



Heart of America Association of Blood Banks

DNA Analysis is Our Ally: Tales from the Immunohematology Frontline

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Immunoematology frontline

Immunoematology “frontline” is the facilitation of “safe” blood transfusion

- **Procedures to accomplish this include:**
 - **Antibody identification**
 - **Antigen typing of patients**
 - **Antigen typing of donors**
 - **Discrepancy resolution in both patients and donors**
 - **Screening for antigen-negative donors**
 - **Cross-matching**

.....**And much more**

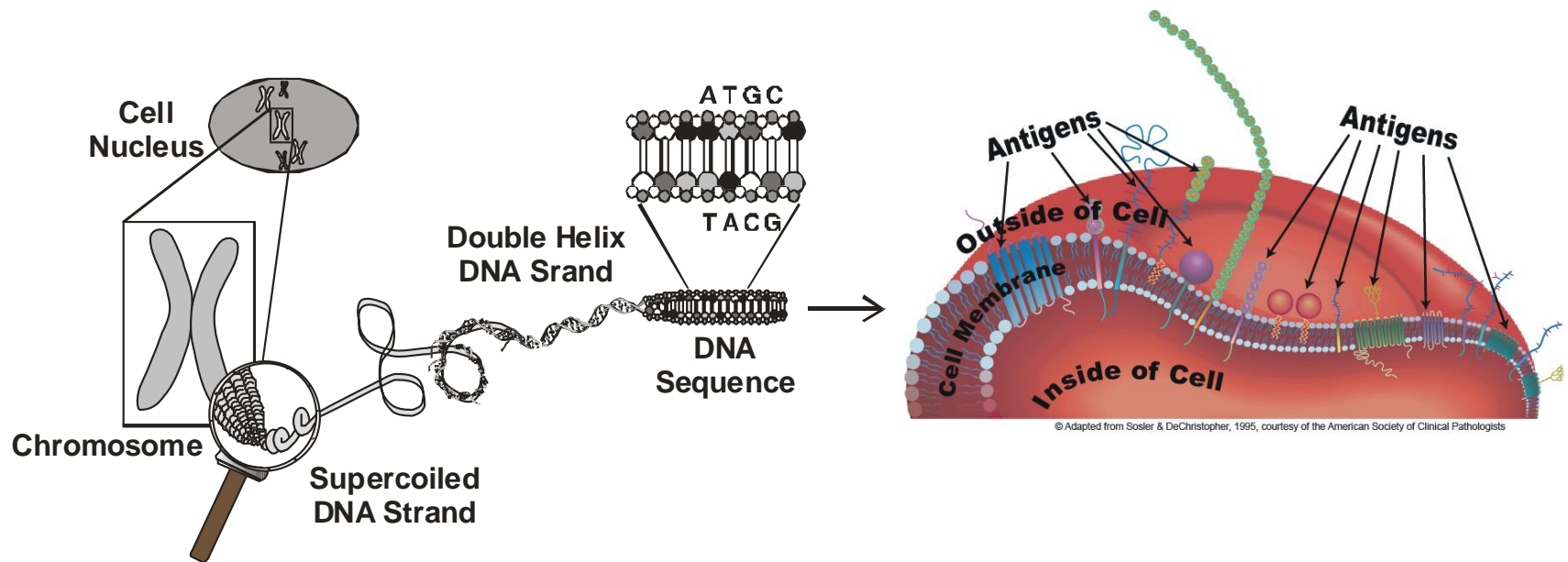
Our traditional arsenal of tools

- **Various test media (e.g., LISS, PEG, Alb, Sal) and phases of reactivity (e.g., 4C, RT, 37C, IS, IAT, DAT)**
- **Absorption and elution**
- **Treatment of panel RBCs with enzymes and chemicals (DTT, EGA)**
- **Null phenotype RBCs for testing with patient's plasma**
- **Inhibition of antibody (natural substances)**
- **Typing of patient's RBCs for common and high/low prevalence antigens (hemagglutination)**
- **Availability of extensively typed RBCs (hemagglutination) [Routine and selected panel(s)]**

Our traditional arsenal of tools is expanding

- Various test media (e.g., LISS, PEG, Alb, Sal) and phases of reactivity (e.g., 4C, RT, 37C, IS, IAT, DAT)
- Absorption and elution
- Treatment of panel RBCs with enzymes and chemicals (DTT, EGA)
- Null phenotype RBCs for testing with patient's plasma
- Inhibition of antibody (natural substances; **recombinant proteins**)
- Typing of patient's RBCs for common and high/low prevalence antigens (hemagglutination; **DNA typing**)
- Availability of extensively typed RBCs (hemagglutination; **DNA typing**) [Routine and selected panel(s)]
- **Molecular technology; an aid for many aspects and levels**

DNA and RBC antigens



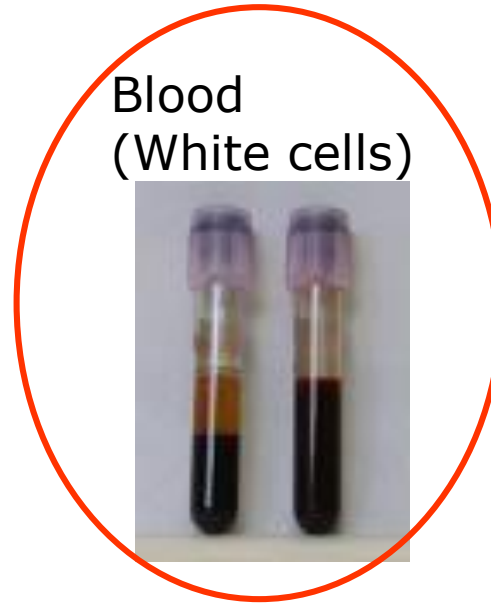
- **Genes encoding the 36 blood group systems have been cloned and sequenced**
- **The molecular bases of most blood group antigens and phenotypes have been determined, with most determined by single nucleotide polymorphisms (SNPs)**

Sample type for DNA

Any nucleated cell

- No sample age requirement
- Patients:
 - Can be post-transfusion
 - Allogeneic stem cell transplant; discrepancy between WBCs and buccal swab

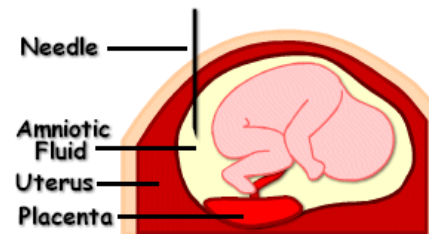
- Donors:
 - Cannot isolate from leukoreduced segments



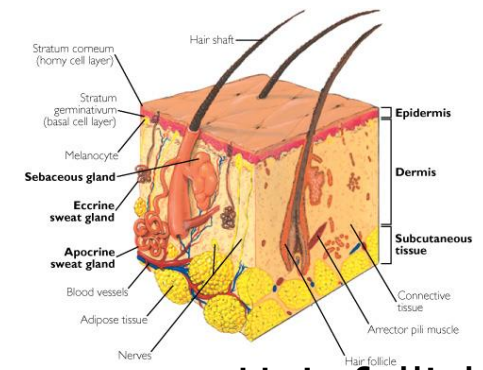
Cheek cells/
Buccal swabs



Amniocytes



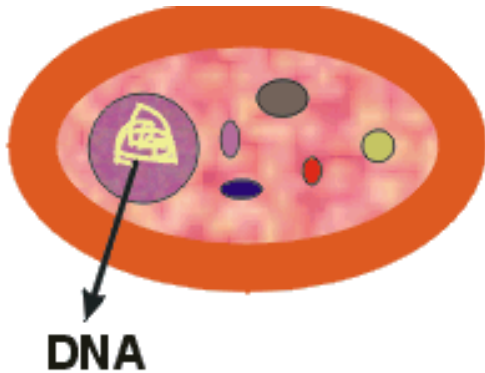
Tissue



Hair follicles

How do you get the DNA?

