Community Blood Center

Resident Rotation:Immunohematology Reference Lab (IRL)

Module 3: Antibody detection & identification

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COMPREHENSIVE CELL SOLUTIONS

NATIONAL CENTER FOR BLOOD GROUP GENOMICS

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Objectives

- Describe the testing performed to detect and identify "unexpected antibodies."
- Discuss how the following results provide clues during the antibody investigation:
  - Phase of reactivity
  - Variability of reactivity
  - Autocontrol result
  - Patient phenotype
  - Use of enzyme-treated reagent RBCs
- Perform rule outs and interpret results of antibody panels.
- In case studies, given testing results, identify antibodies and make appropriate transfusion recommendations.

"Unexpected Antibodies"

- Antibodies other than "naturally occurring" anti-A, anti-B
- Found in ~1-3% of population
  - Depends on cohort
  - Depends on testing methods, enhancement
- Most formed after exposure to foreign antigen:
  - Transfusion
  - HSCT
  - Pregnancy
  - (some examples of antibody formation without exposure event)
- May or may not be clinically significant
  - Informs transfusion recommendations

Pre-transfusion Testing

"Type and Screen" sample received in blood bank

"Type"= ABO/Rh

"Screen"= plasma tested against 2 or 3 reagent RBCs of known phenotype

Negative antibody screen

No detectable "unexpected antibodies"

Immediate spin (IS) or "electronic" crossmatch

Positive antibody screen
Patient plasma reacts with one or more reagent RBC

Antibody identification
Antibody panel

Antibody/ies identified

Full (IAT) antigen-negative (if required) crossmatch

Antibody Screen

https://diahem.com/products/test-cells/

- Group O RBCs: known phenotype
- Screening cells licensed by the FDA must express the following antigens:
  - D, C, E, c, e, M, N, S, s, P1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a and Jk^b
  - Corresponding antibodies in patient plasma should react with one or more screening cell

		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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a
I	R_1R_1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0	+	+	0	0	+	+
II	R_2R_2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	0
III	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	0	+	+

Antibody Screen

https://diaherm.com/products/test-cells/

- **Antigram seen below:**
  - Each row represents antigen typings for 1 vial of RBCs
  - Antigen status (positive or negative) provided for "common red cell antigens"
  - >350 known antigens – antigrams don't show ALL antigen typings

		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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a
I	R_1R_1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+
II	R_1R_2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	0
III	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+

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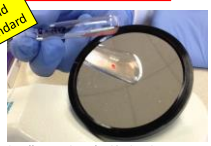
Methods:

https://www.fda.gov/media/128756/download

- Gel column agglutination
- Solid phase red cell adherence assay
- Tube testing

Gold Standard



https://www.youtube.com/watch?v=SbEnOHIU_2E

https://www.trasci.com/article/S1473-0502(12)00070-5/pdf

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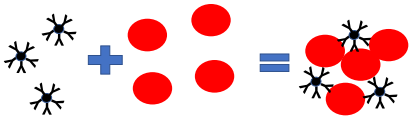
Tube Testing:

- **Direct agglutination:**
  - Antibody + RBCs = visible agglutination
  - Often IgM antibodies
  - Immediate spin/37C phases of testing



Direct agglutinating antibody | **RBCs expressing corresponding antigen** | **Visible agglutination**

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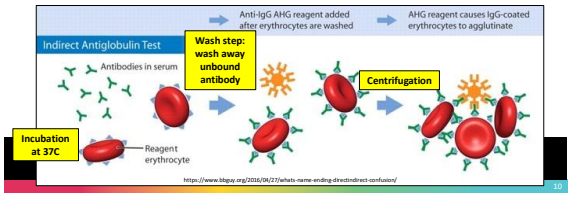
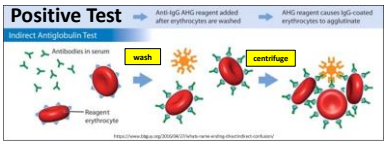
Tube Testing:

- **Indirect Antiglobulin Test (IAT)**
  - Detects IgG antibodies
  - Antibodies and cells incubated at 37C
  - Unbound antibodies washed away
  - Anti-human IgG reagent added
  - Visible agglutination of antibody coated cells

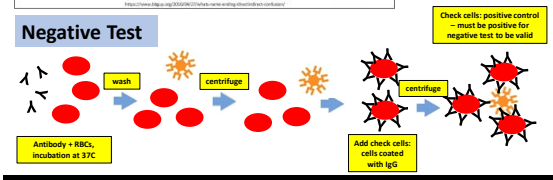


Indirect Antiglobulin Test



Check cells = cells coated with IgG

- Added to all *NEGATIVE TESTS*
- Positive control
- Did you add anti-IgG?
- Did the wash step wash away unbound antibody?



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Tube Testing

Enhancement media you'll be using in the lab this week.

- **Enhancement media**
  - Polyethylene Glycol (PEG)
  - Low Ionic Strength Solution (LISS)
  - Albumin
  - Testing against enzyme-treated cells
    - Papain
    - Ficin

We'll also be discussing & using enzymes

Enhance both clinically significant and clinically insignificant antibodies. **Choose when to use**

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Tube Testing: "Phases" of Reactivity

- **IS= immediate spin**
  - Direct agglutination
  - Antibody + antigen = visible agglutination
  - IgM antibodies and direct agglutinating IgG
- **Enhancement media**
- **37C**
  - Direct agglutination after incubation at 37C
- **IAT (Indirect Antiglobulin Test)**
  - After incubation at 37C, unbound antibody washed away, addition of anti-human IgG
  - Detection of a majority of IgG antibodies

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	IS	LISS
																													37C	IAT
I	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+				
II	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	0	+	0			
III	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	+	+			

Watch an antibody screen

This video demonstrates how to perform an antibody screen on a patient's plasma. You may want to follow along with the *PEG Antibody Screen Procedure* in your handbook.

Performing an antibody screen in tube tests with PEG

https://youtu.be/-XUzXyMFpyQ

Positive Antibody Screen

C	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	IS	LISS
																													37C	IAT
I	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	0	2+
II	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	0	+	0	0	0	2+
III	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	+	+	0	0	0

- In this case…
  - No reactivity seen at IS or 37C
  - IAT: Cells I and II are reactive; Cell III is nonreactive
- **Next step: Antibody panel**

Antibody Panel


https://diahem.com/products/test-cells/

- Test against several reagent RBCs of known phenotype
- Pattern of reactivity aids in identification of antibody
  - "Rule out" antibodies not present
  - "Rule in" antibodies present in patient's plasma

		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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a
1	R_1R_1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+
2	R_1R_2	+	+	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_or'	+	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	0	+	+
6	$r'r$	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+
7	rr	0	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	0	+	+	+	0	+	0
9	rr	0	0	0	+	+	+	+	0	0	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0
10	R_1R_1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	+	0	0	+	+
11	R_oR'	+	+	0	0	+	+	+	0	0	+	+	0	+	0	0	+	+	+	+	0	+	0	+	+	+	+	+	+
Auto																													

Watch an antibody panel

https://youtu.be/BYdn4mqFgr8

Objectives

- Describe the testing performed to detect and identify "unexpected antibodies."
- Discuss how the following results provide clues during the antibody investigation:
  - Phase of reactivity
  - Variability of reactivity
  - Autocontrol result
  - Patient phenotype
  - Use of enzyme-treated reagent RBCs
- Perform rule outs and interpret results of antibody panels.
- In case studies, given testing results, identify antibodies and make appropriate transfusion recommendations.

