*
	V		RH10	
Kell	K/k	698 T>C	KEL1, KEL2	*KEL*01, KEL*02*
	Js^a/Js^b	1910 C>T	KEL6, KEL7	*KEL*06, KEL*07*
	Kp^a/Kp^b	961 T>C	KEL3, KEL4	*KEL*03, KEL*04*
Duffy	Fy^a/Fy^b	125 G>A	FY1, FY2	*FY*01, FY*02*
	GATA (Silencing FY)	-67 T>C**	FY-2	*FY*02N.01*
	$Fy^x/Fy(b+^w)$	265 C>T	FY2W	*FY*02M*
Kidd	Jk^a/Jk^b	838 G>A	JK1, JK2	*JK*01, JK*02*
MNS	M/N	59 C>T	MNS1, MNS2	*GYPA*01, GYPA*02*
	S/s	143 T>C	MNS3, MNS4	*GYPB*03, GYPB*04*
	Silencing S (Uvar, Uneg)	230C>T	MNS-3, 5W, MNS-3,-4,-5	*GYPB*03N.01 or GYPB*03N.02*
		In5 g>t		*GYPB*03N.03 or GYPB*03N.04*
Lutheran	Lu^a/Lu^b	230 A>G	LU1, LU2	*LU*01, LU*02*
Dombrock	Do^a/Do^b	793 A>G	DO1, DO2	*DO*01, DO*02*
	Hy+/Hy-	323 G>T	DO4	*DO*04*
	Jo(a+)/Jo(a-)	350 C>T	DO5	*DO*05*
Landsteiner-Wiener	LW^a/LW^b	308 A>G	LW5, LW7	*LW*05, LW*07*
Diego	Di^a/Di^b	2561 C>T	DI2, DI1	*DI*02, DI*01*
Colton	Co^a/Co^b	134 C>T	CO1, CO2	*CO*01, CO*02*
Scianna	Sc1/Sc2	169 G>A	SC1, SC2	*SC*01, SC*02*

** The GATA mutation listed here has been previously reported at -33 and -46 (ISBT Working Party)[8].

PreciseType HEA Molecular BeadChip Test Package Insert

RHD/RHCE BeadChip Array

- Research Use Only (RUO) Assay
  - Needed own assay due to number of variants
- RHD – 75+ Variants
- RHCE – 44+ Variants

RHD Assay Variant Coverage
Weak D type: 1, 1.1, 2, 3, 5, 14, 17, 29, 34, 40, 41, 47, 51
D negative: RHD deletion, RHDΨ , RHD-CE(3-9)-D, RHD-CE(3-7)-D, RHD-CE(4-7)-D, 48A (W16X), 807G (Y269X), DIIIa-CE(4-7)-D, RHCE(1-3)-D(4-10)
D_{el}: 1227A, IVS3+1G>A
Partial D: DBS0,1,2; DAR, DAR-E, DAU 1,2,3,4,5; DOL 1,2,3; DBT1,2; DIIIa,b,c; DIII type 4,6,7; DIVa,b; DIVa-2, DIV type 3,4,5; DV type 1,2,4,5,6,7,8,9; DVI, DCS1,2; DFR1,2,3,4; DHMi, DNB, DUC2, ceHAR, DFV, Weak D type 4.0, 4.1, 4.3, 11, 15

RHCE Assay Variant Coverage
Detects C, c, E, e, VS, and V antigens along with 44+ RHCE variants including ceAR, ceBI or ceSM, ceCF, CeCW, CeCX, ceEK, cEEW, cEFM, CeFV, ceHAR, ceJAL, cEKH, CeMA, ceMO, ceRA, CeRN, ceRT, ceSL, ceTI, CeVA, r's, ce(1025T), cE(344C), Ce(344G), cE(365T), Ce(365T), cE(602C), Ce(667T), ce(697G,733G), ce(733G), ce(733G,1006T), ce(733G,748A), ce(48C), ce(48C,106A), ce(48C,122G), ce(48C,340T,733G)

PreciseType HEA: Immucor Molecular Overview. Immucor.

