





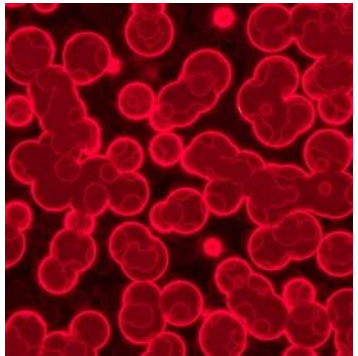

New York Blood Center Enterprises

Allo or Auto?

Real World Application of Blood Group Genomics

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Objectives

- Describe how antigen frequencies in the donor population can determine difficulty of finding units for a patient with multiple antibodies.
- Explain various serologic methods utilized in a case of complex antibodies, including selected cell panels, neutralization, enzyme treatment, and testing rare frozen RBCs.
- Discuss how blood group genotyping can help characterize an unexplained antibody and locate appropriate donors.

Background

- Patient History:
  - 35-year-old female
  - African American
  - Sickle cell disease
  - Multiply transfused, multiple antibodies:
    - **Anti-C**
    - **Anti-E**
    - **Anti-Fy3**
    - **Anti-Jk^{a}**

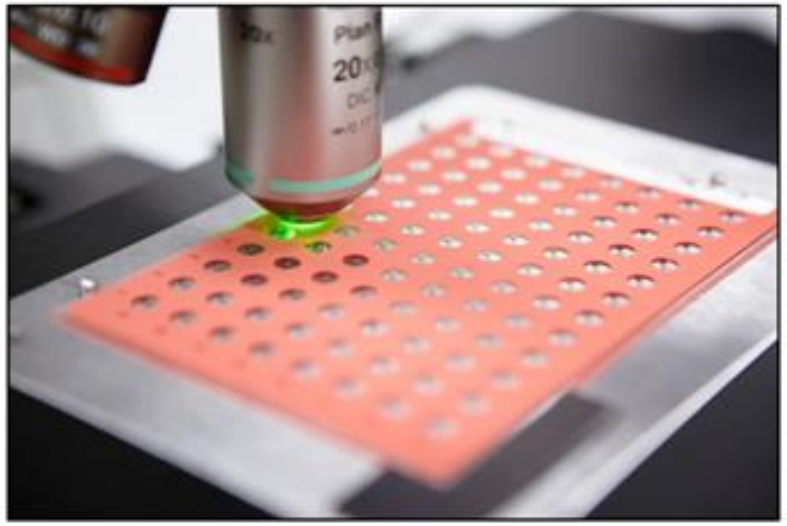
Let's look at each of these antibodies…

- **Anti-C and Anti-E**
  - Current ASH guidelines recommend antigen matching for Rh, K (Chou, et al. *Blood Advances*. 2020;4(2):327-355.)
    - Only *"strong recommendation based on moderate certainty in the evidence about effects"* out of 10 recommendations
  - These antibodies were often first made by patients with SCD
  - Goal to prevent/minimize further alloimmunization

Let's look at each of these antibodies…

- **Anti-Fy3**
  - Fy3 antigen expressed on Fy(a+) and/or Fy(b+) RBCs
    - ~67% of black individuals Fy(a-b-)
    - ~0% of white individual Fy(a-b-)
  - Fy3 antigen resistant to enzyme treatment (papain/ficin)
  - Invariably, black individuals with Fy(a-b-) phenotype have GATA mutation
    - Mutation in promotor region of *FY* gene
    - Evolutionary advantage; protective against some malarial infections
    - *FY*02N.01* = allele encoding null phenotype on erythroid cells only
    - Not at risk for making anti-Fy^b; rarely make anti-Fy3
  - Anti-Fy3 considered clinically significant
    - Give Fy(a-b-) units

Let's look at each of these antibodies…



- **Anti-Jk^a**
  - Common antibody
  - Known for
    - Fixing complement; causing intravascular hemolysis
    - Decreasing in titer quickly; causing DHTR

