Antibody Identification

Part 1: The Basics

A Blood Bank Guy Video Podcast

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Part 1

- Prerequisites
- Geography of a panel
- Antibody ID method
- Case examples

Prerequisites

- Blood Group Overview
  - General facts
  - Podcast from December 2011
- Pretransfusion Testing
  - Testing methodologies
  - Antibody screen
  - Podcast from February 2012

When Do We I.D.?

- Following a positive antibody screen
  - Patients, prenatals, donors
- When testing suggests a new antibody
- To confirm a previously identified antibody (per facility SOP)

Definitions

- Alloantibody
  - Antibody against RBC antigens **not present** on patient's own RBCs
- Autoantibody
  - Antibody against RBC antigens **present** on patient's own RBCs

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What's a Panel?

- Just an expanded antibody screen
- Uses group O reagent RBCs
  - RBCs from 8-20 donors
  - Patient serum or plasma
  - IS / 37 C / AHG if tubes
  - AHG only if gel or solid phase
- Reactions documented on a sheet that outlines every RBC's phenotype

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Geography

- Let's take a close look at a panel
- Important to know your way around
- There are variations, but this is a general guide

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		Rh-hr							Xg	Kell						Duffy		Kidd		P	Lutheran		MNSs				Lewis			Results		
Cell	Rh-hr	D	C	E	c	e	f	C_w	Xg^a	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell			
1	R^1R^1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	1			
2	R^1R^1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	2			
3	R^2R^2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0	3			
4	R^0r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4			
5	r'r	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	5			
6	r''r	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	6			
7	rr	0	0	0	+	+	+	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	7			
8	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0	8			
9	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	0	+	9			
10	rr	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	+	0	10			
11	R^1R^1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	11			
PC																													AC			

A series of 8-20 group O donor reagent red cells, each tested for all main antigens ("phenotyped")

Rh-hr (or "Hr") = Modified Wiener Rh genotype for donor

R_1: DCe	r': dCE
R_2: DcE	r'': dcE
R_0: Dce	r: dce
R_Z: DCE	r^y: dCE

Other stuff:
- -Donor ID
- -Special Antigen Type

Co(b+)

		Rh-hr								Xg	Kell						Duffy	
Cell	Rh-hr	D	C	E	c	e	f	C^w	Xg^a	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b
1	R^1R^1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0
2	R^1R^1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0
3	R^2R^2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	0
4	R^0r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0
5	r'r	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+
6	r''r	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+
7	rr	0	0	0	+	+	+	0	+	+	+	0	+	0	+	0	+
8	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+
9	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0
10	rr	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+
11	R^1R^1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+
PC																	

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- -Special Antigen Type

Pt. phenotype results

		Rh-hr								Xg	Kell						Duffy	
Cell	Rh-hr	D	C	E	c	e	f	C^w	Xg^a	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b
1	R^1R^1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0
2	R^1R^1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0
3	R^2R^2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	0
4	R^0r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0
5	r'r	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+
6	r''r	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+
7	rr	0	0	0	+	+	+	0	+	+	+	0	+	0	+	0	+
8	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+
9	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0
10	rr	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+
11	R^1R^1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+
PC			+							0						+	

D, C, c, E, e, Fy^a, Fy^b, Jk^a, Jk^b, K, k, Le^a, Le^b, M, N, P1, S, s

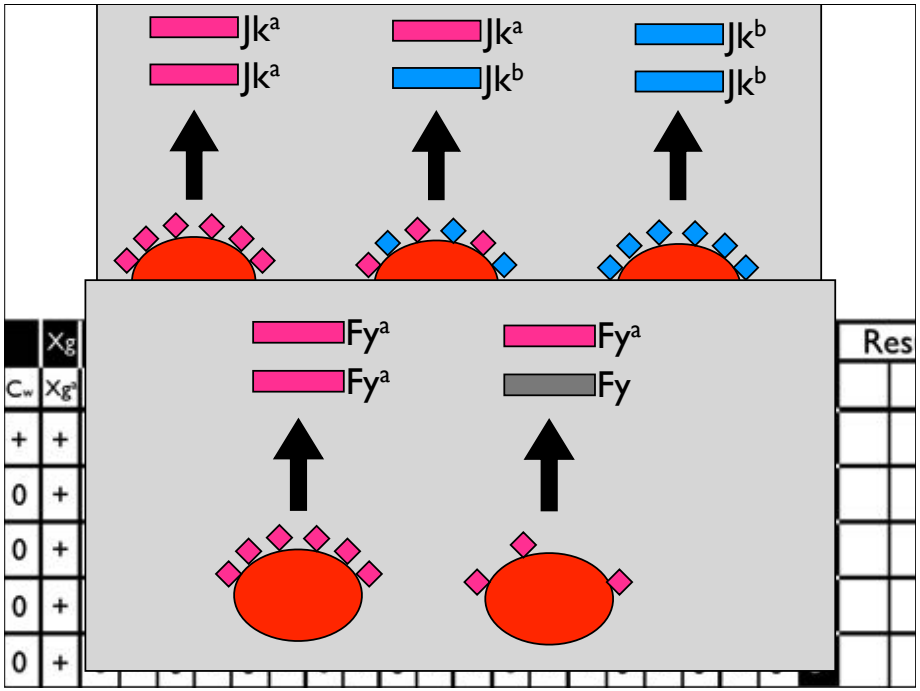
Full donor phenotype

D phenotype for each donor,
C phenotype for each donor,etc...