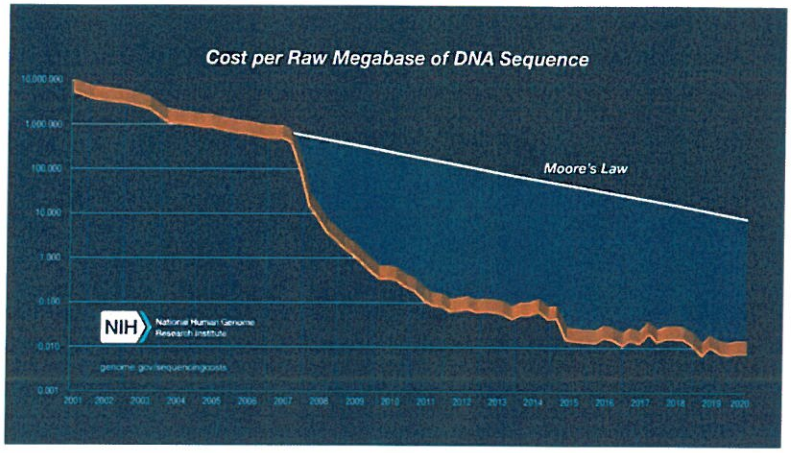
Bead Array Limitations (AABB Conference Case Study)

- 4 yr old sickle cell patient, previously transfused 1 unit
- Admitted for splenectomy and received 2 units matched for Rh and K perioperatively
- Days later Hgb dropped from 10 g/dL to 2.5 g/dL with hyperhemolysis suspected
- HEA predicted C+E-e+,c+ (WT/ce733G variant)
- Possible auto or allo anti-Ce (reacts with R_1 or r' haplotypes) antibody
- RHCE BeadChip Wild Type for Ce
- LDT revealed patient heterozygous for RHCE*CeRN
  - NOTE: Identified in 4% of SCD
- True Genotype: RHCE*CeRN/ce733G variant

Case From: Vege S. Practical Tales of RBC Genotyping: A Molecular Biologist's Perspective. Presented at: AABB 2020 Annual Meeting 2020; Virtual.
Picture From: Halls JB, Vege S, Simmons DP, et al. Overcoming the challenges of interpreting complex and uncommon RH alleles from whole genomes. *Vox Sanguinis. 2020;115(8):790-801.*

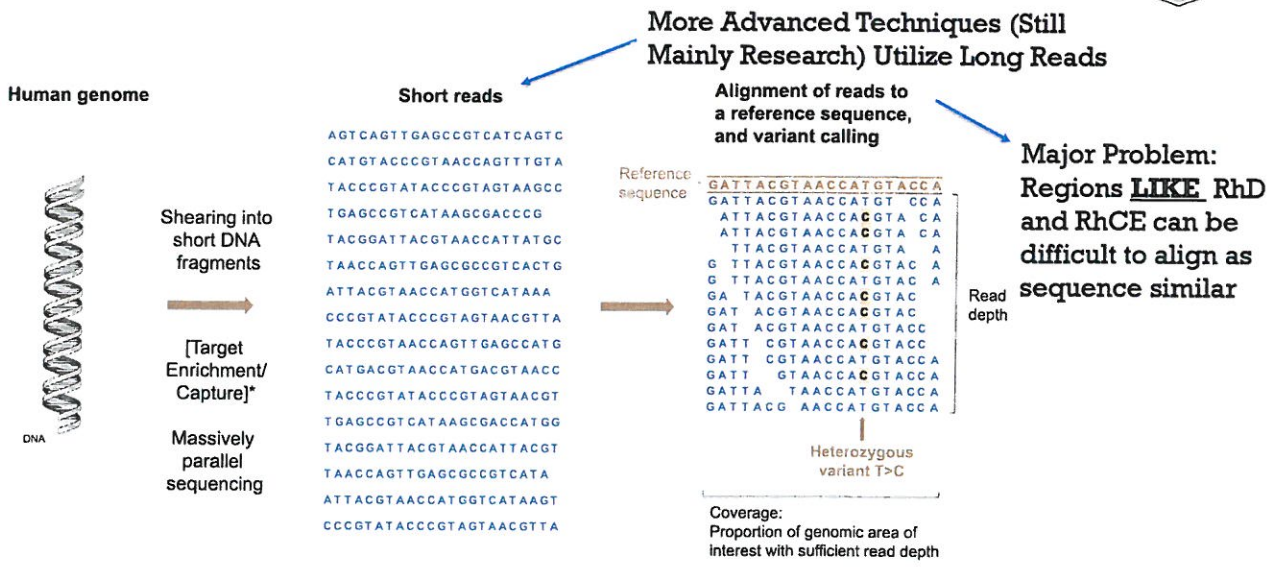
Why not NGS to capture everything? RBC Antigens + RHD/RHCE

