

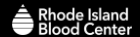
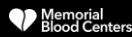
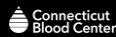


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## A..B..C., Easy as 1..2..3..: A Sickle Cell Patient With Multiple Antibodies

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## Objectives

- Describe the tests used to resolve the most complex serologic workups
- Interpret a variety of test results in order to direct the course of a workup and identify complex antibodies
- Select appropriate units for transfusion



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# The Patient

- **Patient Information:**
  - 38 year old
  - African American
  - Female
- **Diagnosis:**
  - Anemia, Sickle Cell Anemia, Chronic Kidney Disease
- **History:**
  - Anti-E
  - Cold autoantibody
  - Warm autoantibody
  - HLA antibody
- **Hospital Reports:**
  - Positive DAT
  - Antibody Screen: 1-2+ reactivity in all cells



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ABO/Rh						
ABO Group					Rh Type	
	Anti-A	Anti-B	A <sub>1</sub> Cells	B Cells	Anti-D	Control
IS	0	0	4+	4+	3+	0

Direct Antiglobulin Test			
Poly	IgG	C'	Saline
1+	1+	(0) <sup>v</sup>	(0)

- IRL types patient as O Rh positive.
- DAT is positive.

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## Antibody Panel

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results		
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	5' RT	N-HANCE 37C	N-HANCE IAT
1	+	+	0	0	+	+	0	+	W	+	+	0	0	+	0	0	+	0	0	2+ <sup>S</sup>
2	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	(+)
3	0	+	0	+	+	0	+	+	0	+	+	+	0	+	0	+	0	0	0	2+ <sup>S</sup>
4	0	0	0	+	+	0	+	+	+	+	0	+	+	+	0	+	0	0	0	1+ <sup>S</sup>
5	+	0	0	+	+	0	+	0	0	0	+	0	+	+	+	0	+	0	0	(+)
6	+	+	0	0	+	+	+	+	0	+	+	+	0	+	+	0	+	0	0	1+ <sup>S</sup>
7	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+	0	0	0	1+ <sup>S</sup>
8	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	0	0	(+)
9	+	0	0	+	+	0	+	0	0	+	0	0	+	+	+	0	+	0	0	(0) <sup>v</sup>

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## STOP...Check In

- Does it match the history?
- What could it be?
- What next?



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## Acid Eluate

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Eluate
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT
1	+	+	0	0	+	+	0	+	W	+	+	0	0	+	0	0	+	(+)
2	+	0	+	+	0	0	+	+	+	+	0	0	+	0	+	+	+	(+)
3	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	0	+	(+)
4	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) <sup>v</sup>
7	+	+	0	0	+	+	+	+	0	+	+	+	0	+	+	0	+	1+w
8	+	0	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	1+w
9	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+	0	1+w
10	0	0	0	+	+	+	+	0	+	+	0	0	+	0	+	+	+	(+)
11	+	0	0	+	+	0	+	0	0	+	0	0	+	+	+	0	+	(0) <sup>v</sup>

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## STOP...Check In Again

- Does it match the history?
- What could it be?
- What next?



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Blood Group	Antigen	Results	Comments
Rh	c	+	
	C	0	
	e	+	
	E	0	
	V	+	
	VS	+	
Kell	K	0	
	k	+	
	Kpa	0	
	Kpb	+	
	Jsa	+	
	Jsb	+	
Duffy	Fya	0	
	Fyb	(0)*	Not at risk for anti-Fy <sup>b</sup>
Kidd	Jka	+	
	Jkb	0	
MNS	M	+	
	N	+	
	S	0	
	s	+	
	U	+	
Lutheran	Lua	0	
	Lub	+	
Diego	Dia	0	
	Dib	+	
Colton	Coa	+	
	Cob	0	
Dombrock	Doa	0	
	Dob	+	
	Hy	+	
	Jo	+	
Landsteiner-Wiener	LW <sub>a</sub>	+	
	LW <sub>b</sub>	0	
Scianna	Sc1	+	
	Sc2	0	