Problematic Antibody Combination: C-, E-, Fy3-, Jk(a-)

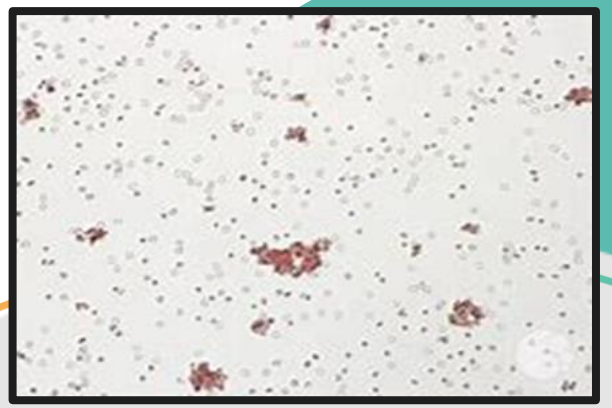
- R_0 (D+, C-, E-) phenotype commonly found in black population (46%)
- Fy3- phenotype commonly found in black population (68%)
- Jk(a-) phenotype less common in black population (8%)
  - Compared to 22% of white population Jk(a-)

0.46 X 0.68 X 0.08 = 2.5% of black donors

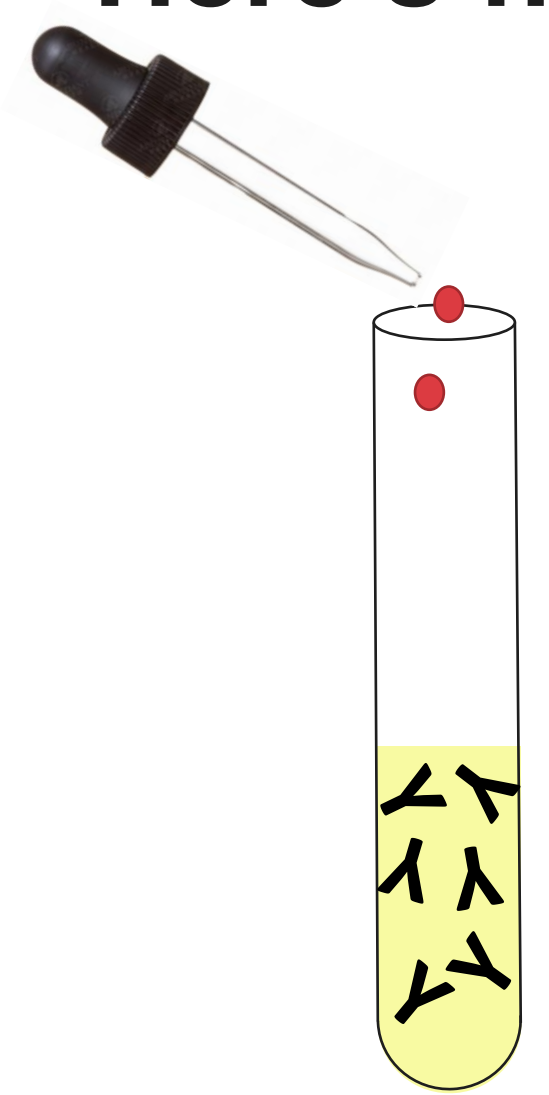
New Sample; New Antibody Identified

Transfusion requirement up until now:
C-, E-, Fy3-, Jk(a-)

- **Anti-Sd^a**
  - Sd^a antigen: 91% frequency in most populations (high prevalence)
    - 96% have Sd^a substance in urine
    - ~4% of population are truly Sd(a-) and can make antibody
  - Sd^a antigen resistant to all chemical/enzyme treatment
  - Anti-Sd^a
    - Characteristic appearance: refractile agglutinates in sea of free flowing RBCs
    - "Mixed field" due to different level of Sd^a antigen on RBCs in circulation
    - Does not react with cord RBCs
    - Neutralized by urine
    - **Not clinically significant** (phew!)