Antibody Identification:

Things to pay attention to:

- Phase of reactivity
  - IS – "cold reacting antibodies;"
    - Usually IgM, usually clinically INSIGNIFICANT
    - Some antibodies characteristically react at cold temperatures:
      - anti-M, Lewis antibodies, anti-P1
  - IAT – detects IgG
    - Usually considered clinically significant
- Enhancement media
  - Different sensitivities

Results		
IS	LISS	
	37C	IAT
0	0	2+
0	0	2+
0	0	(0) √

Parentheses () = tests read *microscopically*
Check mark √ = check cells were reactive & test is valid

Antibody Identification:

Things to pay attention to:

- Variability of reactivity
  - May indicate multiple antibodies

	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	IS	LISS 37C	LISS IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	0	0	0
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	4+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0	0	1+
4	+	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	1+
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	0	0	4+
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0	0
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	0	0	0
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	0	0	0
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	0	0	4+
Auto																0	0	0

Antibody Identification:

Things to pay attention to:

- Variability of reactivity
  - May indicate **dosage**
    - Antigen density on cells may be greater when individual is homozygous for gene encoding the antigen ("double dose" antigen expression)
    - Corresponding antibody may react stronger with double dose

Single dose cell: JK*A/B genotype
Weaker reaction with anti-Jk^a

Anti-Jk^a reactivity

	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	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	1+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0
3	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+	2+

Double dose cell: JK*A/A genotype
Stronger reaction with anti-Jk^a

Antibody Identification:

Things to pay attention to:

- Result of Autocontrol
  - Patient cells + patient plasma
    - Negative result – reactivity is ***allo***antibody
    - Positive result
      - Reactivity is ***auto***antibody, or
      - Serologic evidence of transfusion reaction (mixed field)

	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	IS	LISS 37C	LISS IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	0	0	0
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	4+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0	0	1+
4	+	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	1+
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	0	0	4+
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0	0
Auto																0	0	1+mf

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Antibody Identification:

Things to pay attention to:

- Phenotype (or genotype) of patient's RBCs
  - Not expected to make alloantibody to an antigen on his/her own cells
  - In antibody ID, we use both reactivity in plasma & antigens on RBCs to aid in investigation

	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
Patient RBCs	+	+	0	0	+	0	+	+	0	0	+	+	+	+	+

- Patient cells lack **E**, **c**, **K**, **Fy^b** & **Jk^a** antigens
- Patient could make **anti-E**, **anti-c**, **anti-K**, **anti-Fy^b** & **anti-Jk^a**

Remember: Serologic phenotype unreliable in cases of…
- Recent transfusion
- HSCT patients
- If the direct antiglobulin test (DAT) is positive

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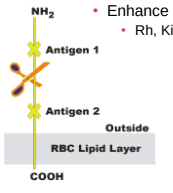
Antibody Identification:

Things to pay attention to:

- Enzyme-treatment of reagent RBCs
  - Ficin/Papain-treat reagent RBCs
    - Destroys some antigens
      - M,N,S,s (variable), Fy^a, Fy^b (and others)
    - Enhance reactivity of some antibodies
      - Rh, Kidd, Lewis (and others)



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Antibody Identification:

Things to pay attention to:

- Enzyme-treatment of reagent RBCs
  - Remember, Fy^a, Fy^b, M, N, S, s (variable) antigens destroyed (shaded in orange)
  - In this example, reactivity **disappeared** when testing enzyme-treated cells
    - Antibody to an antigen destroyed by ficin

	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	Ficin-Treated RBCs IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	3+	0
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	4+	0
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	0	0
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	4+	0
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	0	0
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	0	0
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	3+	0
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	3+	0
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	4+	0
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	0	0
Auto																0	

Antibody Identification:

Things to pay attention to:

- Enzyme-treatment of reagent RBCs
  - Remember, Fy^a, Fy^b, M, N, S, s (variable) antigens destroyed (shaded in orange)
  - In this example, reactivity **is stronger** when testing enzyme-treated cells
    - Antibody enhanced by ficin

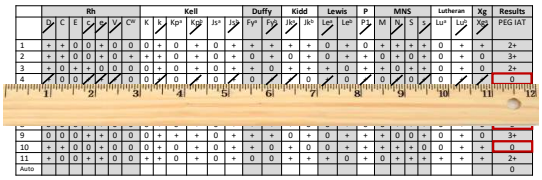
	Rh					Kell		Duffy		Kidd		MNS				Results	
	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	PEG IAT	Ficin-Treated RBCs IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	1+	3+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	1+	3+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	1+	3+
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	1+	3+
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	0	0
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	0	0
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	0	0
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	0	0
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	1+	3+
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	1+	3+
Auto																0	

Objectives

- Describe the testing performed to detect and identify "unexpected antibodies."
- Discuss how the following results provide clues during the antibody investigation:
  - Phase of reactivity
  - Variability of reactivity
  - Autocontrol result
  - Patient phenotype
  - Use of enzyme-treated reagent RBCs
- Perform rule outs and interpret results of antibody panels.
- In case studies, given testing results, identify antibodies and make appropriate transfusion recommendations.

Rule outs (Practicing rule outs #1)

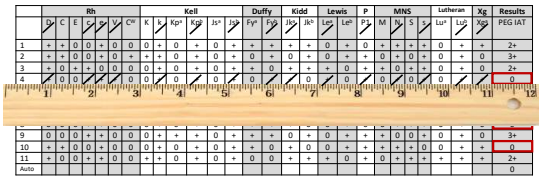
- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma



	Rh							Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	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	$P1^A$	M	N^a	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	2+
2	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	3+
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	2+
4	/	0	0	/	/	/	0	0	/	0	/	0	/	0	/	/	/	0	/	0	/	0	/	0	/	/	/	0	0
9	0	0	0	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	3+
10	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	0	0	+	+	+	0	0
11	+	0	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	+	2+
Auto																												0

Rule outs (Practicing rule outs #1)

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose



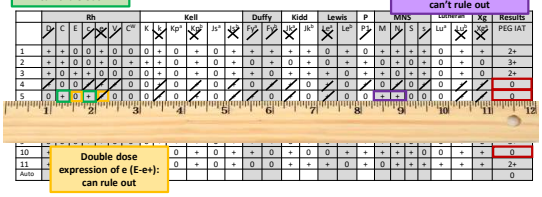
	Rh							Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	$P1^A$	M	N^a	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	2+
2	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	3+
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	2+
4	/	0	0	/	/	/	0	0	/	0	/	0	/	0	/	/	/	0	/	0	/	0	/	0	/	/	/	0	0
9	0	0	0	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	3+
10	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	0	0	+	+	+	0	0
11	+	0	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	+	2+
Auto																												0

Rule outs (Practicing rule outs #1)