		Rh-hr							Xg	Kell						Duffy		Kidd		P	Lutheran		MNSs				Lewis	
Cell	Rh-hr	D	C	E	c	e	f	C^w	Xg^a	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b
1	R^1R^1	+	+	0	0	+	0	+	+				*	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+
2	R^1R^1	+	+	0	0	+	0	0	+	0	+	0	*	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+
3	R^2R^2	+	0	+	+	0	0	0	+	0	+	0	*	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0
4	R^0r	+	0	0	+	+	+	0	+	0	+	0	*	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0
5	r'r	0	+	0	+	+	+	0	+	0	+	0	*	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+
6	r''r	0	0	+	+	+	+	0	+	0	+	0	*	0	+	+	+	+	0	+	0	+	0	+	+	+	0	+
7	rr	0	0	0	+	+	+	0	+	+	+	0	*	0	+	0	+	+	0	+	0	+	0	+	+	+	0	0
8	rr	0	0	0	+	+	+	0	+	0	+	0	*	0	+	+	+	+	0	+	0	+	0	+	+	+	0	+

Jk(a+b–): Assume "double dose" Jk^a
Jk(a+b+): "Single dose" Jk^a and Jk^b
Jk(a–b+): Assume "double dose" Jk^b

	Xg	Kell						Duffy		Kidd		P	Lutheran		MNSs				Lewis			Res	
C^w	Xg^a	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell		
+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	1		
0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	2		
0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0	3		
0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4		
0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	5		
0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	6		
0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	7		



Kidd		P	Lutheran		MNSs				Lewis			Results		
Jk^a	Jk^b	P1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell	IS	37	AHG
+	0	+	0	+	0	+	0	+	0	+	1	0	1+	3+
+	+	0	0	+	0	+	0	+	0	+	2	0	1+	3+
+	0	+	0	+	0	+	+	+	0	0	3	0	1+	3+
+	0	+	0	+	0	+	0	+	0	0	4	0	1+	3+
0	+	+	0	+	+	0	+	0	+	0	5	0	0	0 ✓
+	0	+	0	+	0	+	+	+	+	0	6	0	0	0 ✓
0	+	+	0	+	+	0	+	0	0	+	7	0	0	0 ✓
+	0	+	0	+	+	+	+	0	+	0	8	0	0	0 ✓
+	+	+	0	+	+	+	+	+	0	+	9	0	0	0 ✓
+	+	+	0	+	+	0	0	+	+	0	10	0	0	0 ✓
0	+	0	0	+	+	0	0	+	0	+	11	0	1+	3+
											AC			

IS = Immediate spin
37 = 37 C Incubation
AHG = Anti-human globulin
IAT = Indirect antiglobulin test
(=AHG)

This is tube testing!

- LISS ✓
- Albumin ✓
- Saline ✓
- ~~PEG~~

Kidd		P	Lutheran		MNSs				Lewis			Results		
Jk^a	Jk^b	P1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell	IS	AHG	
+	0	+	0	+	0	+	0	+	0	+	1	0	3+	
+	+	0	0	+	0	+	0	+	0	+	2	0	3+	
+	0	+	0	+	0	+	+	+	0	0	3	0	3+	
+	0	+	0	+	0	+	0	+	0	0	4	0	3+	
0	+	+	0	+	+	0	+	0	+	0	5	0	0 ✓	
+	0	+	0	+	0	+	+	+	+	0	6	0	0 ✓	
0	+	+	0	+	+	0	+	0	0	+	7	0	0 ✓	
+	0	+	0	+	+	+	+	0	+	0	8	0	0 ✓	
+	+	+	0	+	+	+	+	+	0	+	9	0	0 ✓	
+	+	+	0	+	+	0	0	+	+	0	10	0	0 ✓	
0	+	0	0	+	+	0	0	+	0	+	11	0	3+	
											AC			

IS = Immediate spin
~~37 = 37 C Incubation~~
AHG = Anti-human globulin
IAT = Indirect antiglobulin test
(=AHG)

This is also tube testing!
(often with PEG)

Kidd		P	Lutheran		MNSs				Lewis			Results		
Jk^a	Jk^b	P1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell	AHG		
+	0	+	0	+	0	+	0	+	0	+	1	3+		
+	+	0	0	+	0	+	0	+	0	+	2	3+		
+	0	+	0	+	0	+	+	+	0	0	3	3+		
+	0	+	0	+	0	+	0	+	0	0	4	3+		
0	+	+	0	+	+	0	+	0	+	0	5	0 ✓		
+	0	+	0	+	0	+	+	+	+	0	6	0 ✓		
0	+	+	0	+	+	0	+	0	0	+	7	0 ✓		
+	0	+	0	+	+	+	+	0	+	0	8	0 ✓		
+	+	+	0	+	+	+	+	+	0	+	9	0 ✓		
+	+	+	0	+	+	0	0	+	+	0	10	0 ✓		
0	+	0	0	+	+	0	0	+	0	+	11	3+		
											AC			

~~IS = Immediate spin~~
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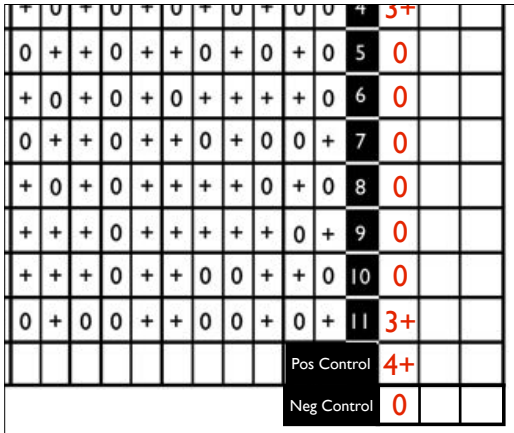
(=AHG)

This could be:

