Community Blood Center

Resident Rotation:Immunohematology Reference Lab (IRL)
Module 5: Antigen Typing

Lynsi Rahorst, MHPE, MT(ASCP)SBB[CM]
Manager, Education and Training IRL/Genomics
NYBC Enterprise
lrahorst@cbckc.org

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Objectives

- List reasons why it might be helpful to know extended antigen profiles on patients and donors.
- Compare and contrast serologic phenotype with molecular genotype.
- Perform calculations determining probability of encountering antigen-negative donor units.

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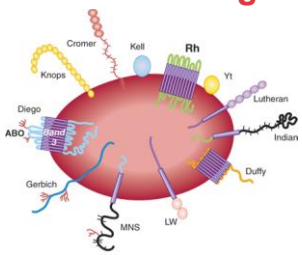
Let's talk about antigens…



Source: H. Franklin Bunn, Jon C. Aster: Pathophysiology of Blood Disorders
www.accessmedicine.com

Serologic phenotype

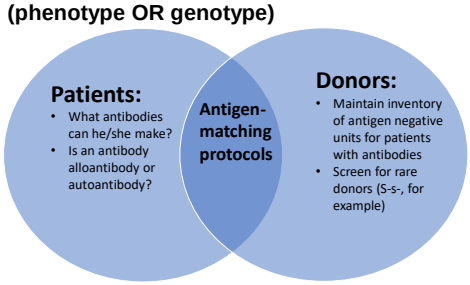
- Includes these antigens:

Rh							Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg
D	C	E	c	e	f	V	C w	K	k	Kp a	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Le a	Le b	P 1	M	N	S	s	Lu a	Lu b	Xg a

- Correspond to clinically significant antibodies

Why would we need to know extended antigen types?

(phenotype OR genotype)



What's antigen-matching?

What does that mean?

- Example:
  - Patient phenotype: D+,E+,C-,c+,e+; K+,k+; Fy(a-b+); Jk(a+b+); S-,s+
  - Which antibodies can the patient make? **Anti-C, anti-Fy^a, anti-S**
  - Give C-negative, Fy(a-), S-negative RBCs
- Some phenotypes would be much more difficult to match {Example: D-,E-,C-,K-, Fy(a-), Jk(b-), S-}

Principle: Don't expose patients to antigens that their RBCs lack

- Prevent alloimmunization

Chronically-transfused patients

Warm autoantibody patients

Daratumumab patients

Maybe someday… All patients?

In the lab…

- Serologic phenotype of your own sample
- How "rare" are you?

Table I. Comparison of antigen frequencies of Indian donors with other ethnic groups

Antigen		Indian (n=3073)		Caucasian[15]	Black[15]	Chinese[15]
Traditional	ISBT	%	95% CI	%	%	%
D	RH1	93.6	92.7-94.5	85.0***	92.0***	99.0***
C	RH2	87.0	85.8-88.2	68.0***	27.0***	93.0***
E	RH3	20.0	18.6-21.4	29.0***	22.0***	39.0***
c	RH4	58.0	56.3-59.7	80.0***	96.0***	47.0***
e	RH5	98.0	97.5-98.5	98.0	98.0	96.0***
K	KEL1	3.5	2.9-4.1	9.0***	2.0***	0*
k	KEL2	99.97	99.9-100	99.8*	100*	100*
Fya	FY1	87.4	86.2-88.6	66.0***	10.0***	99.0***
Fyb	FY2	57.7	56.0-59.4	83.0***	23.0***	9.2***
Jka	JK1	81.4	80.0-82.8	77.0***	92.0***	73.0***
Jkb	JK2	67.6	65.9-69.3	74.0***	49.0***	76.0***
M	MNS1	88.8	87.7-89.9	78.0***	74.0***	79.7***
N	MNS2	65.4	63.7-67.1	72.0***	75.0***	67.4**
S	MNS3	54.8	53.0-56.6	55.0	31.0***	8.7***
s	MNS4	88.7	87.6-89.8	89.0	99.0***	100***

*Proportions are too small to calculate a *P* value
P<0.05, **<0.01, ***<0.001 compared to respective antigen frequency in the present study

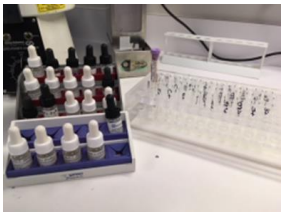
http://www.ijmr.org.in/article.asp?issn=0971-5916;year=2013;volume=137;issue=3;spage=521;epage=526;aulast=Makroo

Antigen typing in the IRL

- QC for each antisera
  - Positive control
  - Negative control
- Choosing positive and negative control cells
  - Example: Anti-E antisera
    - Negative control: E- cell
    - Positive control: E+,e+ cell

Does the antisera detect even the weaker expression of the antigen??