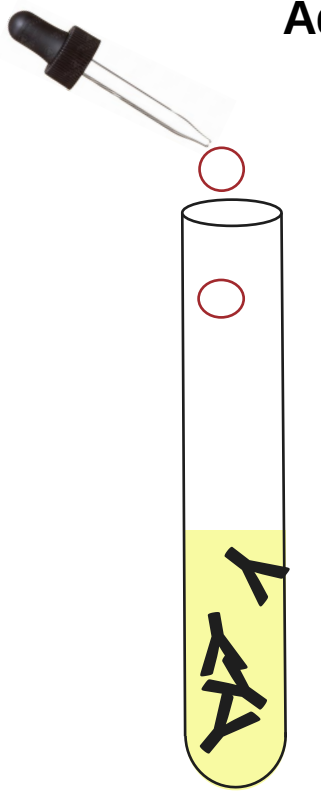
Here’s how neutralization works:



Addition of soluble antigen (pooled urine)

Plasma with antibodies

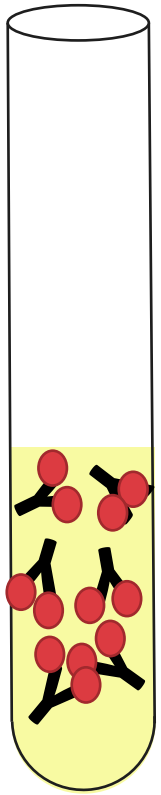
Addition of saline



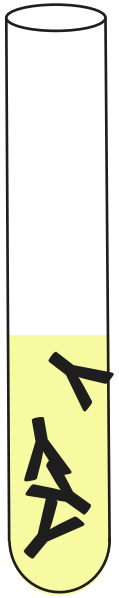
Dilution control

Here’s how neutralization works:

Incubation with soluble antigen (pooled urine)



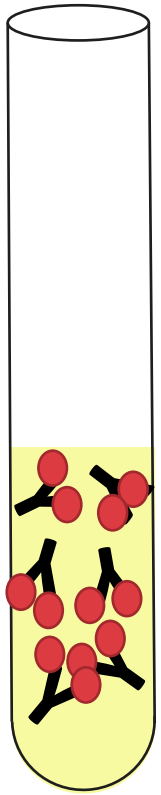
Plasma with neutralized (inhibited) antibodies



Slightly diluted plasma

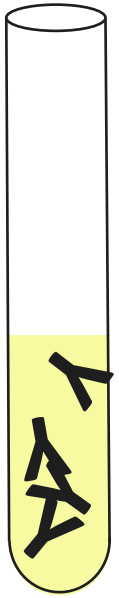
Dilution control

Here’s how neutralization works:



Reactivity of antibody will no longer be detected

		Rh						Kell			Duffy		Kidd		MNSs				P	Lewis		Lu	Co
		C	D	E	c	e	C^w	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P$_1$	Le^a	Le^b	Lu^a	Co^b
1	R1R1	+	+	0	0	+	0	+	0	0	0	+	0	+	+	0	+	0	+w	0	+	+	0
2	R1R1 w	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	0	+	0	0
3	R2R2	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	+	0	0	0
4	R2R2	0	+	+	+	0	0	0	0	0	+	+	+	0	0	+	0	+	+	0	+	0	0
5	Ror	0	+	0	+	+	0	0	+	0	+	0	0	+	+	+	0	+	+w	0	+	0	0
6	rr	+	0	0	+	+	0	0	+	0	+	0	+	0	0	+	+	+	+w	0	+	+	0
7	r'r	0	0	+	+	+	0	+	+	0	0	+	0	+	+	+	+	+	0	0	0	0	0
8	rr	0	0	0	+	+	0	0	+	0	+	+	0	+	+	+	+	+	+w	0	+	0	0
9	rr	0	0	0	+	+	0	0	+	0	+	+	0	+	0	+	+	0	0	+	0	+	+
10	rr	0	0	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	0	0	+	0	0
11	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	+	0	+	+	+w	+	0	0	0
	Auto																						
AI	R1R1	+	+	0	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+w	0	+	+	0
AI I	R2R2	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	+	0	0	0
AI II	rr	0	0	0	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	0	+	+	0