-Tube testing
OR
-Gel testing
OR
-Solid-phase testing



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This could be:
-Tube testing
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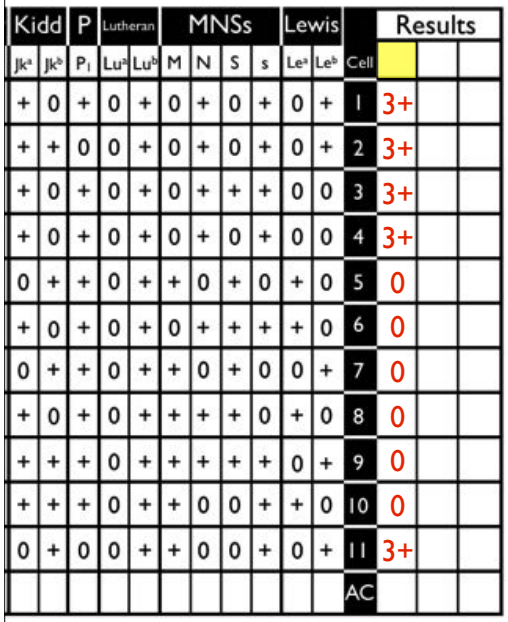
OR
-Solid-phase testing



~~IS = Immediate spin~~
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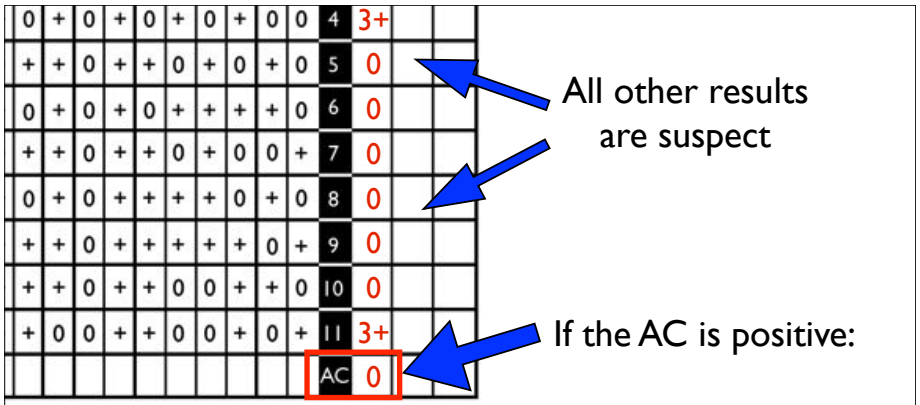
This is almost always:
-Gel testing
OR
-Solid-phase



All other results are suspect

If the AC is positive:

Ya Gotta Have a Plan…

- Consistent approach minimizes error
- Most are simple, but cutting corners increases risk for dumb mistakes
- Use this or your own system, but use the same approach every time!

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General Process

- Check history
- Check autocontrol
- Look at general pattern
- Look at what's NOT there (cross-outs)
- Look at what IS there
- Use special techniques as necessary
- Ensure statistical significance

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History

- Both in real life and on exams
- History can give you a clue and keep you from doing something dumb
- Up to 70% of cases impacted by history

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History

- Clinical history examples:
  - Anti-D in pregnant pt; consider RhIG
  - Recent bacterial infection; consider antibiotic induced warm autoab
  - Recent viral illness; consider auto-anti-I or -i (consider age)
  - Recent transfusion; consider newly developing antibody
  - ITP; consider IV RhIG in D+ patient

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History

- Clinical history examples:
  - Consider racial profiling (in a good way, of course!)
  - African-Americans: Lack of Duffy antigens
  - Asians: Almost all D+
  - Whites: May lack high freq antigens

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History

- Consider serologic history, if known
- Previous phenotyping will help, but be careful!
  - Transplants, transfusions, errors
- In real world, can use this info for targeting the panel and cells chosen

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Cell	Rh-hr	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell			
1	R^1R^1	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	1	3+		
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	2	3+		
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0	3	3+		
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4	3+		
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	5	0		
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	6	0		
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	7	0		
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0	8	0		
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	0	+	9	0		
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	+	0	10	0		
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	11	3+		
AC																												AC	0		

Positive Autocontrol

- More details next time…
- Question 1: Is DAT positive?
- Question 2: What's the patient history?
- Possibilities include:
  - Autoantibodies (warm and cold)
  - Recent transfusion/DHTR
  - Drug-induced issues
  - Passively acquired antibodies

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Pattern

- Are reactions:
  - Uniform or variable?
  - Against all, most, or rare cells?
  - Present in what phases?

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Variability

- Uniform reactions suggest a single antibody
- Variable reactions suggest either:
  - Multiple antibodies OR
  - Single antibody with dosage

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Cells Reactive

- With negative autocontrol…
  - A mixture of reactive and nonreactive cells suggests:
    - A single alloantibody OR
    - Multiple alloantibodies
  - A single reactive cell suggests:
    - A single alloantibody against a low-prevalence antigen

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Cells Reactive

- With negative autocontrol…
  - All or virtually all cells reactive suggests:
    - Multiple alloantibodies
    - A single alloantibody against a high-prevalence antigen