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - ception: rule out Kell antibodies with single d

Single dose of C and c: can't rule out

Single dose of M and N: can't rule out

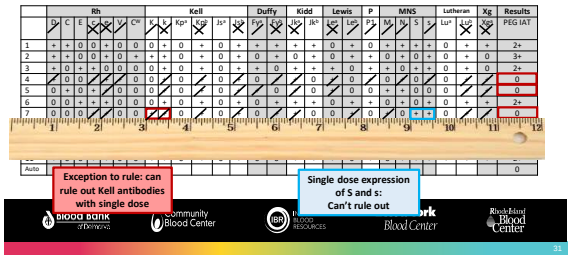


	Rh							Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	$P1^A$	M	N^a	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	2+
2	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	3+
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	2+
4	/	0	0	/	/	/	0	0	/	0	/	0	/	0	/	/	/	0	/	0	/	0	/	0	/	/	/	0	0
5	0	+	0	+	/	0	0	0	/	0	/	0	/	0	/	/	/	0	/	0	+	+	0	0	/	/	/	0	0
10	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	0	0	+	+	+	0	0
11	+	0	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	+	2+
Auto																												0

Double dose expression of e (E-e+): can rule out

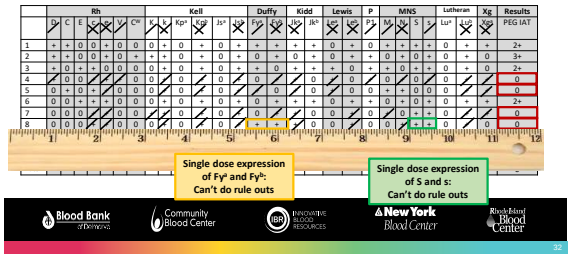
Rule outs (Practicing rule outs #1)

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose



Rule outs (Practicing rule outs #1)

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose



Rule outs (Practicing rule outs #1)

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
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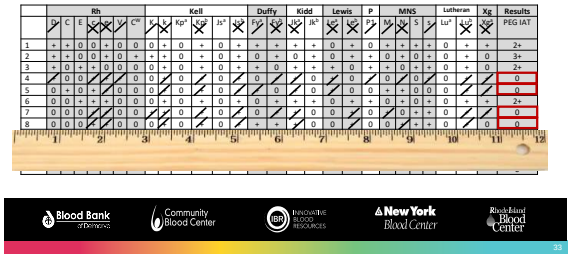
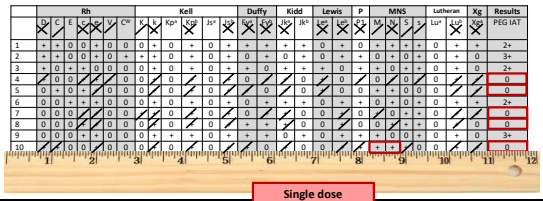
    - Exception: rule out Kell antibodies with single dose



Rule outs (Practicing rule outs #1)

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose

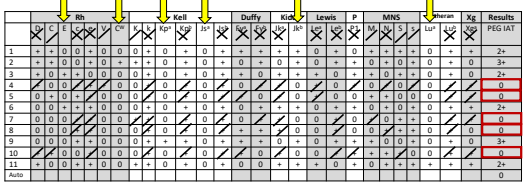


Single dose expression of M & N: Can't do rule outs

Rule outs (Practicing rule outs #1)

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE
    - Exception: rule out Kell antibodies with single dose

Antibody specificities we haven't ruled out, even once

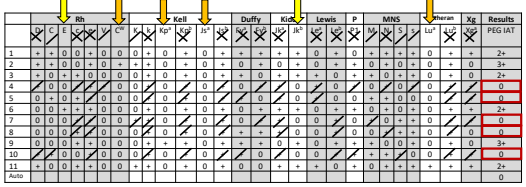


Rule outs finished. Now what?

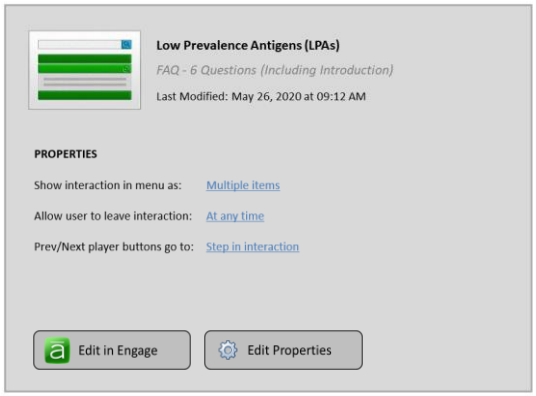
Rule outs (Practicing rule outs #1)

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE
    - Exception: rule out Kell antibodies with single dose

These are low prevalence antigens: won't rule out antibody



Rule outs finished. Now what?



Rule outs (Practicing rule outs #1)

- Rule out sp… ng to antigens present on reagent RBCs that are NO… patient's plasma
  - When … cificities using DOUBLE DOSE
    - Ex… antibodies with single dose

Possible antibody specificity: Anti-E? Anti-Jk^b?

These are low prevalence antigens: won't rule out antibody

	Rh							Kell			Duffy			Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	V	C^w	K	K^p	K^{ea}	Js^a	Fy	Fy^a	Fy^b	Jk	Jk^b	Le	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg	PEG IAT
1	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	2+
2	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	3+
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	2+
4	/	0	0	/	/	/	0	0	/	0	/	0	/	/	/	0	/	0	/	0	/	0	/	0	/	/	/	0
5	0	+	0	+	/	0	0	0	/	0	/	0	/	0	/	0	/	0	0	+	0	+	0	0	0	/	/	0
6	0	0	+	+	+	0	0	+	0	+	0	+	/	0	+	/	+	0	+	+	0	+	0	+	0	+	+	2+
7	0	0	0	/	/	0	0	/	0	/	0	/	0	/	/	0	0	/	0	0	/	0	+	+	0	/	/	0
8	0	0	0	/	/	0	0	0	/	0	/	0	+	+	/	0	0	/	0	0	/	+	+	0	/	0	0	0
9	0	0	0	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	3+
10	/	/	0	0	/	0	0	0	/	0	/	0	/	/	0	/	0	/	0	0	/	+	+	/	0	0	/	/	0
11	+	0	0	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	2+
Auto																											0

Rule outs finished. Now what?

Rule outs (Practicing rule outs #1)

- Rule out sp… ng to antigens present on reagent RBCs that are NO… patient's plasma
  - When … cificities using DOUBLE DOSE
    - Ex… antibodies with single dose

Possible antibody specificity: Anti-E? Anti-Jk^b?