Dilution control: antibody remains detectable

Dilution control

Neutralization Reactions

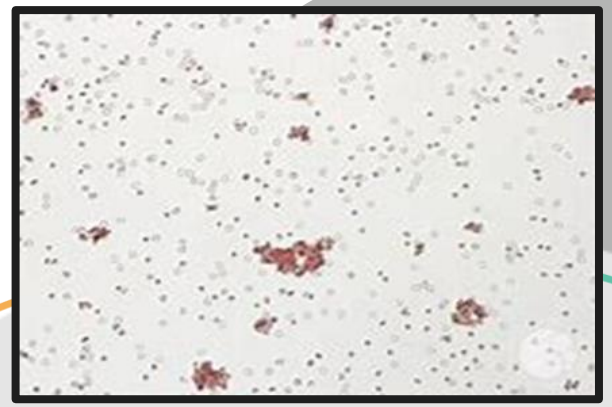
		Rh					Kell		Duffy		Kidd		MNS				Results			
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	PEG IAT		Neut PEG IAT	Dil cont PEG IAT
1	R_0r	+	0	0	+	+	0	+	0	0	0	+	+	+	+	+	$+w^{MF}$		0√	$+w^{MF}$
2	R_0r	+	0	0	+	+	+	+	0	0	0	+	0	+	0	+	$+w^{MF}$		0√	$+w^{MF}$
3	R_0r	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	$+w^{MF}$		0√	$+w^{MF}$
4	R_0r	+	0	0	+	+	0	+	0	0	0	+	+	+	0	+	$+w^{MF}$		0√	$+w^{MF}$
5	rr	0	0	0	+	+	0	+	0	0	0	+	+	+	+	0	$+w^{MF}$		0√	$+w^{MF}$
6	rr	0	0	0	+	+	0	+	0	0	0	+	0	+	0	+	$+w^{MF}$		0√	$+w^{MF}$

Neut= patient plasma neutralized with pooled urine

Dil cont= dilution control; patient plasma + saline

Use neutralized plasma to:

- Rule out additional alloantibodies
- Confirm anti-Sd^a specificity



Another Sample; Another Antibody Identified

Transfusion requirement up until now:
D-???, C-, E-, Fy3-, Jk(a-)

- **Anti-D**
  - Weak/micro Anti-D in neutralized plasma
  - Patient has been receiving D+ units
  - Patient RBCs D+
  - Is anti-D autoantibody or alloantibody?
    - Autocontrol/DAT positive; eluate contains anti-D
    - Reactivity with hypotonic washed autologous RBCs → seems like alloantibody
    - *RHD* variants (partial D expression) prevalent in patients with SCD
    - Determining auto vs allo is important for unit selection

D-, C-, E-, Fy3-, Jk(a-) = 0.4% of Black donors
No units available

Testing Units We Had Frozen For This Patient

	Rh					Kell		Duffy		Kidd		MNS				Results			
	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	PEG IAT		Neut PEG IAT	Dil cont PEG IAT
1	+	0	0	+	+	0	+	0	0	0	+	+	+	+	+	$+w^{MF}$		+w	$+w^{MF}$
2	+	0	0	+	+	+	+	0	0	0	+	0	+	0	+	$+w^{MF}$		0v	$+w^{MF}$
3	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	$+w^{MF}$		+w	$+w^{MF}$
4	+	0	0	+	+	0	+	0	0	0	+	+	+	0	+	$+w^{MF}$		0v	$+w^{MF}$
5	+	0	0	+	+	0	+	0	0	0	+	+	+	+	0	$+w^{MF}$		+w	$+w^{MF}$