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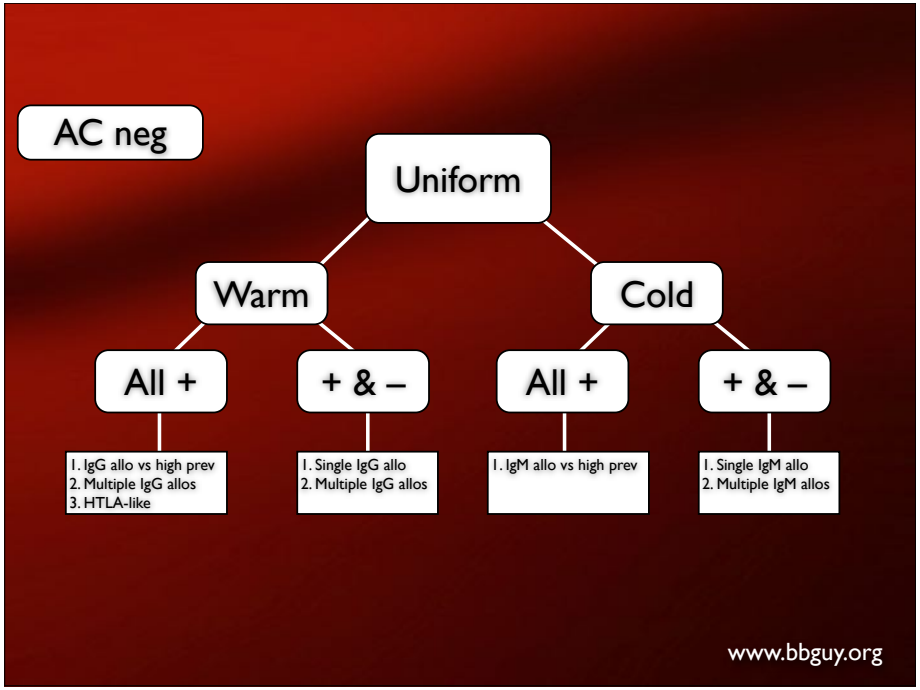
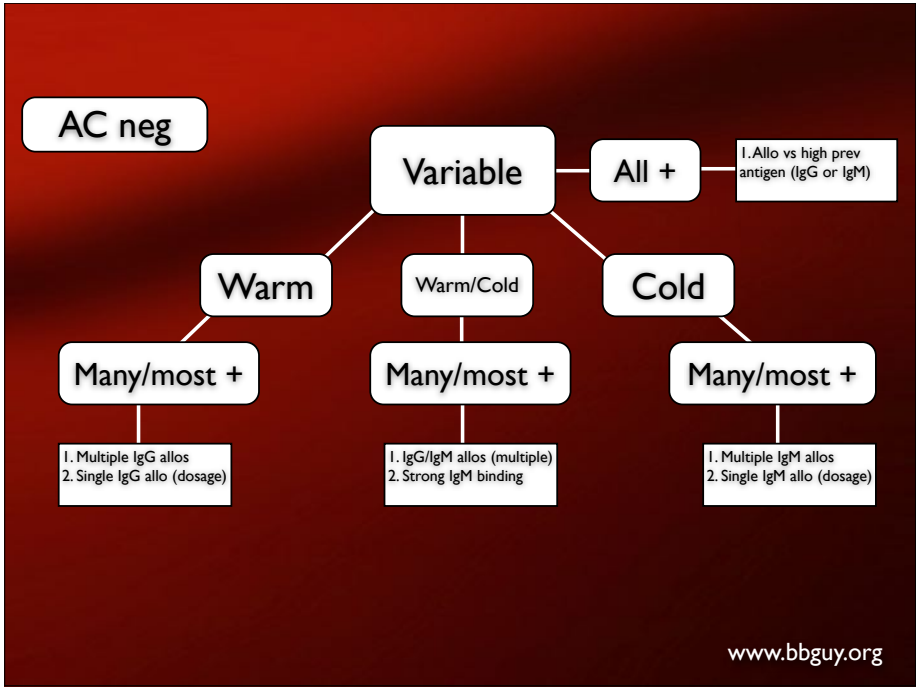
37/AHG:	Rh
AHG:	K, Fy, Jk, S/s
Variable:	Lu, Xg
IS (RT):	Le, MN, P

37 C and AHG	V	AHG Only	RT	Var.	RT	AHG	RT

	Rh-hr							Xg	Kell					Duffy		Kidd		P	Lutheran		MNSs				Lewis		Cell
	D	C	E	c	e	f	C^w	Xg^a	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	
	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	1
	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	2
	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0	3
	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4
	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	5
	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	6
	0	0	0	+	+	+	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	7
	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0	8





		Rh-hr								Kell						Duffy		Kidd		P	Lutheran		MNSs				Lewis			Results		
Cell	Rh-hr	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell			
1	R^1R^1	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	1	3+		
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	2	3+		
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0	3	3+		
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4	3+		
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	5	0		
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	6	0		
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	7	0		
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0	8	0		
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	0	+	9	0		
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	+	0	10	0		
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	11	3+		
AC																													AC	0		

1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Uniform pattern; single antibody most likely

General Process

- Check history
- Check autocontrol
- Look at general pattern
- Look at what's NOT there (cross-outs)
- Look at what IS there
- Use special techniques as necessary
- Ensure statistical significance

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		Rh-hr								Kell						Duffy		Kidd		P	Lutheran		MNSs				Lewis			Results		
Cell	Rh-hr	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell			
1	R^1R^1	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	1	3+		
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	2	3+		
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0	3	3+		
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4	3+		
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	5	0		
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	6	0		
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	7	0		
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0	8	0		
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	0	+	9	0		
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	+	0	10	0		
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	11	3+		
AC																													AC	0		

1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Uniform pattern; single antibody most likely

"Cross-outs"

~~Fy^a~~	Fy^b	IS	37C	IAT
(+)	0	0	0	0
+	+	0	0	0
~~0~~	~~+~~	~~0~~	~~1+~~	~~2+~~

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“Cross-outs”