- Sequencing is beating Moore's Law
- Steep cliff dive '07
- Has plateaued
- NGS should be considerations in future



DNA Sequencing Costs: Data [Internet]. Genome.gov. [cited 2021 Feb 14]. Available from: https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data

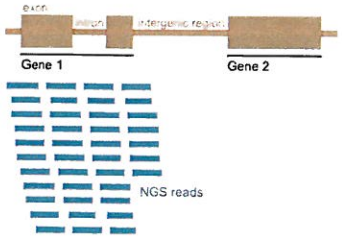
Background: Basic Principle NGS



Gorcenco S, Ilinca A, Almasoudi W, et al. New generation genetic testing entering the clinic. *Parkinsonism & related disorders. 2020;73:72-84.*

Background: NGS Sequencing Technique Comparison

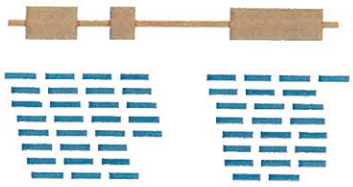
Targeted Sequencing (TS)



Selection (capture):
Panel of genes of interest
(typically 20-200 predefined genes)

Bias! Due to Capture

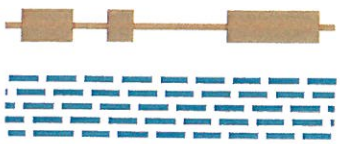
Whole Exome Sequencing (WES)



Selection (capture):
All exons of all known genes
(1.5-2% of all human DNA)

Bias! Due to Capture

Whole Genome Sequencing (WGS)



No selection:
Entire human DNA analyzed
Including introns, RNA genes, etc.

No Capture = No Bias!

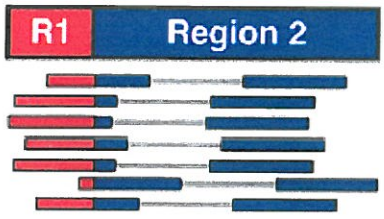
Bias = Difficult Copy Number Detection

Gorcenco S, Ilinca A, Almasoudi W, et al. New generation genetic testing entering the clinic. *Parkinsonism & related disorders. 2020;73:72-84.*