Neut= patient plasma neutralized with pooled urine

Dil cont= dilution control; patient plasma + saline

Neutralized plasma:

- Contains anti-D
- 2/5 D+ units nonreactive
  - Both from same D+ donor

Case Recap

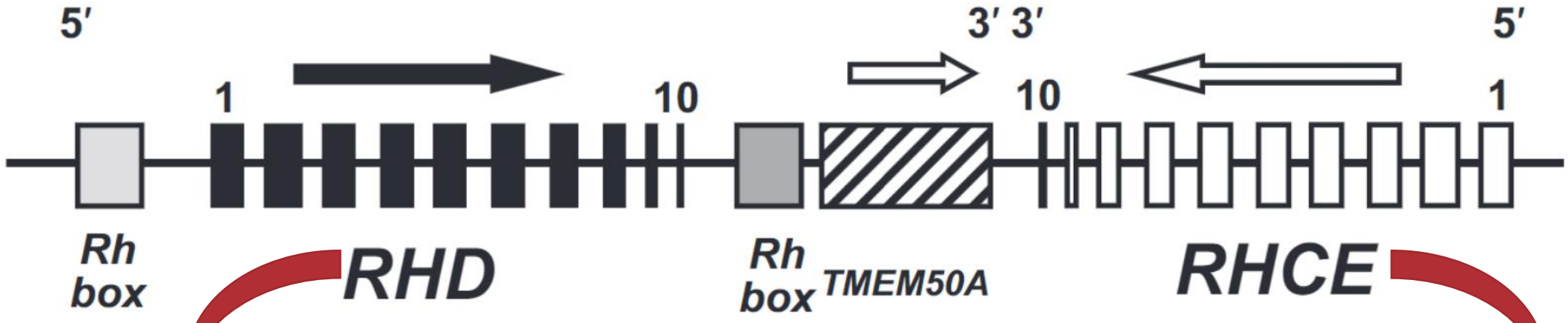
What we know…

- Anti-D
- Anti-C
- Anti-E
- Anti-Fy3
- Anti-Jk^a
- Anti-Sd^a (not clinically significant)

What we don't know…

- Anti-D auto or allo?
- Why are 2 D+ units compatible with neutralized plasma?