These are low prevalence antigens: won't rule out antibody

	Rh							Kell			Duffy			Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	V	C^w	K	K^p	K^{ea}	Js^a	Fy	Fy^a	Fy^b	Jk	Jk^b	Le	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg	PEG IAT
1	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	2+
2	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	3+
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	2+
4	/	0	0	/	/	/	0	0	/	0	/	0	/	/	/	0	/	0	/	0	/	0	/	0	/	/	/	0
5	0	+	0	+	/	0	0	0	/	0	/	0	/	0	/	0	/	0	0	+	0	+	0	0	0	/	/	0
6	0	0	+	+	+	0	0	+	0	+	0	+	/	0	+	/	+	0	+	+	0	+	0	+	0	+	+	2+
7	0	0	0	/	/	0	0	/	0	/	0	/	0	/	/	0	0	/	0	0	/	0	+	+	0	/	/	0
8	0	0	0	/	/	0	0	0	/	0	/	0	+	+	/	0	0	/	0	0	/	+	+	0	/	0	0	0
9	0	0	0	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	3+
10	/	/	0	0	/	0	0	0	/	0	/	0	/	/	0	/	0	/	0	0	/	+	+	/	0	0	/	/	0
11	+	0	0	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	2+
Auto																											0

Pattern of reactivity = Matches anti-Jk^b perfectly

Next steps = Finish rule outs

- Pattern matches anti-Jk^b (2+2 rule for ruling in
- All other antibodies to common antigens rule
  - Anti-C, anti-K, anti-M, anti-S, anti-s only r
  - Anti-E not ruled out even once
    - Don't have to rule out antibodies to low prevalence antigens (anti-C^w, -Kp^a, -Js^a, -Lu^a)

Test selected cells to complete rule outs: Jk(b-) cells, positive for these antigens

	Rh							Kell						Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	V	Cʷ	K	k	Kpᵃ	Kpᵇ	Jsᵃ	Jsᵇ	Fyᵃ	Fyᵇ	Jkᵃ	Jkᵇ	Leᵃ	Leᵇ	P1	M	N	S	s	Luᵃ	Luᵇ	Xgᵃ	PEG IAT
1	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	0	+	+	2+
2	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	3+
3	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	+	0	2+
4	/	0	0	/	/	/	0	0	/	0	/	0	/	0	/	/	0	/	0	/	0	/	0	/	0	/	/	0
5	0	+	0	+	/	0	0	0	/	0	/	0	/	/	0	/	0	/	0	0	+	0	0	0	0	/	/	0
6	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	2+
7	0	0	0	/	/	0	0	/	/	0	/	0	/	0	/	/	0	/	0	/	0	+	+	0	/	/	0
8	0	0	0	/	/	0	0	0	/	0	/	0	/	+	+	/	0	0	/	0	0	/	+	+	0	/	0	0
9	0	0	0	+	+	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	3+
10	/	/	0	0	/	0	0	0	/	0	/	0	/	/	0	/	0	0	/	/	+	+	/	0	0	/	/	0
11	+	0	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	2+
Auto																												0

Next steps = Finish rule outs

- Anti-Jk^b is present
- Need more rule outs for anti-C, anti-E (2x), anti-K anti-M, anti-S & anti-s
  - Jk(b-) cells

We are expecting this reaction to be positive

Additional selected cells	Rh							Kell						Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	f	V	Cʷ	K	k	Kpᵃ	Kpᵇ	Jsᵃ	Jsᵇ	Fyᵃ	Fyᵇ	Jkᵃ	Jkᵇ	Leᵃ	Leᵇ	P1	M	N	S	s	Luᵃ	Luᵇ	Xgᵃ	PEG IAT
1	+	+	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	
3	+	0	+	+	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	+	+	+	+	+	0	+	+	

Cell #2= will rule out anti-C

Cell #2 = will rule out anti-K

Cell #3 = will rule out anti-M

Cells #2 & #3 = will rule out anti-s/anti-S

Cells #3 & #4 = will rule out anti-E (2x)

Next steps = Finish rule outs

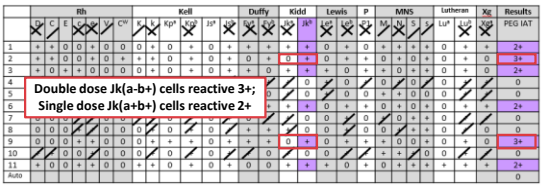
- Suspect anti-Jkb is present
- Need more rule outs for anti-C, anti-E (2x), anti-M, anti-S & anti-s
  - Jk(b-) cells

Additional selected cells	Rh							Kell						Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	f	V	Cʷ	K	k	Kpᵃ	Kpᵇ	Jsᵃ	Jsᵇ	Fyᵃ	Fyᵇ	Jkᵃ	Jkᵇ	Leᵃ	Leᵇ	P1	M	N	S	s	Luᵃ	Luᵇ	Xgᵃ	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	+	3+
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	+	0	0
4	+	0	/	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	+	+	0	+	+	0

Now all rule outs complete

Finishing a workup (Practicing rule outs #1)

- Antibody identified: anti-Jk^b
  - **2 + 2 rule**: 2 Jk(b+) cells reactive, 2 Jk(b-) cells nonreactive
  - All other antibodies to common RBC antigens ruled out x2
- Anti-Jk^b is showing dosage!



Further testing?

- Phenotype patient's RBCs
- Expect patient's cells to be Jk(b-)

	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
Patient RBCs	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+

Transfusion Recommendation?

- Anti-Jk^b clinically significant
- Transfuse
  - Jk(b-) donor units
  - Full IAT crossmatch
  - Requirement for the rest of patient's life

Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	3+
3	+	0	+	+	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	+	+	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	2+
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	0
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	0	+	+	+	0	+	0	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	0	0	+	+	3+
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	0
Auto																													0

Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	3+
3	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	+	0	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	0	+	+	+	0	+	0	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	0	0	+	+	3+
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	0
Auto																													0

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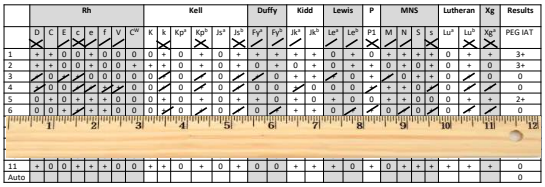
	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	3+
3	+	0	+	+	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	+	+	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	0	0	+	+	3+
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	0
Auto																													0

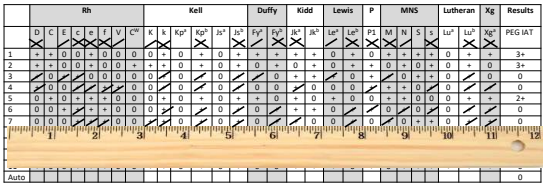
Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose



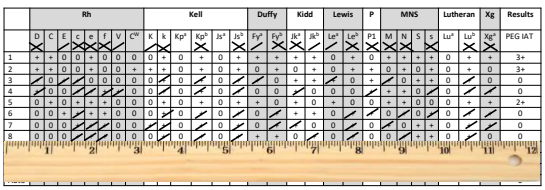
Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose



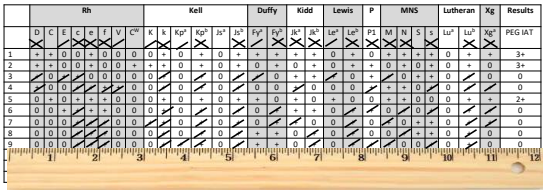
Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose



Practicing rule outs #2

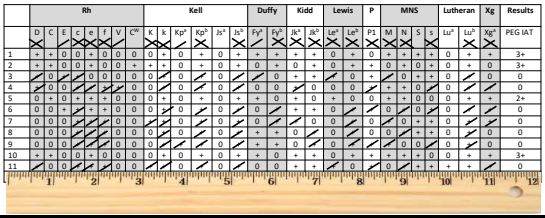
- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose



	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	0	3+
3	/	0	/	+	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	/	/	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	2+
6	0	0	+	/	+	+	0	0	0	/	0	/	0	/	0	/	+	+	0	/	/	0	/	0	/	0	/	/	0
7	0	0	0	/	/	/	0	0	/	/	0	/	0	/	0	/	/	0	0	/	0	/	0	+	+	0	/	/	0
8	0	0	0	/	/	/	0	0	/	/	0	/	0	/	+	+	0	/	0	/	0	/	+	+	0	/	/	0	0
9	0	0	0	/	/	/	0	0	/	/	0	/	0	/	+	+	0	/	0	/	/	/	0	0	/	0	/	0	0

Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE cells
    - Exception: rule out Kell antibodies with single dose



	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	0	3+
3	/	0	/	+	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	/	/	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	2+
6	0	0	+	/	+	+	0	0	0	/	0	/	0	/	0	/	+	+	0	/	/	0	/	0	/	0	/	/	0
7	0	0	0	/	/	/	0	0	/	/	0	/	0	/	0	/	/	0	0	/	0	/	0	+	+	0	/	/	0
8	0	0	0	/	/	/	0	0	/	/	0	/	0	/	+	+	0	/	0	/	0	/	+	+	0	/	/	0	0
9	0	0	0	/	/	/	0	0	/	/	0	/	0	/	+	+	0	/	0	/	/	/	0	0	/	0	/	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	+	3+
11	/	0	0	/	/	/	0	0	/	/	0	/	0	/	0	+	+	/	0	/	/	/	0	/	+	+	/	/	0

Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are NONREACTIVE with the patient's plasma
  - When possible, rule out specificities using DOUBLE DOSE
    - Exception: rule out Kell antibodies with single dose

Antibody specificities we haven't ruled out, even once

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	0	3+
3	/	0	/	+	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	/	/	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	2+
6	0	0	+	/	+	+	0	0	0	/	0	/	0	/	0	/	+	+	0	/	/	0	/	0	/	0	/	/	0
7	0	0	0	/	/	/	0	0	/	/	0	/	0	/	0	/	/	0	0	/	0	/	0	+	+	0	/	/	0
8	0	0	0	/	/	/	0	0	/	/	0	/	0	/	+	+	0	/	0	/	0	/	+	+	0	/	/	0	0
9	0	0	0	/	/	/	0	0	/	/	0	/	0	/	+	+	0	/	0	/	/	/	0	0	/	0	/	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	+	3+
11	/	0	0	/	/	/	0	0	/	/	0	/	0	/	0	+	+	/	0	/	/	/	0	/	+	+	/	/	0
Auto																												0

Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are **NONREACTIVE** with the patient's plasma
  - When possible, rule out specificities using **DOUBLE DOSE**
    - Exception: rule out Kell antibodies with single dose

These are low prevalence antigens: won't rule out antibody

	Rh							Kell			Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	0	+	0	3+
3	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	2+
6	0	0	+	0	+	+	0	0	0	0	0	0	0	0	0	+	0	+	0	0	+	0	+	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	+	+	0	0	0	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	+	3+
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	+	+	+	0	+	0	0
Auto																												0

Antibody specificities not rule out: anti-C & anti-S

Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are **NONREACTIVE** with the patient's plasma
  - When possible, rule out specificities using **DOUBLE DOSE**
    - Exception: rule out Kell antibodies with single dose

These are low prevalence antigens: won't rule out antibody

	Rh							Kell			Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	0	+	0	3+
3	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	2+
6	0	0	+	0	+	+	0	0	0	0	0	0	0	0	0	+	0	+	0	0	+	0	+	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	+	+	0	0	0	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	+	3+
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	+	+	+	0	+	0	0
Auto																												0

Pattern of reactivity = anti-C

Practicing rule outs #2

- Rule out specificities corresponding to antigens present on reagent RBCs that are **NONREACTIVE** with the patient's plasma
  - When possible, rule out specificities using **DOUBLE DOSE**
    - Exception: rule out Kell antibodies with single dose

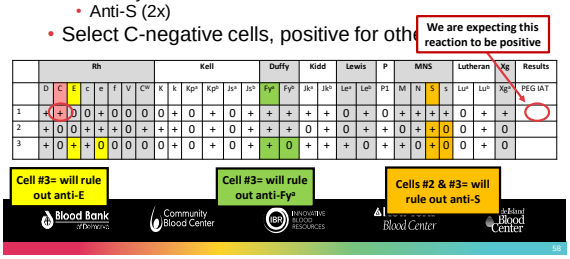
These are low prevalence antigens: won't rule out antibody

	Rh							Kell			Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	PEG IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	0	+	+	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	0	+	0	3+
3	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	2+
6	0	0	+	0	+	+	0	0	0	0	0	0	0	0	0	+	0	+	0	0	+	0	+	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	+	+	0	0	0	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	+	3+
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	+	+	+	0	+	0	0
Auto																												0

Rule outs not complete for: Anti-E, Anti-Fy^a & anti-S (2x)

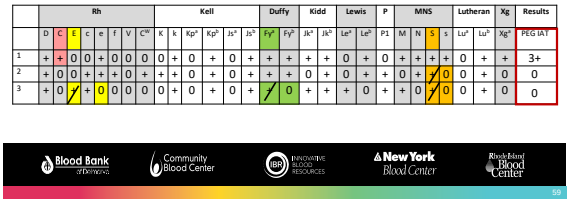
Selected cells: finishing rule outs

- Anti-C identified (2+2 rule)
- Haven't ruled out
  - Anti-E
  - Anti-Fy^a
  - Anti-S (2x)
- Select C-negative cells, positive for oth



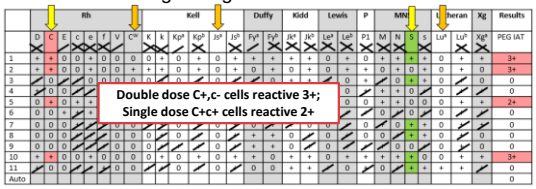
Selected cells: finishing rule outs

- Suspect anti-C
- Haven't ruled out
  - Anti-E
  - Anti-Fy^a
  - Anti-S (2x)
- Select C-negative cells, positive for other antigens



Finishing a workup (Practicing rule outs #2)

- Antibody identified: anti-C
  - **2 + 2 rule**: 2 C+ cells reactive, 2 C- cells nonreactive
  - All other antibodies to common RBC antigens ruled out x2
- Anti-C is showing dosage!



Further testing?

- Phenotype patient's cells
  - Expect patient cells to be C-negative

	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
Patient RBCs	+	0	+	+	+	0	+	+	+	+	0	+	+	+	+

Transfusion Recommendations?