## Human Erythrocyte Antigen (HEA) Phenotype by DNA Analysis Report

- Sample contains GATA mutation resulting in loss of Fy<sup>b</sup> expression on RBCs
  - Individuals not expected to make anti-Fy<sup>b</sup>
- Patient is positive for common high incidence antigens
  - Examples: anti-Js<sup>b</sup> and anti-U

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## Acid Eluate

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Eluate
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	
1	+	+	0	0	+	+	0	+	W	+	+	0	0	+	0	0	+	(+)
2	+	0	+	+	0	0	+	+	+	+	0	0	+	0	+	+	+	(+)
3	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	0	+	(+)
4	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) <sup>v</sup>
7	+	+	0	0	+	+	+	+	0	+	+	+	0	+	+	0	+	1+W
8	+	0	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	1+W
9	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+	0	1+W
10	0	0	0	+	+	+	+	0	+	+	0	0	+	0	+	+	+	(+)
11	+	0	0	+	+	0	+	0	0	+	0	0	+	+	+	0	+	(0) <sup>v</sup>

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## Antibody Panel

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results		
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	5' RT	N-HANCE 37C	N-HANCE IAT
1	+	+	0	0	+	+	0	+	W	+	+	0	0	+	0	0	+	0	0	2+ <sup>S</sup>
2	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	(+)
3	0	+	0	+	+	0	+	+	0	+	+	+	0	+	0	+	0	0	0	2+ <sup>S</sup>
4	0	0	0	+	+	0	+	+	+	+	0	+	+	+	0	+	0	0	0	1+ <sup>S</sup>
5	+	0	0	+	+	0	+	0	0	0	+	0	+	+	+	0	+	0	0	(+)
6	+	+	0	0	+	+	+	+	0	+	+	+	0	+	+	0	+	0	0	1+ <sup>S</sup>
7	0	+	0	+	+	0	+	+	0	0	+	+	+	0	+	0	0	0	0	1+ <sup>S</sup>
8	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	0	0	(+)
9	+	0	0	+	+	0	+	0	0	+	0	0	+	+	+	0	+	0	0	(0) <sup>v</sup>

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## What Next?

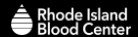
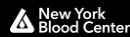
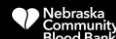
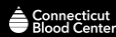
- Warm Autoantibody
- Cold Autoantibody
- High Incidence Antibody
- Multiple Antibodies



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## What Do We Know

- Have a genotype already
  - List of potential antibodies the patient can form
- Patient already has an anti-E
- Antigens to consider:
  - C, K, Fy<sup>a</sup>, Jk<sup>b</sup>, S



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## Selected Cell Panel

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results	Eluate Results
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT	PEG IAT
1	+	+	0	0	+	0	+	0	0	+	0	0	0	+	0	0	+	(0) <sup>v</sup>	(0) <sup>v</sup>
2	+	0	0	+	+	0	+	0	0	+	0	0	+	+	0	+	0	(0) <sup>v</sup>	(0) <sup>v</sup>
3	0	0	0	+	+	0	+	+	0	+	0	0	0	0	+	0	+	2+ <sup>s</sup>	1+
4	0	0	0	+	+	0	+	0	0	0	+	+	0	+	0	0	+	1+ <sup>w</sup>	(0) <sup>v</sup>
5	+	0	+	+	0	0	+	0	0	+	0	+	0	+	0	0	+	Not Tested	(0) <sup>v</sup>
6	+	0	0	+	+	+	+	0	0	+	0	0	+	+	0	0	+	(+)	(0) <sup>v</sup>
7	0	0	0	+	+	0	+	0	+	+	0	0	+	+	0	0	+	2+	1+
8	+	0	0	+	+	0	+	0	0	+	0	0	0	+	0	0	+	(0) <sup>v</sup>	(0) <sup>v</sup>