RH Blood Group System Genes & Proteins



Encodes

Encodes

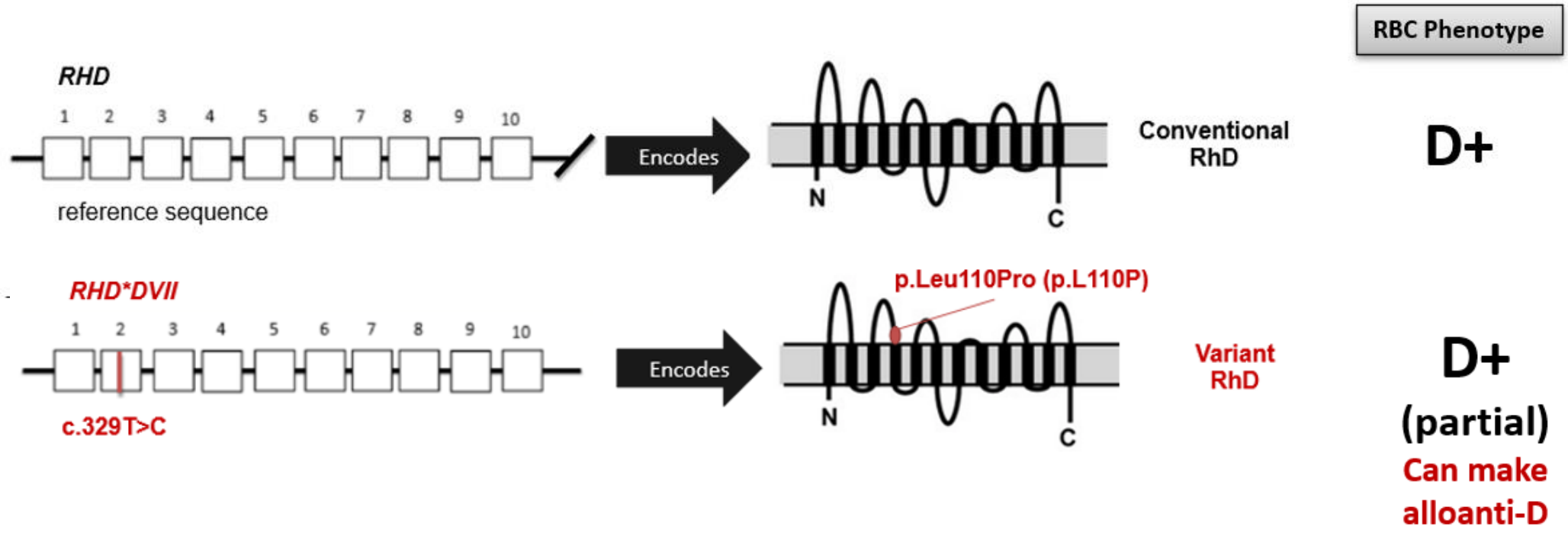
RhD Protein
D antigen

Red Blood Cell

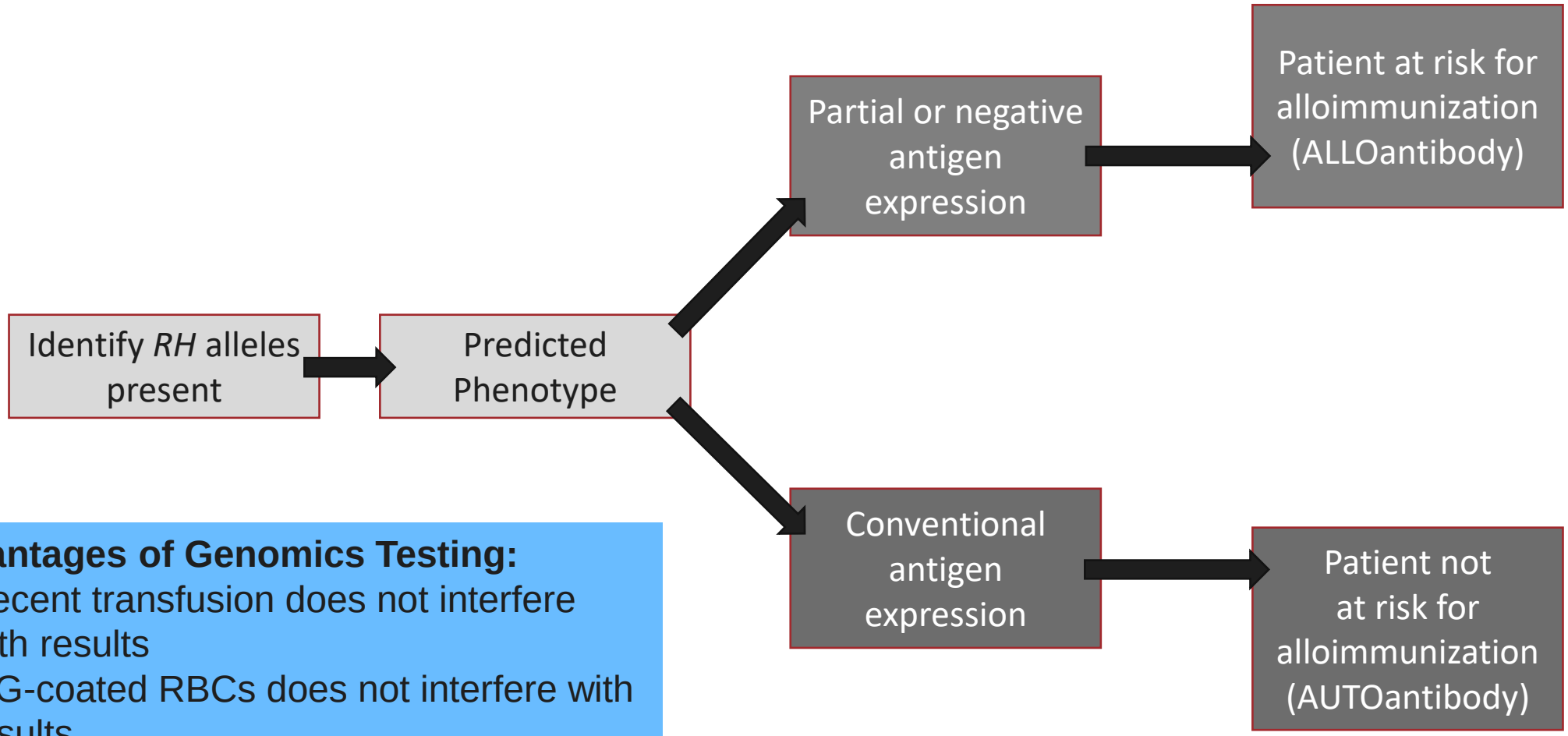
RhCE Protein
C, E, c, e, etc. antigens

Daniels G. *Br J Haematol.* 2013;161:461-470.

Variant Alleles and D Antigen Expression



Differentiating Allo- From Autoantibody



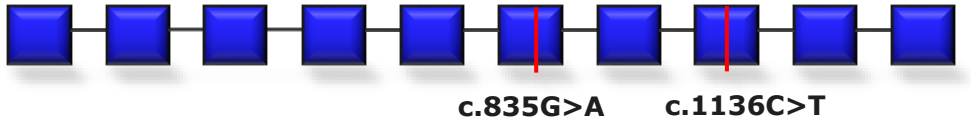
Advantages of Genomics Testing:

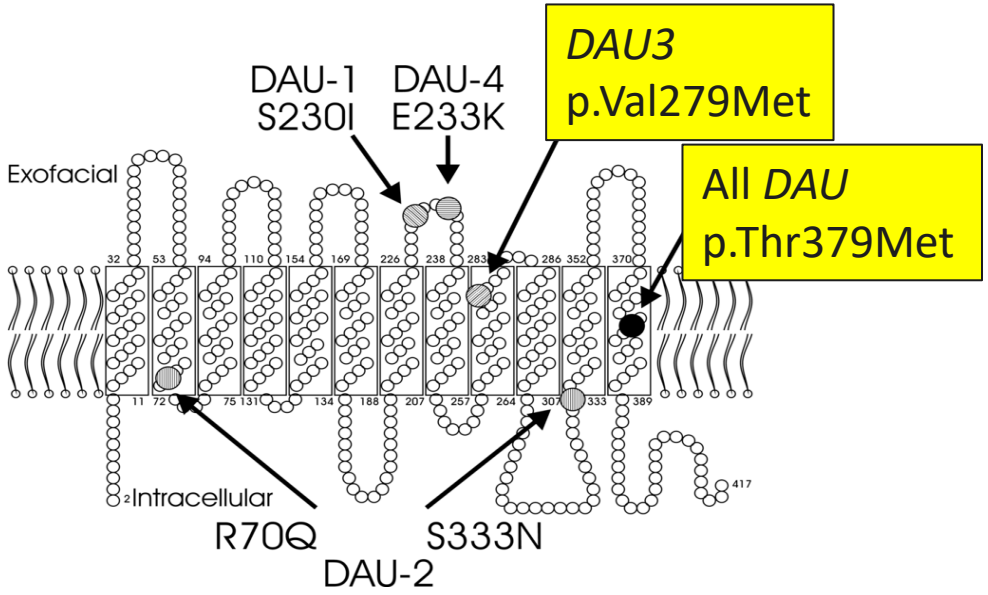
- Recent transfusion does not interfere with results
- IgG-coated RBCs does not interfere with results

RHD Genotyping Results

RHD*DAU3

RHD*DAU3





Patient *RHD* genotyping

***RHD*DAU3* homozygote**

- Allele encodes partial D antigen (double dose)
- Patient is at risk of making alloanti-D
- Anti-D detected likely alloanti-D
- Give D-negative units

Why were units from one D+ donor compatible?