Antigen typing in the IRL



- **Testing for 11 antigens**
  - C,E,c,e; K; Fy^a,Fy^b; Jk^a,Jk^b; S,s
- **Each antisera has unique procedure**
  - RT incubation
  - 37C incubation
  - IAT testing
- **For each antisera, you'll label and set up 3 tubes**
  - Positive control
  - Negative control
  - Patient (your) RBCs

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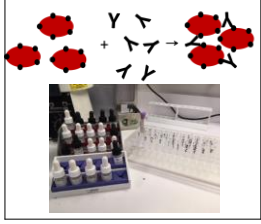
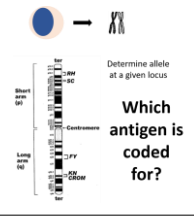
Phenotype vs Genotype

Serology	Genomics
	

Phenotype vs Genotype

Serologic Phenotype

Test RBCs with known antisera by agglutination

Observable expression of antigens on RBCs

Genotype: Predicted phenotype

Test extracted DNA, analyze at given locus

Prediction of antigens based on alleles present

Genotype *BETTER* than a phenotype?

- High throughput system
- Recently transfused patients
- Warm autos
- DARA
- Lack of antisera

Genotyping: a high throughput, low cost system

Phenotype

11 antigens: ~1-2 hours

Great for an individual, patient to be transfused STAT

Genotype

>30 antigens: ~8 hours (with extraction)

But… you c… test >… sam…

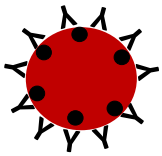
Great for donor testing/ non-STAT patient situations!

Recent Transfusion

- Serology
  - Mixed field agglutination
  - Unknown if patient is positive for antigen and donor is negative, or vice versa
- Genotype
  - Can be performed on recently transfused patients
  - DNA extracted from WBCs
  - DNA can also be extracted from buccal swab sample

Genotype preferred!

Warm autoantibodies: Antigen-blocking



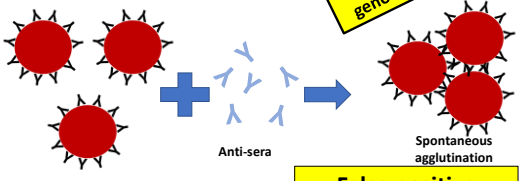
RBC expressing antigen, coated with IgG

Antisera testing for antigen

False negative results!

Warm autoantibodies: spontaneous agglu

Warm autoantibody patients: genotype preferred



Antibody-coated RBCs

Anti-sera

Spontaneous agglutination

False positive results!

DARATUMUMAB

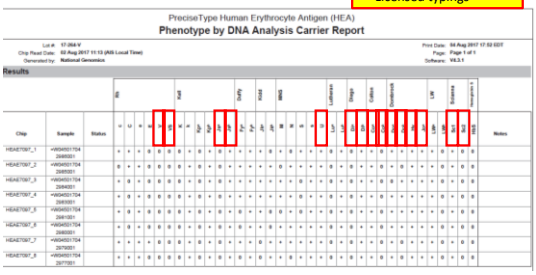
- Treatment for multiple myeloma (and other conditions off-label!)
- Anti-CD38
- Causes weak reactivity in IAT tests
- One strategy: give antigen-matched units

		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	LISS IAT
1	R_1R_1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	1+
2	R_1R_1	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	1+
3	R_1R_2	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	1+
4	R_2r	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	1+
5	r'r	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	1+
6	r"r	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	1+
7	rr	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	1+
8	rr	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	
9	rr	0	0	0	+	+	0	+	+	+	0	+	+	0	0		
10	R_1R_1	+	+	0	0	+	0	+	+	0	+	+	+	+			
11	R_2r	+	0	0	+	+	+	+	0	0	+	+	0				
Auto																	

Pre-DARA workup: genotype preferred

Genotype Panel

Many antigens
- No commercial antisera
- Correspond to clinically significant antibodies
- Licensed typings


PreciseType Human Erythrocyte Antigen (HEA)
Phenotype by DNA Analysis Carrier Report

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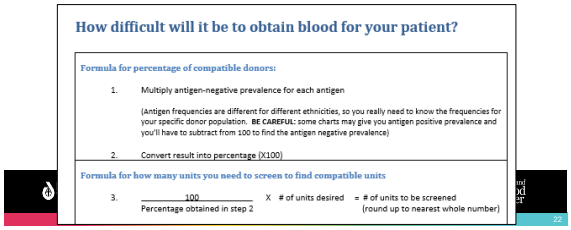
Antigen frequency calculations

- Review material in your handbook for calculations/formulas
- How easy/difficult it is to find certain antigen combinations depends on donor antigen frequencies
- Pay attention to if frequencies are given for antigen-positive or antigen-negative donors



How many units would you have to screen…??

- Excellent board questions!
- Real life:
  - Blood center maintains extensive donor records (historical typings)
  - High-throughput antigen screening of donors by genotyping
  - May use donor ethnicity to screen for certain antigen combinations

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