Sample - cells



Lyse cells



DNA

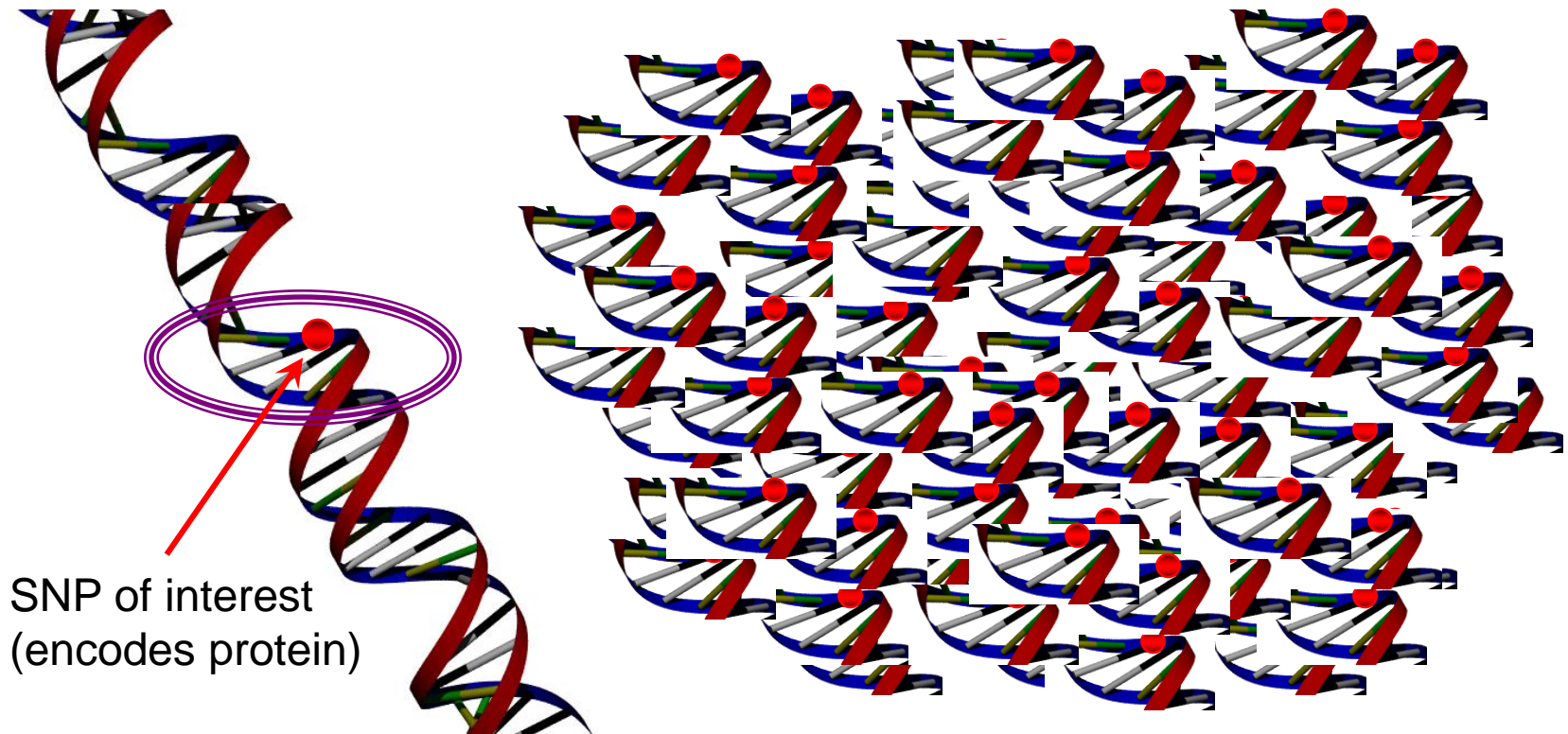


- 🔴 Commercial kits
- 🔴 Automation - BioRobots
 - 🔴 Process 96 samples in 3 hrs



Polymerase Chain Reaction (PCR)

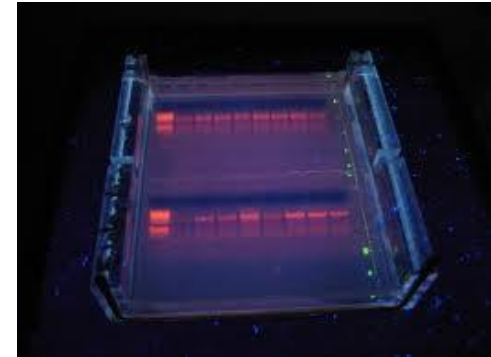
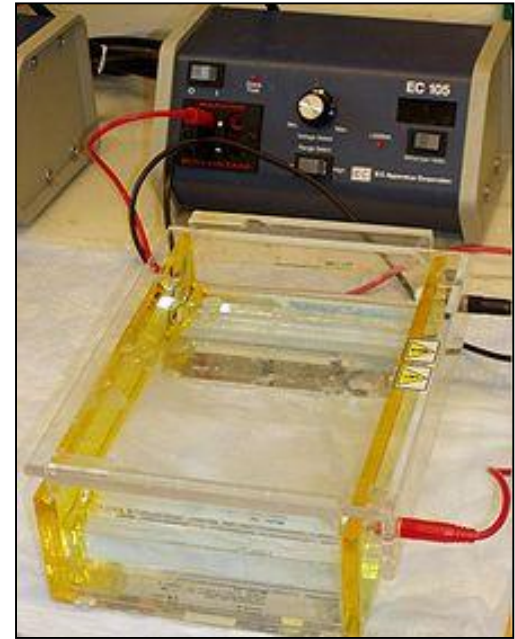
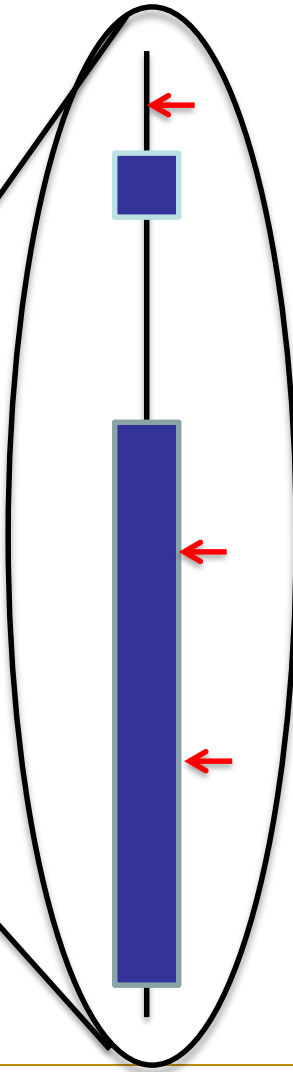
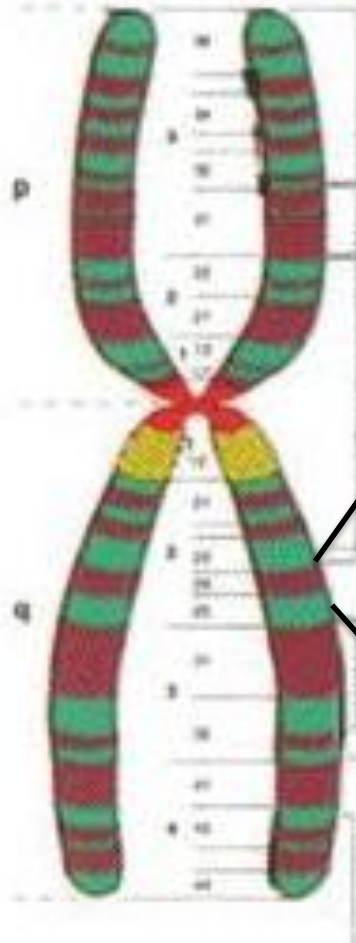
- Amplify a particular segment of DNA that contains SNP or polymorphism(s) of interest
- Generate millions of copies of that segment for further analysis



🔴 For single or few SNPs

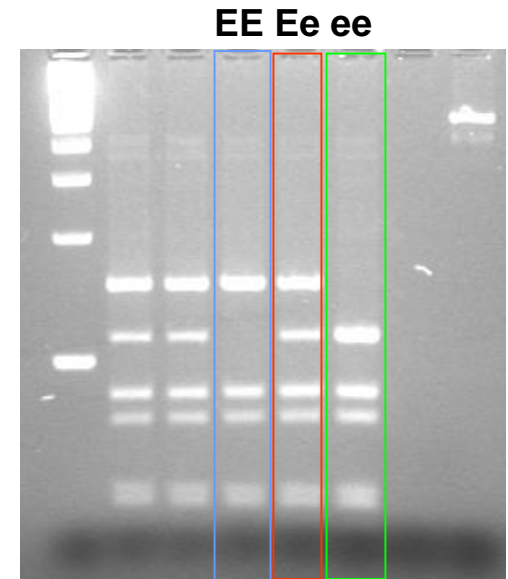
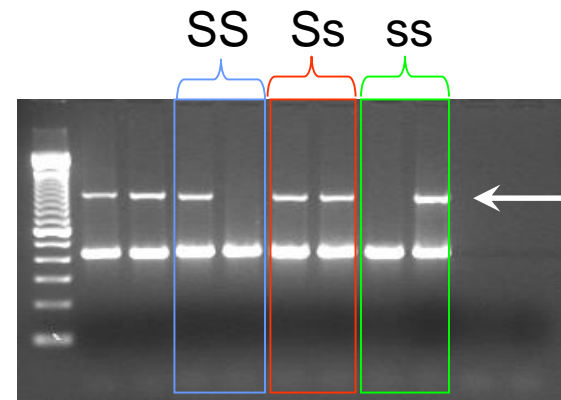
DUFFY