- Anti-C clinically significant
- Transfuse
  - C-negative donor units
  - Full IAT crossmatch
  - Requirement for the rest of patient's life

Objectives

- Describe the testing performed to detect and identify "unexpected antibodies."
- Discuss how the following results provide clues during the antibody investigation:
  - Phase of reactivity
  - Variability of reactivity
  - Autocontrol result
  - Patient phenotype
  - Use of enzyme-treated reagent RBCs
- Perform rule outs and interpret results of antibody panels.
- In case studies, given testing results, identify antibodies and make appropriate transfusion recommendations.

Case 1

- 50 year-old female
  - Lower GI bleeding
  - Current Hgb: 6.5 g/dL
- Transfusion history:
  - Received 2 units of RBCs 6 months ago: hysterectomy
  - Antibody Screen negative at that time
- Order to transfuse 3 units of RBCs
  - Type: Group O, Rh positive
  - Antibody Screen: Negative at 37C & IAT

	Rh									Kell						Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results	
	D	C	E	c	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	LISS 37C	LISS IAT
SC I	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	0
SC II	+	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	0
SC III	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	0	0	0

Crossmatches (immediate spin)			
	Unit #1	Unit #2	Unit #3
Patient plasma	1+	2+	1+

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Case 1

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  - Lower GI bleeding
  - Current Hgb: 6.5 g/dL
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  - Received 2 units of RBCs 6 months ago: hy
  - Antibody Screen negative at that time
- Order to transfuse 3 units of RBCs
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  - Antibody Screen: Negative at 37C & IAT

Notice that the crossmatches tested at ***different phase*** than antibody screen

	Rh									Kell						Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results	
	D	C	E	c	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	LISS 37C	LISS IAT
SC I	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	0
SC II	+	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	0
SC III	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	0	0	0

Crossmatches (immediate spin)			
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Antibody Panel: Case 1

- Test at phase where reactivity is detected
  - Screen negative (37C & IAT)
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	Rh									Kell						Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
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1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	1+	0	0
2	+	+	0	0	+	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	0	0	0
4	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	0	+	0	+	1+	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	0	+	+	0	0	0	+	+	1+	0	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	0	0	0
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	2+	0	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	0	0	0	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+	+	0	0	+	0	+	0	2+	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	+	0	0	+	+	1+	0	0
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	0	0
Auto																													0	0	0

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Antibody Panel: Case 1

- Test at phase where reactivity is detected
  - Screen negative (37C & IAT)
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Similar reactivity to antibody screen & crossmatches: reactivity at IS, no reactivity at 37C/IAT

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
	D	C	E	c	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	IS	LISS 37C	LISS IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	1+	0	0
2	+	+	0	0	+	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	0	0
4	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+	0	+	+	1+	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	1+	0	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	+	2+	0	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0	+	+	+	0	+	0	0	0	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	2+	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	+	1+	0	0
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	0	0	0
Auto																													0	0	0

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Antibody Panel: Case 1

- Test at phase where reactivity is detected
  - Screen negative (37C & IAT)
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Similar reactivity to antibody screen & crossmatches: reactivity at IS, no reactivity at 37C/IAT

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
	D	C	E	c	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	IS	LISS 37C	LISS IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	1+	0	0
2	+	+	0	0	+	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	0	0
4	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+	0	+	+	1+	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	1+	0	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	+	2+	0	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0	+	+	+	0	+	0	0	0	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	2+	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	+	1+	0	0
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	0	0	0
Auto																													0	0	0

If you did rule outs at IAT phase, all antibodies to common RBC antigens would be ruled out!

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Antibody Panel: Case 1

- Test at phase where reactivity is dete
  - Screen negative (37C & IAT)
  - Crossmatches positive (IS)

Which antibodies tend to be ***cold-reacting?*** (usually NOT clinically significant)

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
	D	C	E	c	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	IS	LISS 37C	LISS IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	1+	0	0
2	+	+	0	0	+	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	0	0
4	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+	0	+	+	1+	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	1+	0	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	+	2+	0	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0	+	+	+	0	+	0	0	0	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	2+	0	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	+	1+	0	0
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	0	0	0
Auto																													0	0	0

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Antibody Panel: Case 1

- Test at phase where reactivity is det
  - Screen negative (37C & IAT)
  - Crossmatches positive (IS)

Which antibodies tend to be ***cold-reacting?*** (usually NOT clinically significant)

	Rh								Kell				Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
	D	C	E	c	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	IS	LISS 37C	LISS IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	1+	0	0
2	+	+	0	0	+	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	0	0
4	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+	0	+	+	1+	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	1+	0	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	+	2+	0	0
8	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	0	0	0
9	0	0	0	+	+	+	0	0	+	+	+	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	2+	0	0
10	+	+	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	+	0	+	+	1+	0	0
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	0	0	0
Auto																													0	0	0

Does the pattern of reactivity match one of these cold-reacting antibodies?

Antibody Panel: Case 1

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  - Screen negative (37C & IAT)
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Which antibodies tend to be ***cold-reacting?*** (usually NOT clinically significant)

	Rh								Kell				Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
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2	+	+	0	0	+	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	0	0
4	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+	0	+	+	1+	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	1+	0	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	+	2+	0	0
8	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	0	0	0
9	0	0	0	+	+	+	0	0	+	+	+	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	2+	0	0
10	+	+	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	+	0	+	+	1+	0	0
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	0	0	0
Auto																													0	0	0

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1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	1+	0	0
2	+	+	0	0	+	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	0	0
4	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+	0	+	+	1+	0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	1+	0	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	+	2+	0	0
8	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	0	0	0
9	0	0	0	+	+	+	0	0	+	+	+	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	2+	0	0
10	+	+	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	+	0	+	+	1+	0	0
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	0	0	0
Auto																													0	0	0

Is anti-M identified by *2+2 rule?*

Yes!
≥2 M+ cells reactive
≥2 M- cells nonreactive

Further testing: Case 1

- Test patient's cells
- Expect patient cells to be M-negative

	Anti-M
Patient cells	0

- Do our results make sense?
  - Anti-M often "cold reacting"
    - Reactivity at IS, no reactivity at 37C/IAT
    - Incompatible XMs – tested at IS
      - ~78% of donors M+
      - All incompatible units were probably M+

Transfusion Recommendation?