Structural Variants Detection By NGS

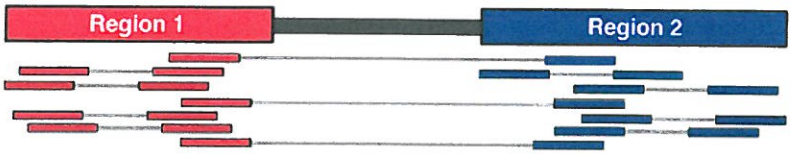
Split Reads

Ex: RHD Ex: RHCE



Discordant Paired Reads

Ex: RHD Ex: RHCE

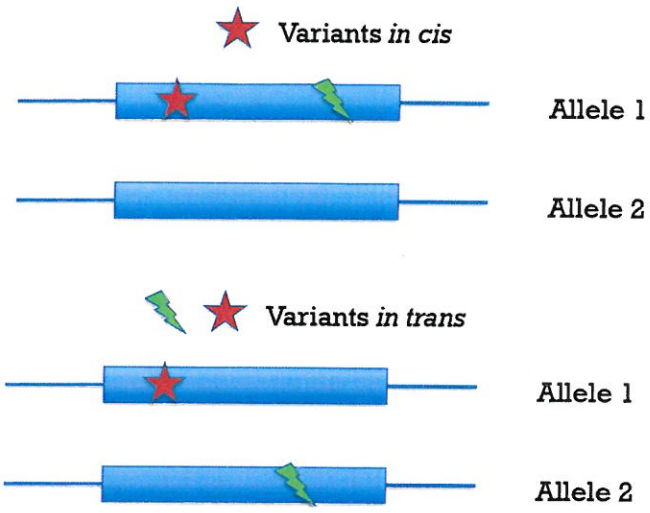


Halls JB, Vege S, Simmons DP, et al. Overcoming the challenges of interpreting complex and uncommon RH alleles from whole genomes. *Vox Sanguinis. 2020;115(8):790-801.*

Pirooznia M, Goes FS, Zandi PP. Whole-genome CNV analysis: advances in computational approaches. *Frontiers in genetics. 2015;6:138.*

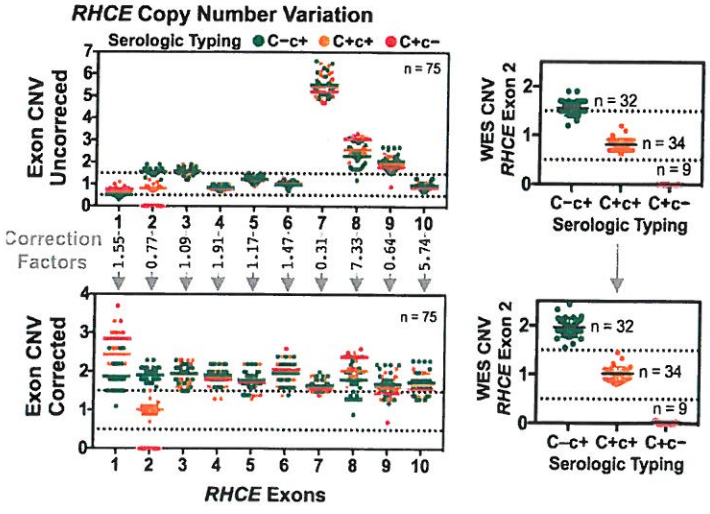
Variant Phasing may be Difficult to Detect

- Molecular techniques for RBC may report "most likely" genotype
  - Due to fact variant phasing is not truly known
- With no phasing information
  1. Knowledge database of variants required
  2. Truly novel variants may be missed
- Short range sequencing may lack phase information



Whole Exome Struggles with RHCE: Copy Number Detection Needed to Be Corrected

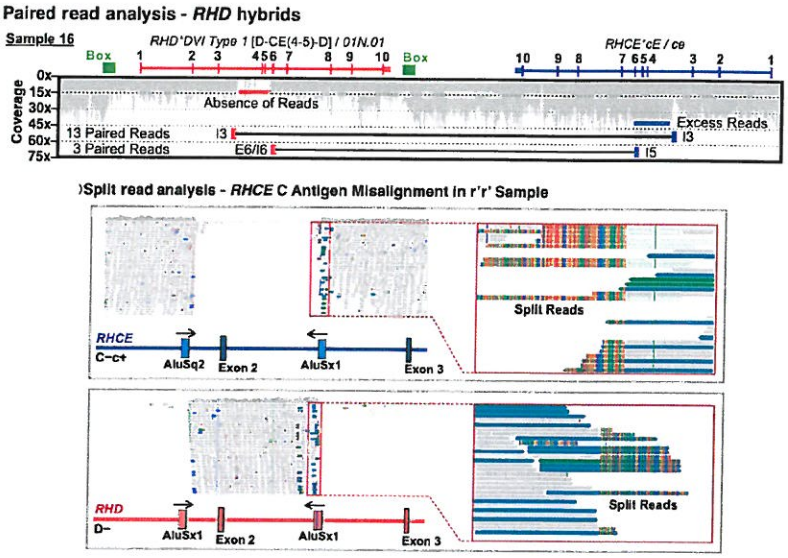
- Sequenced 75 individuals and compared with whole exome Sequencing
  - Problems with C antigen detection
- Why do you need copy number detection?
  - RHCE exon 2 w/ C variant = RHD exon 2
    - Ancestral 4kb gene conversion
  - Due to short reads by NGS copy number only way to detect!