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## What Do We Know



- Eluate
  - Reactivity with:
    - Fy(a+b-) and Fy(a-b+) cells
- Plasma
  - Reactivity with:
    - Fy(a+b-) and Fy(a-b+) cells
    - Jk(a-b+) cells
    - K+k+ cells

## Next Steps..

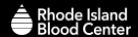
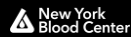
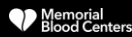
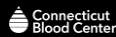


- Plasma Testing
  - Rule in anti-Jk<sup>b</sup>
  - Rule in anti-K
  - Determine what antibody in the Duffy blood group system is causing reactivity
    - Anti-Fy<sup>a</sup>, Anti-Fy<sup>b</sup>, Anti-Fy<sup>3</sup>, Anti-Fy<sup>5</sup>
  - Rule out all other clinically significant antibodies
    - Anti-C and anti-S

## Rule-In and Out

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT
1	+	0	0	+	+	0	+	0	0	+	0	0	0	+	+	+	0	(0) <sup>v</sup>
2	+	+	0	0	+	0	+	0	0	+	0	0	0	+	0	0	+	(0) <sup>v</sup>
3	+	0	0	+	+	0	+	0	0	0	+	0	0	+	0	0	+	2+
4	+	0	0	+	+	0	+	0	0	+	0	0	+	+	0	0	+	(0) <sup>v</sup>
5	+	0	0	+	+	0	+	0	0	+	0	+	0	+	0	0	0	(0) <sup>v</sup>

- No K+k+ cells available that are E-negative, Fy(a-b-), Jk(b-)



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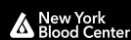
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## Enzyme Treatment

- Ficin treated RBCs tested with patient's plasma

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma		
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT	Ficin	
																			30'37C	IAT
1	0	0	0	+	+	0	+	+	0	+	0	0	0	0	+	0	+	2+ <sup>S</sup>	1+	@
2	0	0	0	+	+	0	+	0	+	+	0	+	0	0	+	0	+	2+	1+	@
3	+	+	0	0	+	0	+	+	0	+	0	0	+	+	+	0	+	2+ <sup>S</sup>	1+	@
4	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	0	+	1+ <sup>S</sup>	1+	@

@ = agglutinated after washing



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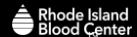
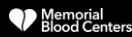
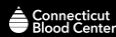
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# Duffy Blood Group System

- Comprised of 5 antigens:
  - Fy1 (Fy<sup>a</sup>)
  - Fy2 (Fy<sup>b</sup>)
  - Fy3
  - Fy4 (obsolete)
  - Fy5
- Third allele, *FY*, more abundant than *FYA* and *FYB*
  - *FY* produces no Duffy glycoprotein
    - Phenotype: Fy(a-b-)
    - RBCs are also Fy:-3,-5

## Fy<sup>a</sup> and Fy<sup>b</sup> Antigens

- Sensitive
  - Proteolytic enzymes (papain, ficin, bromelin)
- Resistant
  - Trypsin
  - DTT
  - 2ME



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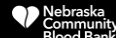
## Fy3



- Fy3 Antigen
  - Resistant to proteolytic enzymes
  - Present on Fy(a+) and Fy(b+) cells
  - **Present** on Rh<sub>null</sub> cells
- Anti-Fy3
  - **Negative** with cord cells
  - Positive with Rh<sub>null</sub> cells

## Fy5

- Fy5 Antigen
  - Resistant to proteolytic enzymes
  - Present on Fy(a+) and Fy(b+) cells
  - Present on cord cells
  - **Absent** on Rh<sub>null</sub> cells
- Anti-Fy5
  - Positive with cord cells
  - **Negative** with Rh<sub>null</sub> cells



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# Rh Null and Cord Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma	Eluate
		D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT	PEG IAT
1	Cord Cells	0	0	0	+	+	0		+	+	+	0					0	+	3+	1+ <sup>S</sup>
2	Cord Cells	+	0	0	+	+	0		0	+	+	0					+	+	1+	(0) <sup>V</sup>
3	Rh null	0	0	0	0	0	0	+	+	+	+	0	0	+	+	+	0	+	3+	1+
4	Rh null	0	0	0	0	0	0	+	+	+	+	0	0	+	+	+	+	+	Not Tested	1+

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# Are We Done?