MANUAL ASSAYS



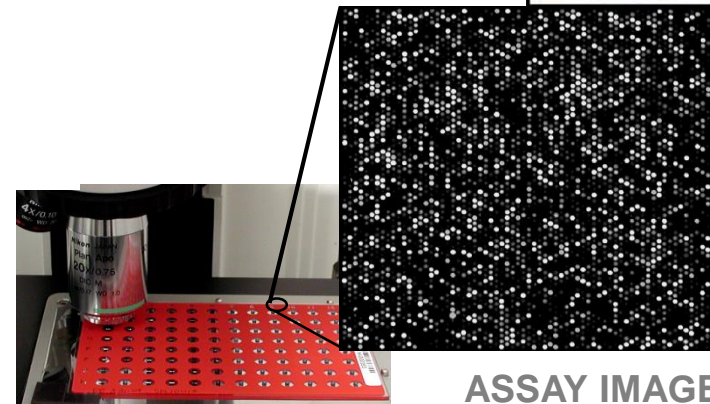
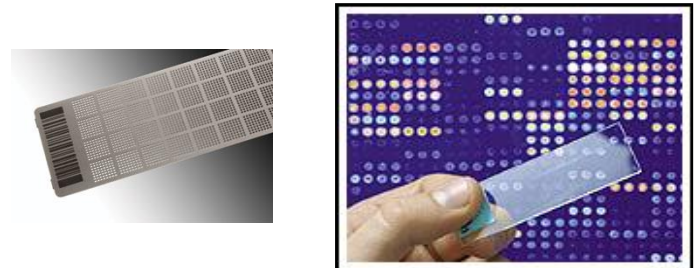
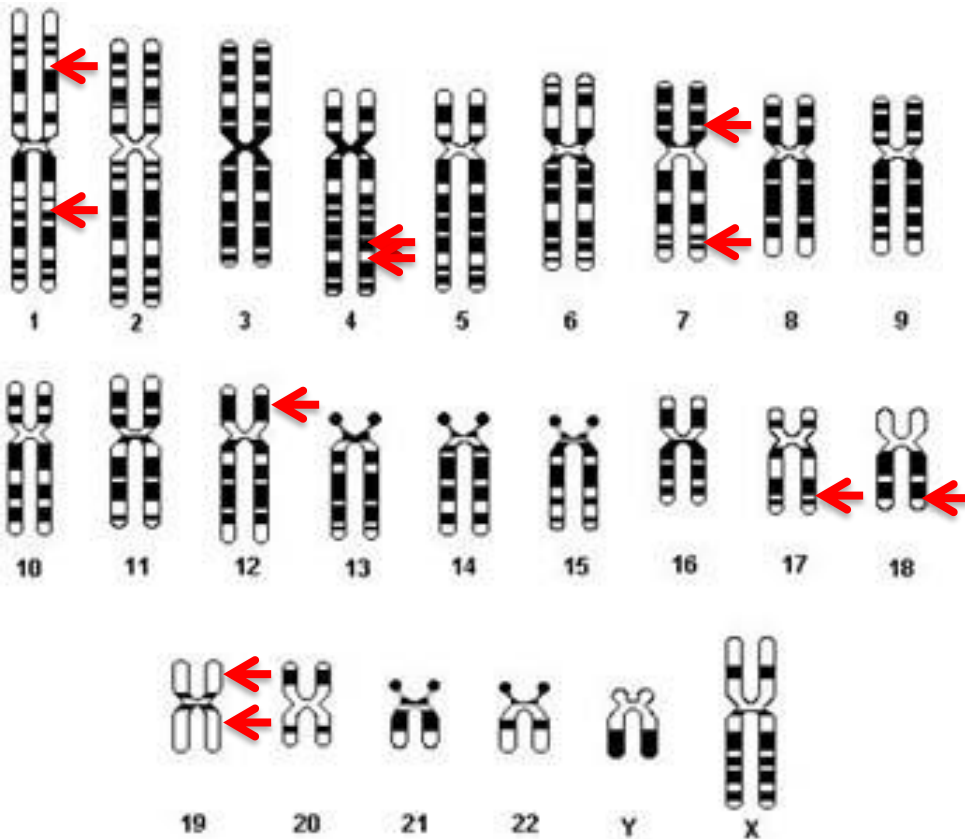
Manual DNA Assays

- Allele-specific PCR (AS-PCR)
 - Primers are specific for SNP/allele
- PCR-Restriction Fragment Length Polymorphism (RFLP)
 - PCR is digested with restriction enzyme and alleles are identified by resulting pattern
- Require electrophoresis for results
 - Gels stained with ethidium bromide
- Time consuming
- Interpretations are done manually



Many SNPs in many genes

- DNA arrays
 - One multiplex PCR
 - Fluorescent read-out for each SNP of interest
 - Interpretation by software
 - Results ~ 5 hours (+ time to extract DNA)



Hemagglutination versus DNA-based Assays

Hemagglutination-based assays:

- **Directly determine** the presence or absence of an antigen through agglutination, or lack thereof, when antibody and RBCs are combined

DNA-based assays:

- Test for the presence or absence of a nucleotide or a sequence of nucleotides within a gene
- **Indirectly predict** the likely presence or absence of an antigen
- Provide a “snapshot” of a gene at a single location; as mostly only a few selected nucleotides are tested for
- **Does not require special reagents**



Case Studies



Case 1

The “Invisible Antibody”

Case 1

- **59 year old female diagnosed with AIHA**
- **Multiple transfusions; unable to phenotype**
- **History of anti-E and anti-K**
- **All units incompatible**
- **Repeated alloabsorptions with R₁R₁, R₂R₂, rr RBCs**
- **No new antibodies demonstrated**
- **However, patient has overt post-transfusion hemolysis**

No answer from serology.....



DNA typing to the rescue

Case 1: DNA testing predicts the RBC phenotype

- Patient sample submitted for DNA typing
- Probable genotype:
 - *RHD, RHCE*C/c, RHCE*e/e, KEL*2/2, JK*A/A, GYPB*S/s, FY*A/B* (with wild type GATA box)
- Predicted RBC phenotype:
 - **D+C+E–c+e+, K–k+, Jk(a+b–), S+s+, Fy(a+b+)**
- Was the post-transfusion hemolysis caused by (serologically undetectable) alloanti-Jk^b?
- Transfused with E–K–Jk(b–) blood
- No post-transfusion hemolysis

Patients with autoimmune hemolytic anemia

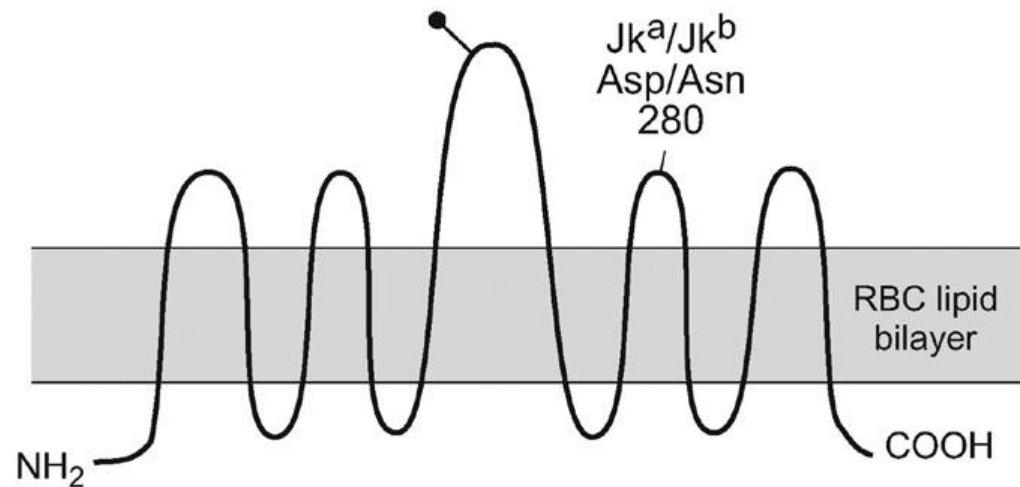
- **Challenge to find appropriate RBC units for transfusion**
- **Due to the presence of a strong autoantibody:**
 - **All RBC samples on the antibody screening and identification panels will be agglutinated**
 - **Difficult to detect and exclude underlying alloantibodies**
 - **Adsorption techniques, either allo or auto, cannot be done in all facilities and are time-consuming**
- **20% to 40% of patients have underlying clinically significant alloantibodies**