~~Fy^a~~	Fy^b	IS	37C	IAT
+	0	0	0	0
+	+	0	0	0
~~0~~	~~+~~	~~0~~	~~1+~~	~~2+~~

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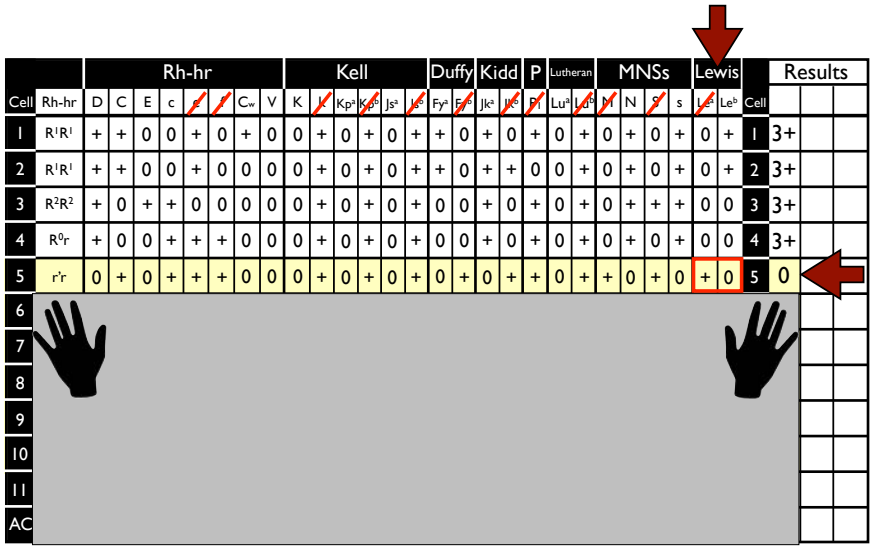