- Anti-M detected, not reactive at 37C/IAT
- NOT clinically significant
  - Doesn't react at 37C
- Typed and labeled M-negative units NOT required
- XM compatible units

Case 1 Highlights:
- Pay attention to PHASE of reactivity
- "Cold-reacting" anti-M not usually clinically significant

Case 2

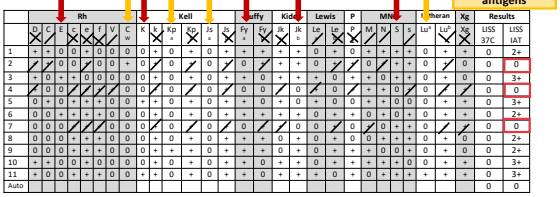
- 60 year-old male
- Diagnosis: leukemia
- Hgb: 6.0 g/dL
- Transfusion history:
  - Transfused 4 months ago, 2 units RBCs
  - Antibody screen negative at the time
- 2 units for transfusion ordered
  - Type: Group A, Rh positive
  - Screen: positive at IAT with 2/3 screening cells

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results	
	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	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	LISS 37C	LISS IAT
SC I	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	2+
SC II	+	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	0
SC III	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	0	0	3+

Antibody Panel: Cas

Antibodies to common RBC antigens that haven't been ruled out:

Low prevalence antigens



	Rh							K	k	Kp	Kp	Js	Js	Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results	
	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	M	N	S	s	Lu a	Lu b	Xg	LISS 37C	LISS IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	2+
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	3+
4	/	0	0	/	/	/	/	0	0	/	0	/	0	/	0	0	/	0	/	0	/	+	+	0	/	0	/	/	0	0
5	0	+	0	+	+	+	0	0	+	+	0	+	+	+	0	+	+	0	+	0	0	+	+	0	0	0	+	+	0	3+
6	0	0	+	+	+	+	0	0	0	+	0	+	+	+	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	2+
7	0	0	0	/	/	/	0	0	0	/	0	/	0	/	0	/	/	0	0	/	0	/	0	+	+	0	/	/	0	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	0	+	+	0	+	0	0	2+
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	+	0	+	0	0	+	0	0	2+
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	0	0	+	+	0	0	3+
11	+	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	3+
Auto																													0	0

Another Useful Tool:

We haven't ruled out:

- anti-E
- anti-K
- anti-Fy^a
- anti-Jk^b
- ~~anti-S~~

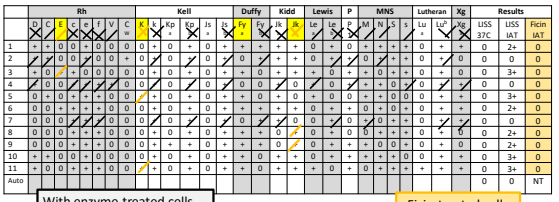
Patient phenotype:

Patient wouldn't be expected to make anti-S

	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
Patient RBCs	+	0	0	+	+	0	+	0	+	+	0	+	+	+	+

Case 2: Testing enzyme-treated cells

- **Ficin:** Destroys some antigens (Fy^a, Fy^b, M, N, S, s (variable), others)
  Enhances some antibodies (Rh, Kidd, others)



	Rh							K	k	Kp	Kp	Js	Js	Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
	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	M	N	S	s	Lu a	Lu b	Xg	LISS 37C	LISS IAT	Ficin IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	2+	0
2	/	/	0	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	3+	0
4	/	0	0	/	/	/	/	0	0	/	0	/	0	/	0	0	/	0	/	0	/	+	+	0	/	0	/	/	0	0	0
5	0	+	0	+	+	+	0	0	+	+	0	+	+	+	0	+	+	0	+	0	0	+	+	0	0	0	+	+	0	3+	0
6	0	0	+	+	+	+	0	0	0	+	0	+	+	+	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	2+	0
7	0	0	0	/	/	/	0	0	0	/	0	/	0	/	0	/	/	0	0	/	0	/	0	+	+	0	/	/	0	0	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	/	0	+	0	0	+	0	+	+	0	+	0	0	2+	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	+	0	+	0	0	+	0	0	2+	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	0	0	+	+	0	0	3+	0
11	+	0	0	+	+	+	0	0	/	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	3+	0
Auto																													0	0	NT

With enzyme-treated cells, can't rule out anti-Fy^a, -Fy^b, -M, -N, -S, -s

Ficin-treated cells all nonreactive

Pattern?

- Reactivity fits anti-Fy^a
- Reactivity disappears with ficin-treated RBCs
- Patient cells test Fy(a-)

How will we finish ruleouts? Test more Fy(a-) cells

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results	
	D	C	E	c	e	f	V	Cw	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	LISS 37C	LISS IAT	Ficin IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	2+	0
2	/	/	0	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	3+	0
4	/	0	0	/	/	/	0	0	/	0	/	0	/	0	0	/	0	/	0	/	/	+	+	0	/	0	/	0	0	0
5	0	+	0	+	+	+	0	0	/	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	0	+	+	0	3+	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	0	+	+	0	2+	0
7	0	0	0	/	/	/	0	0	/	0	/	0	/	0	/	/	0	0	/	0	/	0	/	+	0	/	/	0	0	0
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	/	0	+	0	0	+	+	+	0	+	0	0	2+	0
9	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	0	/	0	+	+	+	0	0	+	0	+	0	0	2+	0
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	0	0	+	+	0	3+	0
11	+	0	0	+	+	+	0	0	/	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	+	0	3+	0
Auto																												0	0	NT

Conclusion of Case 2

- Anti-Fy^a detected in plasma
- Anti-Fy^a is clinically significant
- Transfusion recommendation:
  - Fy(a-) donor units
  - Full IAT crossmatch
  - Requirement for the rest of the patient's life

Case 2 Highlights:
- Use of enzyme-treated cells (destroys some antigens, enhances some antibodies)
- Use of patient full phenotype to determine what antibodies patient can make

Case 3

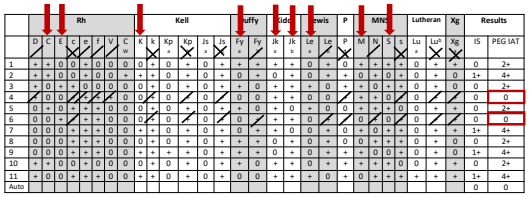
- 47 year-old female
- Diagnosis: breast cancer, pre-surgery
- Transfusion history: 4 units RBCs 1 year ago due to bleeding during a total abdominal hysterectomy
  - Antibody screen negative at that time
- Today's results:
  - Type: Group B, Rh positive
  - Screen: Positive 2/3 cells

Notice:
- Phases of reactivity
- Variable reactivity strength

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results	
	D	C	E	c	e	f	V	Cw	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	IS	PEG IAT
SC I	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	2+
SC II	+	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	1+	4+
SC III	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	0	+	0	+	0	0	0

Antibody Panel: Case 3

Common specificities that can't be ruled out



	Rh							Kell				Duffy		Kidd		Lewis		P	MNS			Lutheran		Xg	Results	
	D	C	E	c	e	f	V	Cw	K	k	Kp	Js	Js	Fy	Fy	Jk	Jk	Le	Le	P	M	N	S	s	Lu	Lu	Xg	IS	PEG IAT
	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	+	+	0	2+	
1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	1+	4+	
2	+	0	+	0	0	0	0	0	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	+	0	0	2+		
3	0	0	+	0	+	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	+	0	+	0	2+	
5	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0		0
6	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0	2+	
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	1+	4+	
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	2+	
9	0	0	0	+	+	+	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	1+	4+	
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	0	+	0	2+	
11	+	0	0	+	+	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	+	0	+	+	+	+	1+	4+	
Auto																											0	0	

Patient phenotype: Case 3

	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
Patient RBCs	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+

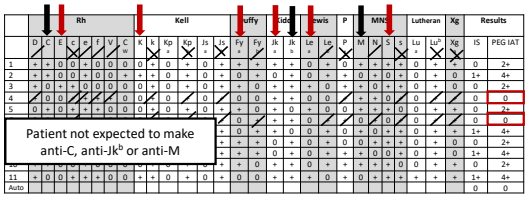
What antibodies can the patient make?