RHD*DAU3

Deleted *RHD*

Donor: *RHD* genotyping

***RHD*DAU3* hemizygote**

- Same allele encodes partial D antigen (single dose)
- D antigen missing same epitopes as patient
- Compatible with anti-D made by patient with same variant allele

Perfect donor:

D+(partial), C-, E-, Fy(a-b-), Jk(a-); compatible with patient's neutralized plasma

2 units transfused successfully

Blood center continues to freeze this donor's units specifically for this patient

Case Conclusions

Opportunities

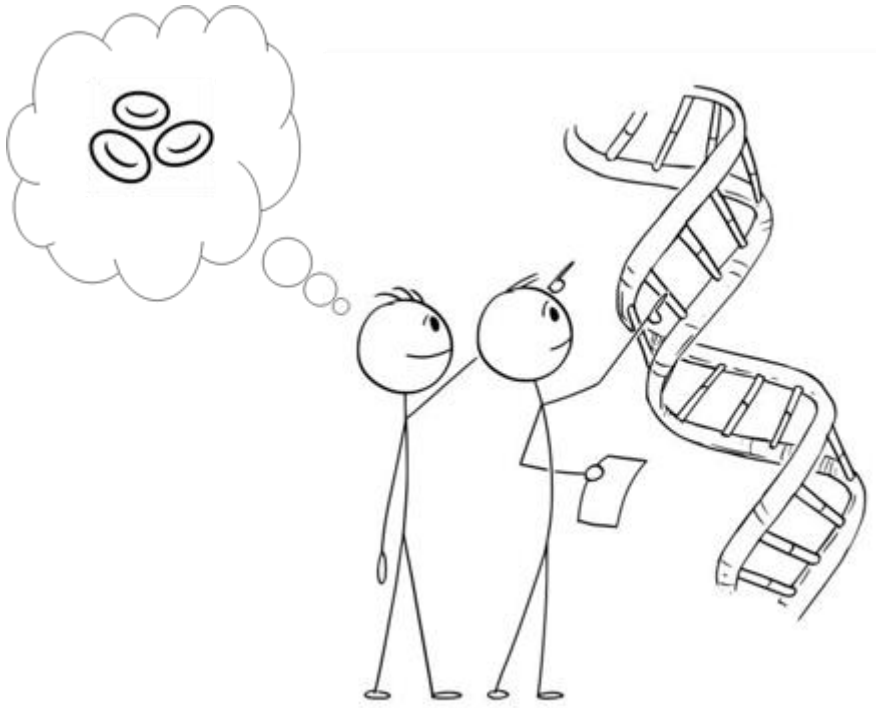
- RH genotyping for variants to determine allo vs autoantibody
- Example of *RH* genotype matching
  - Transfuse units from donor with same *RHD* genotype (same partial D antigen)
- Broadens donor pool if no D-,C-,E-,Fy(a-b-),Jk(a-) units available

Challenges

- Would need to *RH* genotype many donors to identify donors with same variant alleles
- Limitations of computer systems to store allele information
- Would you be able to transfuse D+ units to a patient with anti-D in your institution?

Key Points

- Multiply transfused patient with difficult antibody combination
- Difficult serologic reactivity requiring special techniques (neutralizations)
- Serology & genomics testing work together to resolve reactivity & locate compatible donor.



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Thank you! Questions?