Patients with AIHA: Prediction of RBC Phenotype

- **Should determining patient's phenotype and providing prophylactic antigen-matched RBCs become routine?**
- **Provides flexibility in for transfusion management, but maintains safety and avoids or simplifies pre-transfusion adsorption studies**
- **DNA-based assays make prediction of RBC phenotype feasible**
- **Level of antigen-matching to be decided!**



No Kidding around with Kidd



Case 2: history

- 49 yo Hispanic woman; congestive heart failure
- Last transfused in 2005
- Hbg: 9.5g/dL HCT: 28.5%
- Hospital suspects anti-Jk^a

IRL results

- Group O Rh-positive
- DAT: + weak with polyspecific, anti-IgG, anti-C3
- Anti-Jk^a by albumin IAT, PEG IAT and IgG gel test
- Selected panels ruled out other specificities
- Warm autoantibody (no specificity)

Testing for Jk antigens: serology and DNA

- DNA extracted from whole blood
- HEA array performed for common red cell antigens

Array Results:

Detail	c	C	e	E	K	k	Kpa	Kpb	Jsa	Jsb	Jka	Jkb	Fya	Fyb	M	N	S	s	Lua	Lub	Dia	Dib	Coa	Cob	Doa	Dob	Joa	Hy
VS+V+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	0	+	+	+	+

Partial e
Could make allo anti-e

GATA mutation
Not at risk for anti-Fyb

Predicted Jk(a+)

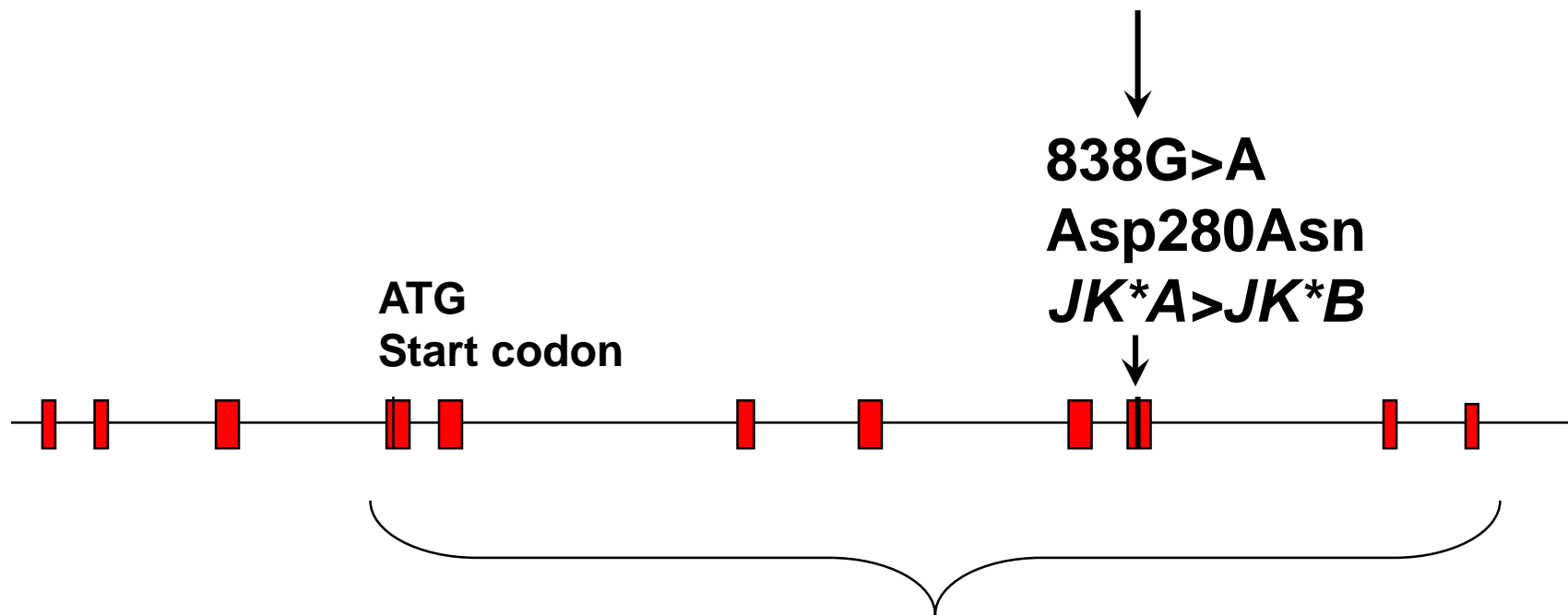
Anti-Jk^a in plasma

RBCs	Jk ^a	Jk ^b
Patient EGA treated	3+	3+
Jk(a+b+) control	3+	3+

JK gene

11 exons

HEA BeadChip targets

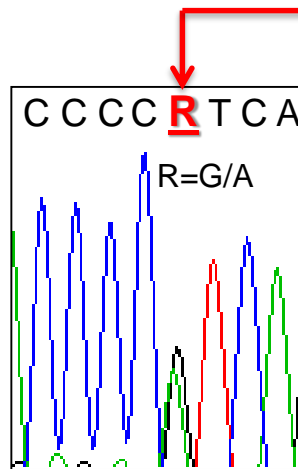


Exons 4 to 11 encode mature protein

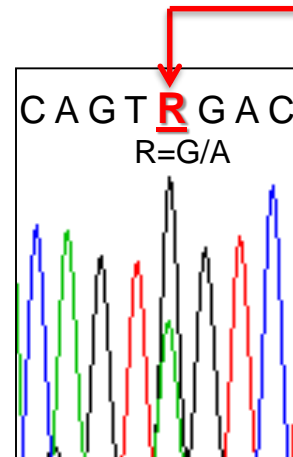
Exons 4 to 11 to be sequenced

Case 2: sequencing of *JK**A and *JK**B

- Sequenced exons 4 to 11
- Confirmed *JK**A/*JK**B as predicted by HEA
- Exon 5 sequence:
 - Heterozygous for 226G>A (Val76Ile) and 303G>A (silent)



226G>A
Val76Ile



303G>A
No change in
amino acid

- Allele was previously reported: *JK**01W.04
- Predicted phenotype for patient: Jk(a^wb⁺)
- *JK**A (*JK**01W.04) encodes partial Jk^a antigen
- Patient's anti-Jk^a likely alloantibody; transfuse Jk(a⁻) units

Case 3: history (first admission)

Patient:

- 33 year old Filipino male with sepsis and cirrhosis
- Transfusion urgently required
- Positive antibody screen; DAT+
- Transfused 4 months ago; negative antibody screen at that time

Plasma contained:

- Anti-E, **anti-Jk^b**, warm autoantibody

Patient's RBCs:

- **Jk(a+b⁻) by serology**

DNA predicts Jk(a+b⁺) by HEA
Additional testing initiated

Transfusion:

- Patient transfused with Jk(b⁻) E⁻ RBCs

Case 3: HEA array analysis

USA

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

- Predicted to be Jk(a+b+)
- Silenced *JK*B* suspected based on serology
- Additional DNA testing initiated

Case 3: first admission – serological results

RBC testing

- **DAT 1+ IgG**
- **An eluate made from the patient's RBCs :**
 - Reacted weakly (1+) in the IAT with all panel cells tested
 - Reacted with the EGA-treated autocontrol indicating probable warm autoantibody
- **EGA-treated RBCs typed Jk(a+b–) with Immucor polyclonal reagents**
- **Untreated RBCs also typed Jk(a+b–) with Ortho BioClone reagents**
- **The Jk^a typing with both reagents was weaker than with Jk(a+b+) control RBCs**