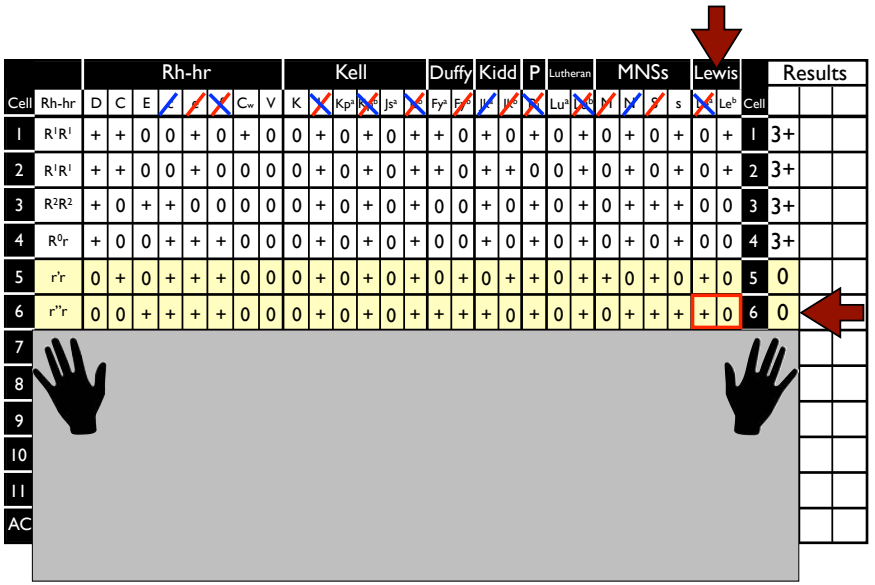

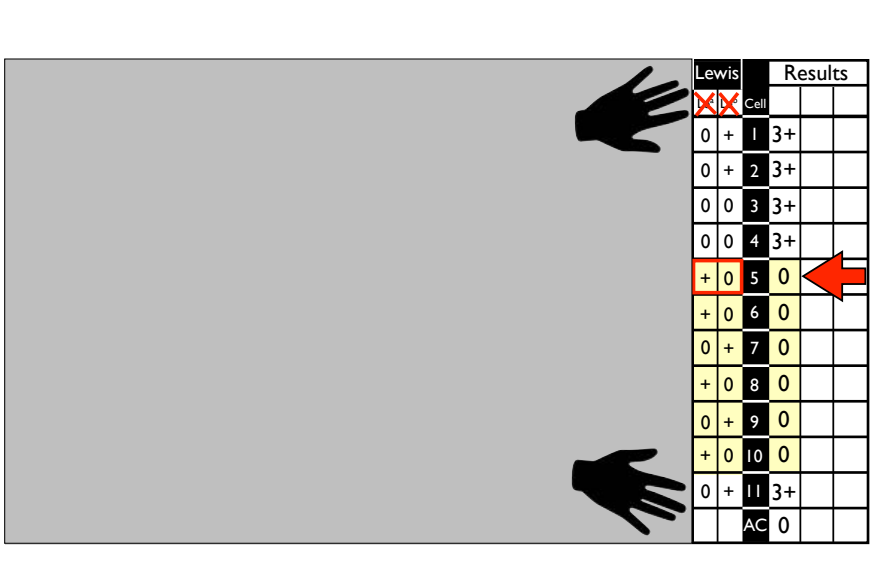

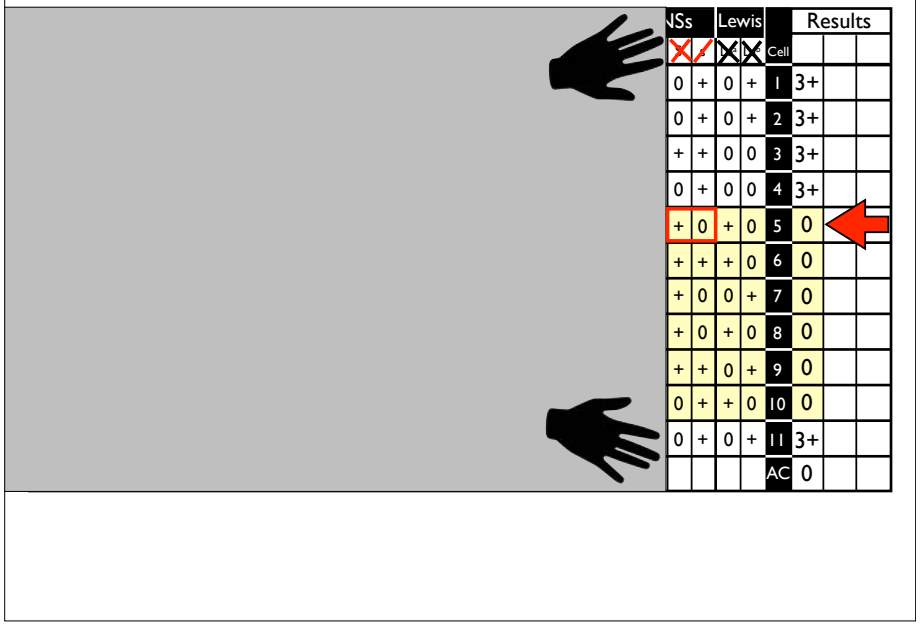

Cell	Rh-hr	D	C	E	Kell	Duffy	Kidd	P	Lutheran	MNSs	Lewis	Cell	Results
1	R^1R^1	+	+	0	0 + 0 + 0 + 0	+ 0	+ 0	+	0 +	0 + 0 +	0 +	1	3+
2	R^1R^1	+	+	0	0 + 0 0 0	+ 0	+ +	0	0 +	0 + 0 +	0 +	2	3+
3	R^2R^2	+	0	+	+ 0 0 0 0	0 0	+ 0	+	0 +	0 + 0 +	+ +	3	3+
4	R^0r	+	0	0	+ + + 0 0	0 0	+ 0	+	0 +	0 + 0 +	0 0	4	3+
5	r'r	0	+	0	+ + + 0 0	0 +	0 +	+	0 +	+ 0 + 0	+ 0	5	0
6	r''r	0	0	+	+ + + 0 0	+ +	+ 0	+	0 +	0 + 0 +	+ +	6	0
7	rr	0	0	0	+ + + 0 0	+ 0	+ +	+	0 +	+ 0 + 0	0 +	7	0
8	rr	0	0	0	+ + + 0 0	0 +	0 +	+	0 +	+ + + 0	+ 0	8	0
9	rr	0	0	0	+ + + 0 0	+ 0	+ +	+	0 +	+ + + +	0 +	9	0
10	rr	0	0	0	+ + + 0 0	+ +	+ +	+	0 +	+ + 0 0	+ 0	10	0
11	R^1R^1	+	+	0	0 + 0 0 0	+ 0	+ 0	0	0 +	+ 0 0 +	0 +	11	3+
AC												AC	0

1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Uniform pattern; single antibody most likely

General Process

- Check history
- Check autocontrol
- Look at general pattern
- Look at what's NOT there (cross-outs)
- Look at what IS there
- Use special techniques as necessary
- Ensure statistical significance

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General Process

- Try first for a single antibody to explain all reactions

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D C ~~E~~ C_w V ~~K_p^a~~ ~~Js^a~~ ~~Lu^a~~

Cell	Rh-hr	D	C	E				C_w	V			Kell				Duffy		Kidd		P	Lutheran			MNSs				Lewis		Cell	Results
1	R^1R^1	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	1	3+
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	2	3+
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0	3	3+
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4	3+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	5	0	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	6	0		
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	7	0
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	8	0
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+	9	0	
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	+	0	10	0		
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	0	+	0	+	11	3+	
AC																														AC	0

1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Uniform pattern; single antibody most likely

General Process

- Try first for a single antibody to explain all reactions
- Failing that...
- Depending on pattern, hypothesize:
  - Two antibodies in same phase

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D C E C_w V ~~K_p^a~~ ~~Js^a~~ ~~Lu^a~~

Cell	Rh-hr	D	C	E				C_w	V			Kell				Duffy		Kidd		P	Lutheran			MNSs				Lewis		Cell	Results
1	R^1R^1	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	1	4+
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	2	4+
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	0	3	3+		
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4	3+		
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	5	2+		
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	6	0		
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	7	0
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	8	0
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+	9	0	
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	+	0	10	0		
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	0	+	0	+	11	4+		
AC																														AC	0

1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Variable pattern; multiple antibodies most likely

General Process

- Try first for a single antibody to explain all reactions
- Failing that...
- Depending on pattern, hypothesize:
  - Two antibodies in same phase
  - One warm and one cold antibody

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		Rh-hr								Kell							Duffy		Kidd		P	Lutheran		MNSs				Lewis		Results		
Cell	Rh-hr	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b		Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell	IS	37	IAT
1	R^1R^1	+	+	0	0	+	0	+	0	+	+	0	+	0	+		+	0	+	0	+	0	+	0	+	0	+	0	+	1	0	0	0
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+		+	0	+	+	0	0	+	+	0	0	+	0	+	2	0	0	0
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+		0	0	+	0	+	0	+	0	+	+	0	0	0	3	0	0	0
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+		0	0	+	0	+	0	+	0	+	0	+	0	0	4	0	0	0
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+		0	+	0	+	+	0	+	+	0	+	0	+	0	5	1+	1+	3+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+		+	+	+	0	+	0	+	0	+	+	+	+	0	6	1+	1+	1+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+		0	+	0	+	+	0	+	+	0	+	0	0	+	7	0	0	3+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+		+	0	0	+	+	0	+	+	+	+	0	+	0	8	1+	1+	0
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+		+	0	+	+	+	0	+	+	+	+	+	0	+	9	0	0	0
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+		+	+	+	+	+	0	+	+	0	0	+	0	10	1+	1+	1+
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+		0	+	0	+	0	0	+	+	0	0	+	0	+	11	0	0	3+
AC																													AC	0	0	0