Lane WJ, Vege S, Mah HH, et al. Automated typing of red blood cell and platelet antigens from whole exome sequences. *Transfusion. 2019;59(10):3253-3263.*

Whole Genome Sequencing Much Better for Complex Variants

- 22 complex variants tested and successfully detected
- Utilized
  - Pair Reads, Split Reads, and Read Depth to Identify Variants
- Found ancestral breakpoint in dCe/dCe (r'r') individual using split reads
  - Improved C antigen calls



Halls JB, Vege S, Simmons DP, et al. Overcoming the challenges of interpreting complex and uncommon RH alleles from whole genomes. *Vox Sanguinis. 2020;115(8):790-801.*

RBC Genotyping Methods (Non-NGS)

	Custom One Variant Tests (RFLP, TaqMan, etc)	SNP Array	Sanger Sequencing
PRO	- Cheap for single test - Can be utilized for gaps in larger methods	- FDA approved assays - Commonly Used	- Longer range (1000 bp) than typical NGS - better variant phase info
CON	-low throughput	- May miss rare RHD and RHCE variants	- Expensive if assess more than 1 gene

RBC NGS Genotyping Methods

	Targeted NGS	Exome	Genome	Long Read Sequencing
PRO	-most sensible solution for smaller lab	-less pre-planning necessary -well supported panel	- Best CNV detection - Better detects rare variants - Less pre-planning	- The "Holy Grail" - Complete characterization (Phasing -> can characterize very rare variants)
CON	- Vendor support for panel - Still may miss some variants - Need to be very careful with CNV, significant validation needed	-Cost > Targeted - Need to be very careful with CNV, significant validation needed	-Cost > Exome - Massive economy of scale, at best $1000 a run if significant utilization	- Cost - Custom informatics packages necessary - Needs modified methods or less common NGS sequencers - Poor call accuracy with some sequencers (Nanopore)

Learning Objectives

- Describe appropriate uses of red blood cell genotyping
- Demonstrate an understanding of different red blood cell genotyping techniques both commonly utilized as well as emerging methods
- Explain the why the complete assessment of RHD and RHCE by molecular techniques is difficult

Learning Objectives

- Describe appropriate uses of red blood cell genotyping
  - Individuals with or at risk for alloimmunization (ie Hemoglobinopathies)
    1) Help antibody workup
    2) Obtain antigen matched units to prevent any or further immunization
  - Donor Screening/Matching
  - Paternal/Prenatal Testing
  - Candidate for RhIg
  - Transplantation
    - Ex: A_2 subgroup from massive transfused deceased donor
  - Discrepant Serologic Results
    - Ex:
      - Antibody against Antigen Positive By Phenotype
      - "Weak" D
  - Significant serological interference
  - Recent Transfusion

Learning Objectives

- Demonstrate an understanding of different red blood cell genotyping techniques both commonly utilized as well as emerging methods
  - See Previous Summary Table

Learning Objectives

- Explain the why the complete assessment of RHD and RHCE by molecular techniques is difficult
  - There are a significant number of RHD and RHCE variants
    - Especially in African American population who have highest incidence of hemoglobinopathies
  - A complete SNP array panel for all variants is difficult
  - Even removing costs, caution needs to be applied when simply replacing array method with NGS
    - RHD and RHCE are very similar genes next to each other
    - Prone to gene conversion events
      - Ex: C antigen is gene conversion of exon 2 of RHD into exon 2 RHCE
    - Due to this reads may be misaligned -> danger of wrong genotype assigned

Further Reading

- Westhoff CM. Blood group genotyping. *Blood. 2019;133(17):1814-1820.*
- Jackups Jr R. Impact of Genotyping on Selection of Red Blood Cell Donors for Transfusion. *Hematology/oncology clinics of North America. 2019;33(5):813-823.*

Questions?