Case 3: the patient returns

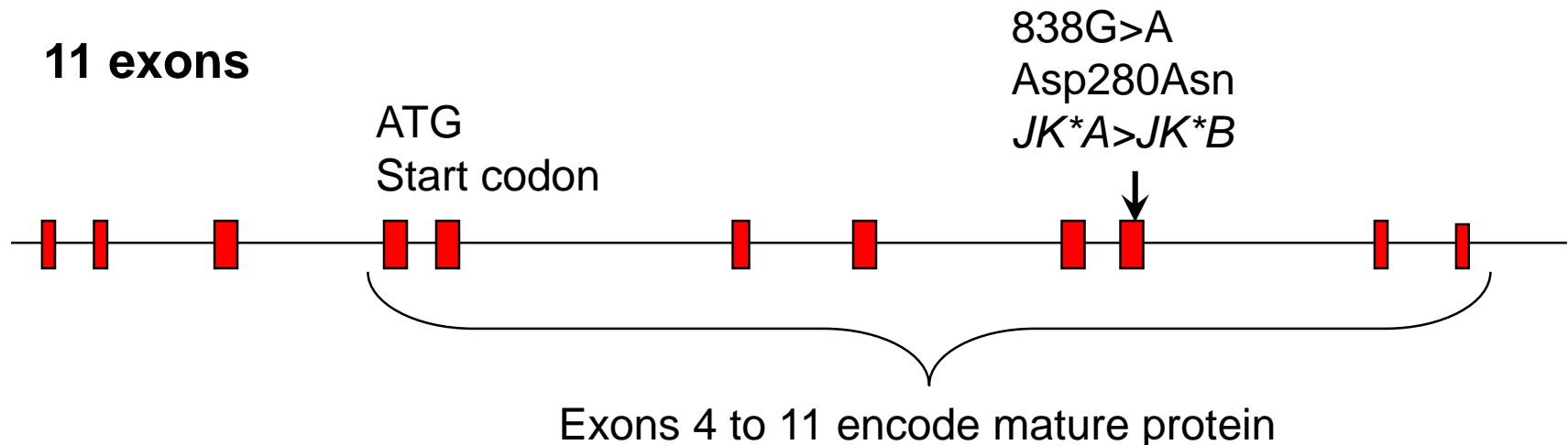
- **Patient subsequently readmitted**
 - Had been multiply transfused with Jk(a+b–) RBCs
 - Plasma reacted weakly micro in the IAT with Jk(a+b–) RBCs
 - **DAT 1+ IgG**
- **An acid eluate reacted weakly (+/- to 1+) with all panel cells tested**
 - Jk(a+b–) RBCs reacted 1+
 - Jk(a–b+) RBCs reacted +/-
- **However, the eluate was non-reactive with Jk(a–b–) RBCs**
 - **Does the eluate contain anti-Jk3?**
- **The patient's sample was exhausted and QNS for further testing**

Case 3: two weeks later.....

- **Plasma reactivity strength increased**
 - 1+^s to 2+^w with all panel cells tested
 - Jk(a+b-) and Jk(a-b+) reacted equally
 - Jk(a-b-) RBCs non-reactive (n=2)
 - Is the plasma antibody anti-Jk3?
- **Adsorption and elution studies of the patient's plasma undertaken to define specificity/ies**
 - The patient's plasma contained separable anti-Jk^a and anti-Jk^b
 - Ruled out anti-Jk3 in plasma
- **Due to lack of sample, adsorption/elution studies could not be performed on eluate to look for anti-Jk3**

Case 3: DNA testing results

- HEA testing predicted Jk(a+b+)
- Silenced *JK*B* suspected based on Jk(b-)serology
 - HEA does not target silenced *JK* or variant *JK*
- *JK* gene sequencing initiated to determine molecular basis of the apparent silenced *JKB*



Case 3: gene sequencing of *JK* exons 4 and 6

- Exon 9: c.838G/A confirmed *JK**A/*B
 - Exon 4: Heterozygous c.130G/A predicting Glu44Lys
 - associated with weak/variant Jk^a
 - has now been found in several populations
 - Exon 6
 - Intron 5, heterozygous IVS5-1 g>a
 - associated with skipping of exon 6 and a silenced (null) *JK**B allele
 - Predicts a Jk(b–) phenotype
 - Not uncommon in Polynesians
 - Patient's *JK* genotype: *JK**01W.01/*JK**02N.01
 - Predicted RBC phenotype: Jk(a+^wb–)
-
- Wester, E.S., et al., 2011. Characterization of Jk(a+^{weak}): a new blood group phenotype associated with an altered *JK**01 allele. *Transfusion* 51, 380–392
 - Whorley T, et al. *Transfusion* 2009; 49S: 48A Abstract (S1 6-040E)

Case 3: transfusion

- Patient was initially transfused Jk(a–b–) RBCs
 - Exceedingly rare phenotype, mostly found in Polynesians, Filipinos and Finns
- Use of Jk(a+b–) RBCs was considered because of the unknown clinical significance of the anti-Jk^a made when *JK**A 130G>A change is present
 - Patient appeared to tolerate Jk(a+b–) blood as reflected by no acute transfusion reaction reported before the anti-Jk^a was identified
- Patient's family was tested for possible donors

Case 3: Family Study

Sample	Jk ^a	Jk ^b	JK* 01 (JK*A)	JK*02 (JK*B)	JK genotype
Proband	1+	0	Exon 4: c.130G>A Glu44Lys	Exon 6/intron 5 IVS5-1g>a skipping of exon 6	<i>JK*01W.01/*02N.01</i>
Father	3+	3+	Exon 4: c.130G>A Glu44Lys	No changes Consensus <i>JK*B</i>	<i>JK*01W.01/*02</i>
Mother	1+	0	Exon 4: c.130G>A Glu44Lys	Exon 6/intron 5 IVS5-1g>a skipping of exon 6	<i>JK*01W.01/*02N.01</i>
Brother	3+	0	Exon 4: c.130G>A Glu44Lys (Homozygous)	No <i>JK*B</i>	<i>JK*01W.01/*01W.01</i>

- ABO-compatible mother and brother are expected to be suitable donors
- DNA sequencing revealed compatible donors that would have been considered unsuitable based only on RBC testing with anti-Jk^a/Jk^b

When is a positive not a positive?



DNA analysis helps to explain an antigen typing discrepancy

Case 4: what's up with E?

- Caucasian female blood donor, Group O+
- 10 prior donations
- On 2 donations RBCs typed D+C+E-c+e+
- Unit labeled and shipped as E-
- Re-typing by the hospital indicated E+

Testing with anti-E reagents

	Gamma clone (GAMA402)	SeraClone (MS60/12)	ALBA clone (DEM1)	In-house polyclonal
Donor RBCs	4+	4+	2+ ^{mf}	very weak

E+ with four strongly reactive with two

	Ortho BioClone (C2)	Immucor polyclonal	Immucor Series I (MS12)	In-house polyclonal
Donor RBCs	0	0	0	0

E- with four

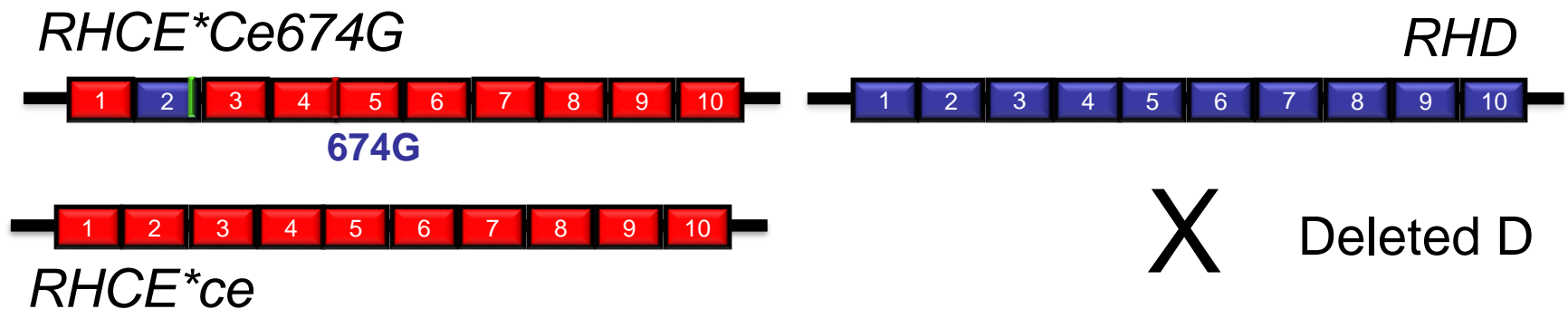
Case 4: DNA results

- HEA Beadchip:
 - Negative for RH^*E
 - Genotype: $RHCE^*Ce$ and $RHCE^*ce$
- RHCE Beadchip:
 - Negative for RH^*E
 - Genotype: $RHCE^*Ce$ and $RHCE^*ce$
 - Predicted phenotype C+**E**-c+e+
- Manual PCR-RFLP for E/e:
 - 676G/G, predicted **E**-e+
- $RHCE$ exon 5 sequencing:
 - 676G/G, predicted **E**-e+
 - Novel nt 674C>G (Ser225Cys)

Is the change present on the $RHCE^*Ce$ or $RHCE^*ce$?