1. Tube testing for sure
2. No autoantibody obvious
3. Highly variable pattern, both warm and cold

"Colds": Le^a, Le^b, M, N, P

Fy^b

		Rh-hr								Kell							Du		Kidd		P	Lutheran		MNSs				Lewis		Results		
Cell	Rh-hr	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b		Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell	IS	37	IAT
1	R^1R^1	+	+	0	0	+	0	+	0	+	+	0	+	0	+		+	0	+	0	+	0	+	0	+	0	+	0	+	1	0	0	0
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+		+	0	+	+	0	0	+	+	0	0	+	0	+	2	0	0	0
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+		0	0	+	0	+	0	+	0	+	+	0	0	0	3	0	0	0
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+		0	0	+	0	+	0	+	0	+	0	+	0	0	4	0	0	0
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+		0	+	0	+	+	0	+	+	0	+	0	+	0	5	1+	1+	3+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+		+	+	+	0	+	0	+	0	+	+	+	+	0	6	1+	1+	1+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+		0	+	0	+	+	0	+	+	0	+	0	0	+	7	0	0	3+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+		+	0	0	+	+	0	+	+	+	+	0	+	0	8	1+	1+	0
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+		+	0	+	+	+	0	+	+	+	+	+	0	+	9	0	0	0
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+		+	+	+	+	+	0	+	+	0	0	+	0	10	1+	1+	1+
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+		0	+	0	+	0	0	+	+	0	0	+	0	+	11	0	0	3+
AC																													AC	0	0	0

1. Tube testing for sure
2. No autoantibody obvious
3. Highly variable pattern, both warm and cold

"Colds": Le^a, Le^b, M, N, P

Fy^b, Le^a

		Rh-hr								Kell							Duffy		Kidd		P	Lutheran		MNSs				Lewis		Results		
Cell	Rh-hr	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b		Fy^a	Fy^b	Jk^a	Jk^b	P_1	Lu^a	Lu^b	M	N	S	s	Le^a	Le^b	Cell	IS	37	IAT
1	R^1R^1	+	+	0	0	+	0	+	0	+	+	0	+	0	+		+	0	+	0	+	0	+	0	+	0	+	0	+	1	0	0	0
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+		+	0	+	+	0	0	+	+	0	0	+	0	+	2	0	0	0
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+		0	0	+	0	+	0	+	0	+	+	0	0	0	3	0	0	0
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+		0	0	+	0	+	0	+	0	+	0	+	0	0	4	0	0	0
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+		0	+	0	+	+	0	+	+	0	+	0	+	0	5	1+	1+	3+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+		+	+	+	0	+	0	+	0	+	+	+	+	0	6	1+	1+	1+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+		0	+	0	+	+	0	+	+	0	+	0	0	+	7	0	0	3+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+		+	0	0	+	+	0	+	+	+	+	0	+	0	8	1+	1+	0
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+		+	0	+	+	+	0	+	+	+	+	+	0	+	9	0	0	0
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+		+	+	+	+	+	0	+	+	0	0	+	0	10	1+	1+	1+
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+		0	+	+	+	0	0	+	+	0	0	+	0	+	11	0	0	3+
AC																													AC	0	0	0

1. Tube testing for sure
2. No autoantibody obvious
3. Highly variable pattern, both warm and cold

General Process

- Try first for a single antibody to explain all reactions
- Failing that...
- Depending on pattern, hypothesize:
  - Two antibodies in same phase
  - One warm and one cold antibody
  - Consider multiple warms and colds
    - Next podcast!!

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General Process

- Check history
- Check autocontrol
- Look at general pattern
- Look at what's NOT there (cross-outs)
- Look at what IS there
- Use special techniques as necessary
- Ensure statistical significance

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Phenotyping

- Helps confirm identification of alloantibody by demonstrating lack of antigen
- Is a TOOL in confirmation, not sole measure of confirmation

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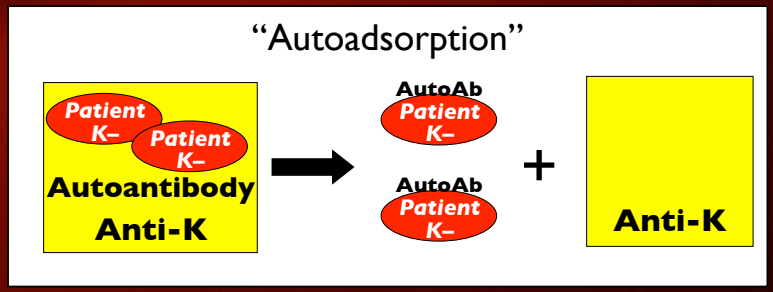
DCE C_w ~~V~~ ~~Kp^a~~ ~~Js^a~~ ~~Lu^a~~

		Rh-hr								Kell								Duffy		Kidd			P	Lutheran		MNSs				Lewis			Results		
Cell	Rh-hr	D	C	E				C_w	V			Kp^a		Js^a				Fy^a	Fy^b					Lu^a									Cell			
1	R^1R^1	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	1	4+		
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	2	4+		
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	3	3+		
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	4	3+		
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	5	2+		
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	6	0		
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0	+	0	0	+	7	0		
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	0	+	+	+	+	0	+	8	0		
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	9	0		
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	+	+	0	+	0	+	+	0	0	+	+	0	10	0		
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	11	4+		
AC		0	0																													AC	0		