Anti-E, Anti-c, Anti-K, Anti-Fy^a, Anti-Jk^a & Anti-S

Antibody Panel: Case 3

What antibodies can the patient make?

Anti-E, Anti-c, Anti-K, Anti-Fy^a, Anti-Jk^a & Anti-S

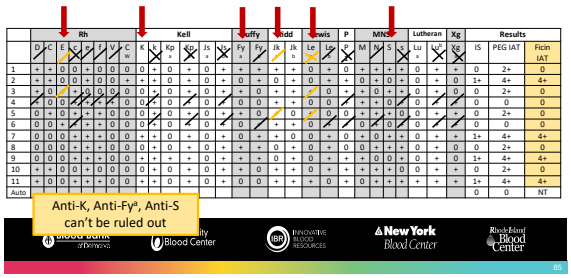


	Rh							Kell				Duffy		Kidd		Lewis		P	MNS			Lutheran		Xg	Results	
	D	C	E	c	e	f	V	Cw	K	k	Kp	Js	Js	Fy	Fy	Jk	Jk	Le	Le	P	M	N	S	s	Lu	Lu	Xg	IS	PEG IAT
	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	+	+	0	2+	
1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	1+	4+	
2	+	0	+	0	0	0	0	0	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	+	0	0	2+		
3	0	0	+	+	0	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	+	0	+	0	2+	
5	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0		0
6	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0	2+	
7	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	1+	4+	
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	2+	
9	0	0	0	+	+	+	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	1+	4+	
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	0	+	0	2+	
11	+	0	0	+	+	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	+	0	+	+	+	+	1+	4+	
Auto																											0	0	

Patient not expected to make anti-C, anti-Jk^b or anti-M

Case 3: Testing enzyme-treated cells

- More negative cells, more rule outs
- Can't rule out specificities that are destroyed by ficin



Case 3: Testing enzyme-treated cells

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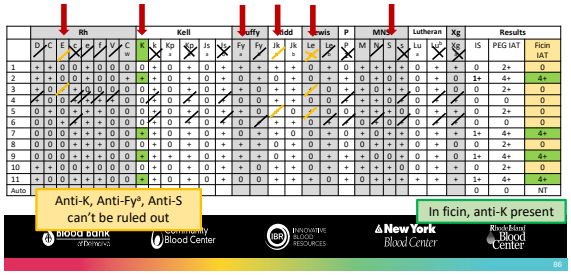

Case 3: Testing enzyme-treated cells

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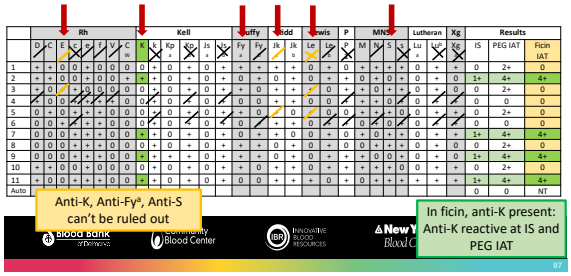
- Can't rule out specificities that are destroyed by ficin



Selected cells: Case 3

- K+, Fy(a-), S-
- K-, Fy(a+), S-
- K-, Fy(a-), S+

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	f	V	Cʷ	K	k	Kpᵃ	Kpᵇ	Jsᵃ	Jsᵇ	Fyᵃ	Fyᵇ	Jkᵃ	Jkᵇ	Leᵃ	Leᵇ	P1	M	N	S	s	Luᵃ	Luᵇ	Xgᵃ	PEG IAT
1	+	+	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	
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	0	+	+	+	0	0	+	+	
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	0	+	+	

Selected cells: Case 3

- K+, Fy(a-), S-
- K-, Fy(a+), S-
- K-, Fy(a-), S+

	Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results
	D	C	E	c	e	f	V	Cʷ	K	k	Kpᵃ	Kpᵇ	Jsᵃ	Jsᵇ	Fyᵃ	Fyᵇ	Jkᵃ	Jkᵇ	Leᵃ	Leᵇ	P1	M	N	S	s	Luᵃ	Luᵇ	Xgᵃ	PEG IAT
1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	+	+	0	+	0	+	+	0	+	0	+	+	4+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	4+
3	+	0	+	+	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	+	+	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	0	+	+	+	0	0	+	+	2+
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	0	+	+	2+

Back to Initial Panel: Case 3

Anti-K reactive at IS??
Doesn't immediate spin reactivity mean NOT clinically significant?
Newly forming antibodies often IgM

- Anti-K reactive at IS, PEG IAT, Ficin IAT
- Anti-S reactive at PEG IAT

	Rh								Kell						Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results		
	D	C	E	c	e	f	V	Cʷ	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	IS	PEG IAT	Ficin IAT
1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	2+		0
2	+	+	0	0	+	0	0	+	w	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	1+		4+	4+
3	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	0	0		2+	0
4	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	+	+	0	+	0	+	+	0		0	0
5	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	0	0	+	+	0		2+	0
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	0	+	+	0		2+	0
7	0	0	0	+	+	+	0	0	w	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	0	+	+	1+		4+	4+
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0	+	+	+	0	+	0	0		2+	0
9	0	0	0	+	+	+	0	0	w	+	+	+	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	1+		4+	4+
10	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	0	+	+	0		2+	0
11	+	0	0	+	+	+	0	0	w	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+	+	1+		4+	4+
Auto																													0		NT	

Conclusion of Case 3

- Anti-K and anti-S
  - Clinically significant
- Transfusion recommendations:
  - K-negative, S-negative donor units
  - Full IAT crossmatch
  - Requirements for the rest of the patient's life

Case 3 Highlights:
- Multiple antibodies, reactive at different strengths, different phases
- Use of enzyme-treated cells
- Use of patient full phenotype to determine what antibodies patient can make

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Review of helpful tools for antibody ID

- Rule outs!
  - Rule out specificities that are present (double dose) on reagent cells that are **nonreactive** with patient plasma
- Phases of testing
  - IS, IAT, enzymes
- Strength of reactivity
  - Variable reactivity can be dosage or multiple antibodies
- Patient phenotype
  - Which antibodies can patient make?

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Additional clues…

If reactivity is…	Suspect…
• Some cells positive, some cells negative • Reactivity same strength and phase of reactivity	Single antibody
• All or some cells positive • Different strengths • Different phases of reactivity	Multiple antibodies
• One cell positive • All specificities ruled out	Antibody to a low prevalence antigen
• All cells positive except autocontrol • Same strength and phase of reactivity	Antibody to a high prevalence antigen

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Objectives

- Describe the testing performed to detect and identify "unexpected antibodies."
- Discuss how the following results provide clues during the antibody investigation:
  - Phase of reactivity
  - Variability of reactivity
  - Autocontrol result
  - Patient phenotype
  - Use of enzyme-treated reagent RBCs
- Perform rule outs and interpret results of antibody panels.
- In case studies, given testing results, identify antibodies and make appropriate transfusion recommendations.

What's next?

- Perform antibody screens/antibody panels in lab
- Practice interpreting "dry panels" in handbook (green sheets)