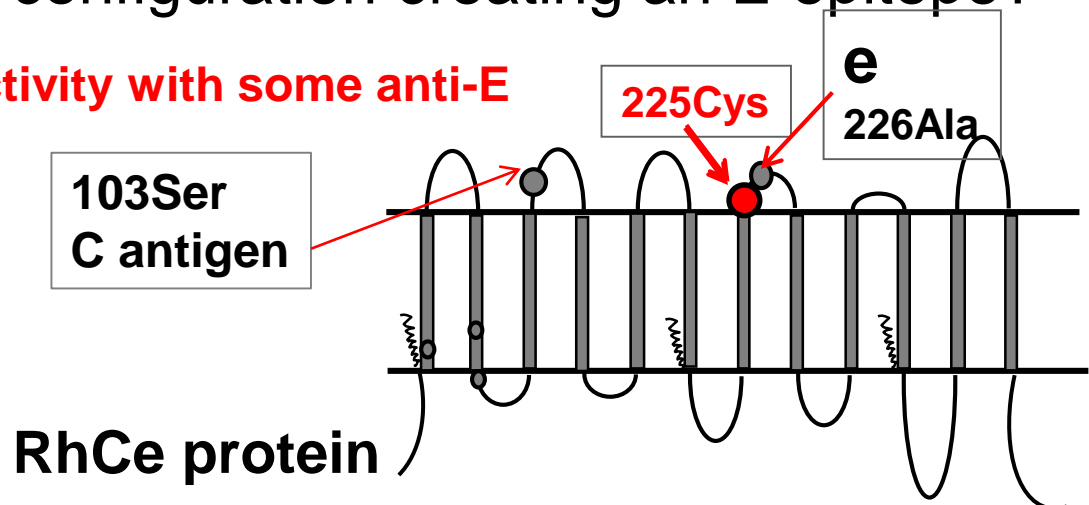
Case 4: Robust (variable) E expression on RhCe

- RNA/cDNA sequence showed 674C>G is on *RHCE*Ce*



- Must alter the protein configuration creating an E epitope?

225Cys in Ce protein = reactivity with some anti-E



Case 4: more questions than answers?

- As donor, unit should be E+ or E-?
- If crossmatched for patient with anti-E, will it be incompatible?
- Possible clue: reactivity with polyclonal anti-E
- Might it stimulate anti-E in a E- patient?
- If patient, should he/she be considered E+ or E-?

	Gamma clone (GAMA402)	SeraClone (MS60/12)	ALBA clone (DEM1)	In-house polyclonal
Donor RBCs	4+	4+	2+ ^{mf}	very weak

	Ortho BioClone (C2)	Immucor polyclonal	Immucor Series I (MS12)	In-house polyclonal
Donor RBCs	0	0	0	0

E antigen typing discrepancy reveals a novel 674C>G change (Ser225Cys) on RhCe responsible for expression of *E* epitopes. S Vege, C Lomas-Francis, Z Hu, K Hue-Roye, P Patel, C M. Westhoff 2012 Transfusion Abstract

Name that antibody

What is the specificity?

Patient is a White 35 year old woman
Transfused 6 month ago

Cell	Rh-hr					Kell		Kidd		Duffy		MNSs				PEG IAT	Papain IAT	
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	M	N	S	s			
1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0		2+	3+
2	+	+	0	0	+	+	+	+	0	+	0	+	+	0	+		2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+		0 ^v	0 ^v
4	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0		0 ^v	0 ^v
5	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0		2+	3+
6	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+		2+	3+

Panel indicates presence of anti-e (allo or auto?)

Additional testing has ruled out other underlying antibodies

What is the specificity?

Patient is a White 35 year old woman
Transfused 6 month ago

	Rh-hr					Kell		Kidd		Duffy		MNSs					PEG	Papain
Cell	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	M	N	S	s		IAT	IAT
1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0		2+	3+
2	+	+	0	0	+	+	+	+	0	+	0	+	+	0	+		2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+		0 ^v	0 ^v
4	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0		0 ^v	0 ^v
5	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0		2+	3+
6	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+		2+	3+
Auto	+	0	+	+	0	0	+	+	0	0	0	+	+	0	+		0 ^v	0 ^v

Patient's RBCs are e-
Panel indicates presence of alloanti-e

What is the specificity?

Patient is a White 72 year old man
Never transfused

DAT
PS: 2+ IgG: 2+ C3: 0

Cell	Rh-hr					Kell		Kidd		Duffy		MNSs				PEG	Papain
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	M	N	S	s	IAT	IAT
1	+	+	0	0												2+	3+
2	+	+	0	0												2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	0 ^v	0 ^v
4	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	0 ^v	0 ^v
5	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	2+	3+
6	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	2+	3+
auto	+	+	0	0	+	0	+	+	0	0	0	+	+	0	+	3+ ^{**}	4+ ^{**}

Autoanti-e, of course!

** EGA-treated RBCs

Patient's RBCs are E-e+
Panel suggests presence of anti-e
Autoanti-e?

Autoadsorption removed all reactivity

Case 5: what is the specificity?

67 year old African American female
 Hgb/HCT: 9.0/27.2
 Chest pain
 Hospital suspects autoanti-e

Initial panel suggests anti-e
No indication of autoantibody
 Additional testing ruled out other
 underlying antibodies

	Rh-hr					Kell		Kidd		Duffy		MNSs					PEG	Papain
Cell	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	M	N	S	s		IAT	IAT
1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0		2+	3+
2	+	+	0	0	+	+	+	+	0	+	0	+	+	0	+		2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+		0 ^v	0 ^v
4	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0		0 ^v	0 ^v
5	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0		2+	3+
6	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+		2+	3+
auto	+	0	0	+	+	0	+	+	0	0	0	+	+	0	+		0 ^v	0 ^v

How can this e+ patient make an apparent alloanti-e?

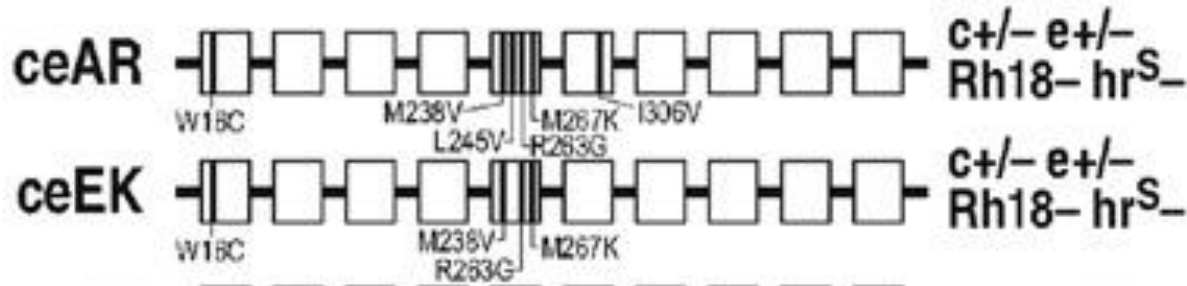
Partial RHCE Antigens

- Analogous to RhD, altered forms of RHCE proteins express partial antigens
- Revealed when:
 - Antigen-positive patient makes the corresponding antibody, for example, alloanti-e or alloanti-C or alloanti-c in plasma of patients with e+ or C+ or c+ RBCs, respectively
 - Variable results are obtained when antigen typing
- Many altered RHCE alleles have been reported
- Distinguishing between auto- and alloantibody in a transfused patient or in the presence of warm autoantibodies can be difficult
- Analysis of *RHCE* genes can provide valuable insight

Case 5: result of DNA analysis

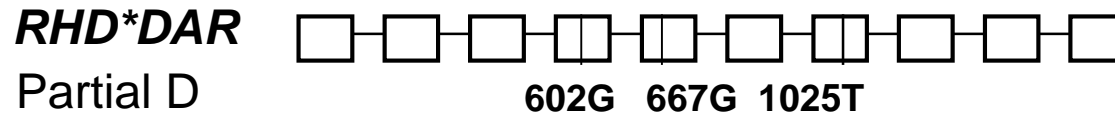
RHCE: 2 altered *RHCE*ce* alleles**

Compound heterozygote: *RHCE*ceAR* with *RHCE***ceEK***



Often partial RHCE phenotypes paired with partial D

RHD: Homozygous for an allele that encodes partial D



Genotype:

RHDDAR*-*RHCE***ceAR*/*RHD***DAR*-*RHCE***ceEK***

Potential to make: Anti-D, -C, -E, -e/hr^S (-c, -f), -Rh18

Case 5: testing with reagent anti-e

Anti-e reagent (clone/s)	RhceAR	RhceEK
Gamma-Clone (MS16, MS21, MS63)	3+	4+
Ortho Bioclone (MS16)	4+	4+
Biotest/Bio-Rad (Seraclone) (MS16, MS21, MS63)	4+	4+

Some partial e phenotypes give strong reactions with monoclonal anti-e
Difficult to recognize with routine reagents

Case 5: transfusion

- R_2R_2 RBCs suitable until patient makes anti-E, anti-D, etc.
- Donor screening with anti-hr^S or patient plasma
- RH genotype any donors identified as hr^S-
 - Very few D- hr^S- donors
- Search for donors with similar *RHD* and *RHCE* genotype
- Often Rh-negative (rr) RBCs can be transfused
- Lack of documented experience with regard to the clinical significance of most anti-e-like antibodies
- Autologous donation if patient's clinical state permits

Patient 6: History

- **54-year-old female orthopedic patient**
- **Hgb 8.9**
- **Recently transfused 3 units**
- **Previous antibody history (Hosp ID):**
 - Anti-C, anti-E, WAA, unspecified antibody**
- **Request for antibody identification**

Case 6: initial RBC testing

- Reactions suggested the antibody was directed at a high prevalence antigen
- Antibody to an Rh antigen was suspected
- The plasma was non-reactive with –D– RBCs
- The patient's RBCS were hr^{B+} and hr^{S–}
- 4 of 8 C– E– hr^{S–} units were compatible

Case 6: initial results for RHCE DNA Analysis

- Predicted C-E+c+e+
- Serology results: C-E-c+e+
- Molecular testing confirmed with manual PCR-RFLP for E/e and exon 5 sequencing
- Race indicated as White
- After investigation, patient is Hispanic
- Possible silenced RHCE*cE allele or altered allele with very weak antigen expression

Case 6: additional results

- RHCE beadchip:
 - Negative for cE variants (EI, EIII, EIV, and EKH)
- PCR-RFLP for 907delC: silenced cE found in Hispanics §
 - Heterozygous for 907 deletion
 - Predicts E-
- Other allele: *RHCE*ceEK*
 - partial c and e, Rh18-, hr^S-
 - Associated with production of alloanti-e, -ce(f), -hr^S and/or -Rh18



Case 6: compatible hr^{S-} cells

- Compatible with 4 of 8 hr^{S-} samples
- Many *RHCE* backgrounds give the hr^{S-} phenotype
- Full RH genotype known on 3 of the samples
 - *RHD***DAR* – *RHCE***ceAR* homozygous
 - *RHD***DAU0* – *RHCE***ceMO* homozygous
 - *RHD***DAU0* – *RHCE***ceMO* / *RHD***DOL* – *RHCE***ceBI*

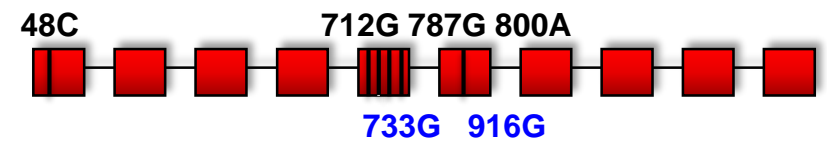
Units transfused

*RHCE***ceEK*

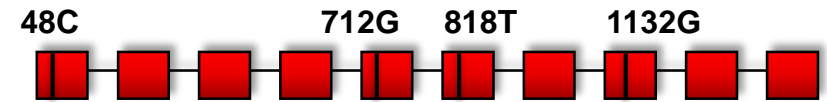


PATIENT

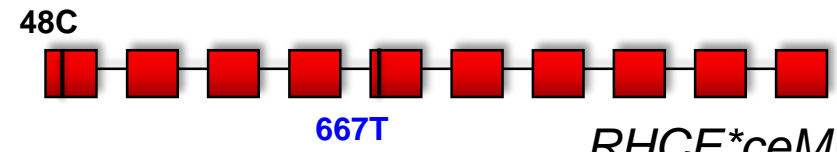
*RHCE***ceAR*



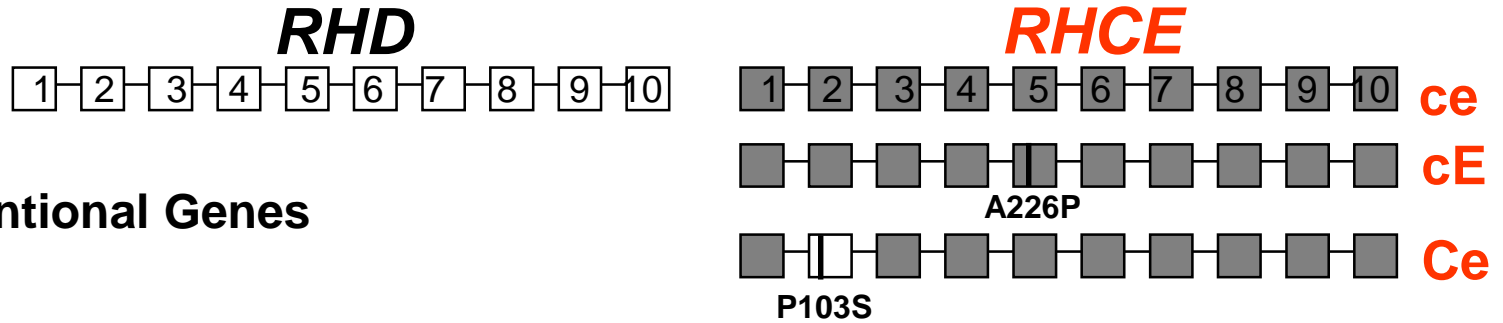
*RHCE***ceBI*



*RHCE***ceMO*

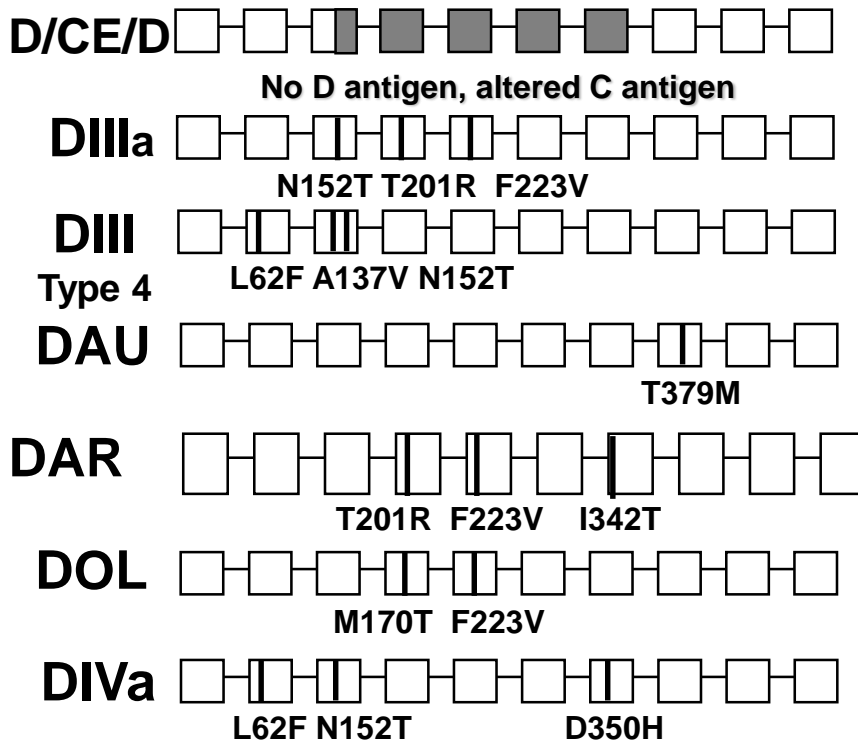


Variant RHD and RHCE genes common in African-Americans (and some Hispanics)