1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Variable pattern; multiple antibodies most likely

Adsorption

- Removing antibodies from sample by incubation with antigen-positive RBCs



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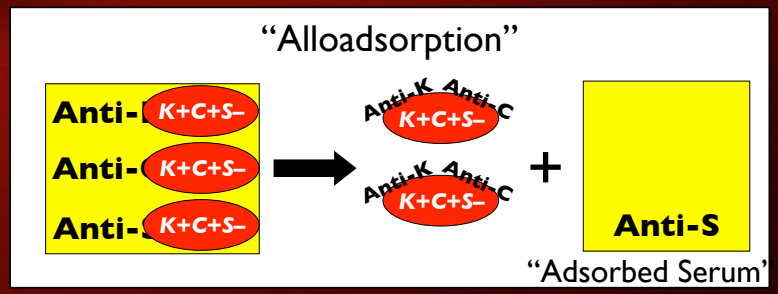
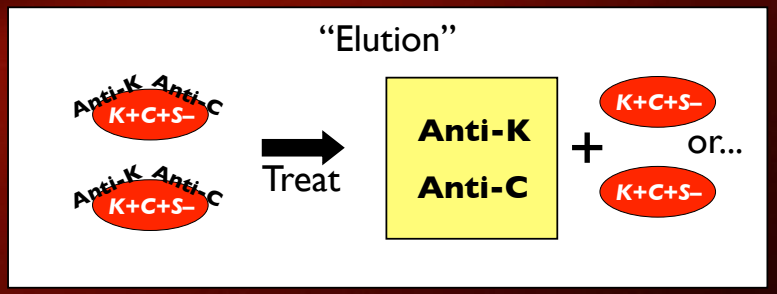
Adsorption

- Removing antibodies from sample by incubation with antigen-positive RBCs



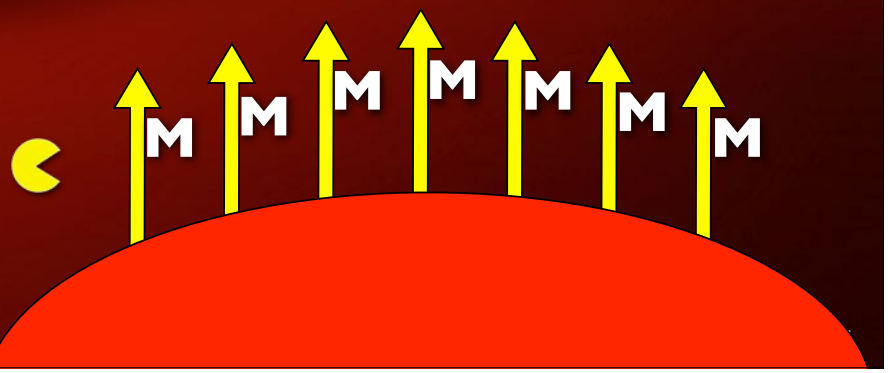
Elution

- Removal of RBC-bound antibodies
- Heat, cold, chemical (glycine)



Proteolytic Enzymes

- Enzymes such as ficin and papain may change Ag expression/Ab binding



General Process

- Check history
- Check autocontrol
- Look at general pattern
- Look at what's NOT there (cross-outs)
- Look at what IS there
- Use special techniques as necessary
- Ensure statistical significance

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Probability

- Basic idea: Ensure what you are seeing is not pure chance
- Traditional interpretation:
  - Ag present: 3 positive reactions
  - Ag absent: 3 negative reactions
- AABB Standards for IRLs, 7th ed:
  - 5.3.3 requires only 2 of each reaction to assign specificity

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Probability

- For practical purposes, preliminary ID only requires ONE double-dose rule out to establish lack of identity
  - This does NOT mean your work is done, however
  - Still need to use selected cells to rule in/out specific Abs (per OP)

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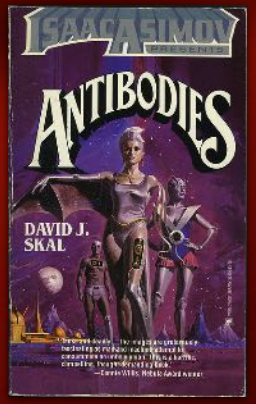
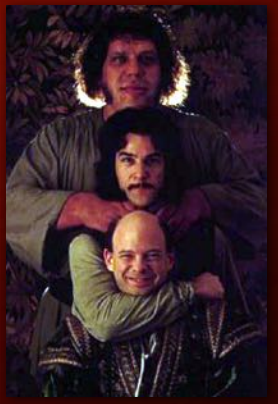
D C E C_w V Kp^a Js^a Lu^a

		Rh-hr								Kell								Duffy		Kidd		P	Lutheran		MNSs					Lewis			Results
Cell	Rh-hr	D	C	E				C_w	V			K^a		J^a				Fy^a	Fy^b	Jk^a	Jk^b		Lu^a		M	N	S			Le^a	Le^b		Cell	
1	R^1R^1	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	1	3+
2	R^1R^1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	2	3+
3	R^2R^2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	3	3+
4	R^0r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	4	3+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0	5	0		
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	6	0		
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	7	0		
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0	+	0	8	0		
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+	9	0		
10	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	+	+	0	+	+	0	0	+	+	0	10	0		
11	R^1R^1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	11	3+		
AC																															AC	0		

1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Uniform pattern; single antibody most likely

Let's do some together!





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Thanks!

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- Kevin Elman (N. Co. Med Center)
- Cami Melland (Bonfils)
- The immortal Connie Howard (Walter Reed)

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