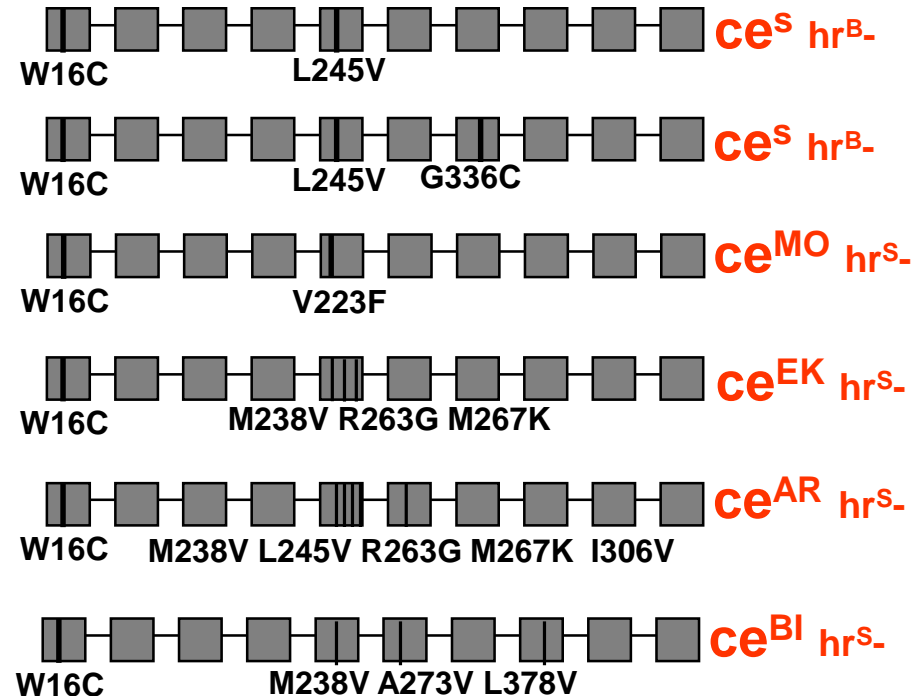


A. Conventional Genes

B. Variant RHD Genes



C. Variant RHce Genes



DNA typing to predict if a fetus is at risk for anemia of the fetus and newborn

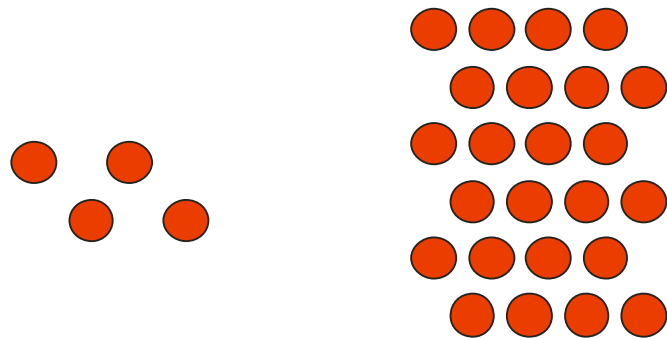
Anti-K in pregnancy

- **Titer of anti-K: not predictive**
 - **Low titer: severely affected fetus**
 - **High titer: K– fetus**
- **Bilirubin level in amniotic fluid: not predictive**
- **Different mechanism from HDFN due to anti-D**

Suppression of erythropoiesis

Erythroid Progenitors (BFU-E & CFU-E) from

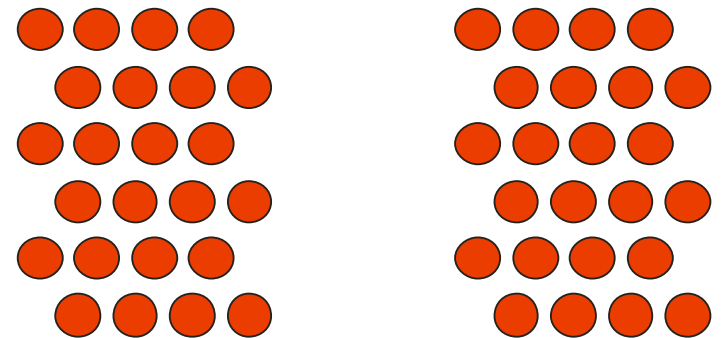
K+ D+ cord blood



Anti-K

Anti-D

K- D+ cord blood



Anti-K

Anti-D

Anti-K, but not anti-D, suppressed their growth
“Anemia of the fetus and newborn” more
appropriate designation

Use DNA testing to determine if fetus at risk

Vaughan, et al., *N. Engl. J. Med.* 1998;338:798

DNA analysis for Kell antigens in pregnancy

- **A valuable tool to determine if fetus is at risk for anemia of the fetus and newborn**
- **Points to remember:**
 - **Maternal anti-K may have been stimulated through transfusion, not pregnancy**
 - **Test of the baby's father strongly recommended**
 - **Only serological testing of father may be sufficient?**
 - **Only DNA analysis of father may be sufficient?**

Case 7: a cautionary tale

- **Pregnant woman with anti-K**
- **Sample from baby's father submitted for Kell genotyping**
 - *KEL*01/02* (HEA DNA array)
 - Predicts the RBCs will be K+k+
 - Predicts 50% probability the baby's RBCs will be K+
- **Sample also submitted for K antigen typing**
 - K+k-
 - Predicts 100% probability the baby's RBCs will be K+
 - Father has a silenced *k* allele
- **An example of the power of combining serological and DNA testing**
- ***Select test method based on the question being asked***

Some applications of DNA analysis

- To predict antigen type of recently transfused patient
- When RBCs are coated with IgG (+DAT)
- To distinguish allo from auto antibodies
- To detect weakly expressed antigens (e.g., Fy^b with Fy^x phenotype); where patient is unlikely to make antibodies to transfused antigen-positive RBCs
- Determine origin of engrafted leukocytes in a stem cell recipient
- Determine origin of lymphocytes in patient with graft-versus-host disease

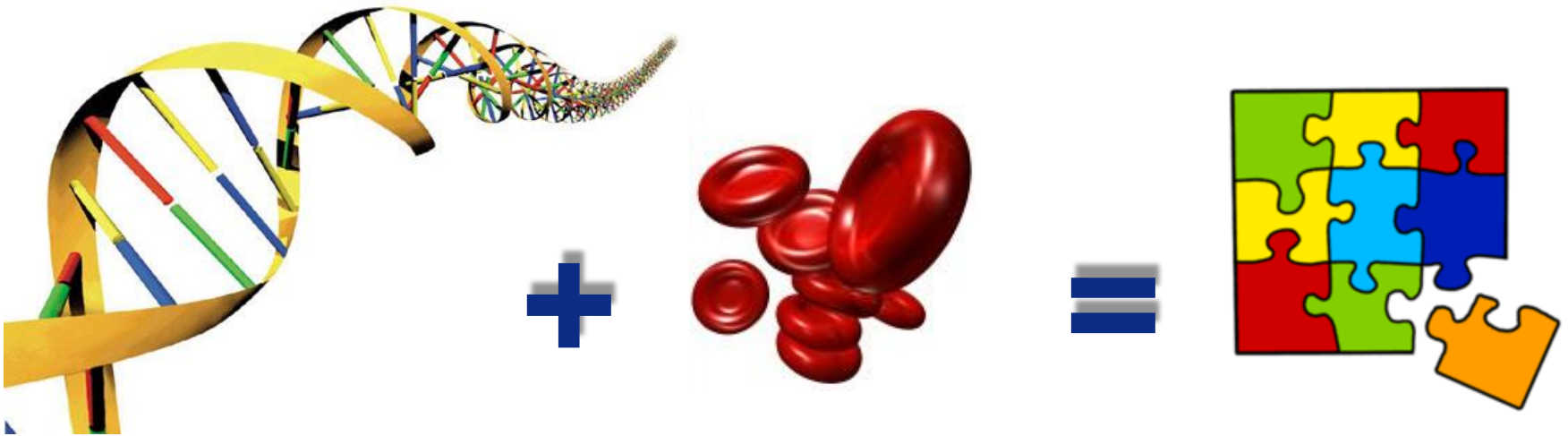
More applications of DNA analysis

- Determine zygosity, particularly *RHD*
- Resolve discrepancies, e.g., A, B, D, C, c, e
- To aid in the resolution of complex serological investigations
- To fill a reagent void to determine antigen type of patient or donor when an antibody is weak or not available, e.g., anti-Do^a, anti-Do^b anti-Jsa, anti-V, anti-VS
- To identify molecular basis of unusual serological results, especially Rh variants

DNA analysis often is the key to unlocking the mysteries of serological testing



Both DNA analysis and serology are essential to put the puzzle together



Acknowledgements

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Immunohematology and Genomics Laboratory**



Thank you for your attention