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## Still Need to Determine If Anti-Fy3 or Anti-Fy5

**WHY?**



## Final Steps

### Plasma

- Perform Adsorption
  - Adsorb out the anti-Fy3 or anti-Fy5
  - Use papain treated E-K-Jk(b-) RBCs
- Test Adsorbed Plasma
  - Anti-Fy3 or anti-Fy5 reactivity removed
  - Can rule in anti-K

### Adsorbing Cells

- Perform Elution
  - From first set of RBCs used in the plasma adsorption
- Test Eluate
  - Have just anti-Fy3 or anti-Fy5 in eluate

## AlloAdsorbed Plasma

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Neat Plasma	2x Alladsorbed Plasma*
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT	PEG IAT
1	0	0	0	+	+	0	+	+	0	+	0	0	0	0	+	0	+	1+ <sup>S</sup>	1+
2	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	0	+	2+	1+

	Rh					Kell		Duffy		Kidd		Lewis		MNS				4x Alladsorbed Plasma*	3x Alladsorbed Plasma <sup>^</sup>
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT	PEG IAT
1	0	0	0	+	+	0	+	+	0	+	0	0	0	0	+	0	+	1	1+
2	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	0	+	1+	1+

\* = adsorption with papain treated RBCs

<sup>^</sup> = adsorption with untreated RBCs

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## Eluate from Adsorbing Cells

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Eluate
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT
1	0	0	0	+	+	0	+	+	0	+	0	0	0	0	+	0	+	1+ <sup>w</sup>
2	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	0	+	1+ <sup>w</sup>
3	0	+	0	+	+	0	+	+	0	+	+	+	0	+	0	+	0	1+ <sup>w</sup>
4	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	1+ <sup>w</sup>
5	0	0	0	+	+	0	+	0	0	0	+	+	0	+	0	0	+	(0) <sup>v</sup>
6	+	0	0	+	+	0	+	0	0	+	0	0	0	0	+	0	+	(0) <sup>v</sup>
7	+	0	0	+	+	0	+	0	0	0	+	0	0	+	0	0	+	(0) <sup>v</sup>

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## Eluate from Adsorbing Cells: Rh Null and Cord Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Eluate
		D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	PEG IAT
1	Cord Cells	0	0	0	+	+	0		+	+	+	0					0	+	1+ <sup>S</sup>
2	Cord Cells	+	0	0	+	+	0		0	+	+	0					+	+	1+
3	Rh null	0	0	0	0	0	0	+	+	+	+	0	0	+	+	+	0	+	(0) <sup>∇</sup>
4	Rh null	0	0	0	0	0	0	+	+	+	+	0	0	+	+	+	+	+	(0) <sup>∇</sup>

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- Anti-Fy3
  - **Negative** with cord cells
  - Positive with Rh<sub>null</sub> cells
- Anti-Fy5
  - Positive with cord cells
  - **Negative** with Rh<sub>null</sub> cells

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## Report


- History:
  - Warm autoantibody
  - Cold autoantibody
  - Anti-E
  - HLA antibody
- Eluate
  - Probable anti-Fy5
  - Unable to exclude anti-Fy<sup>a</sup> or anti-Fy<sup>b</sup>
- Plasma
  - Anti-Fy5
  - Anti-Jk<sup>b</sup>
  - Unable to exclude anti-Fy<sup>a</sup>, anti-Fy<sup>b</sup> or anti-K

## Transfusion

- Recommend:
  - E-negative, K-negative, Fy(a-b-), Jk(b-) blood negative with the patient's plasma.
- Transfused:
  - 2 E-,C-,K-,Fy(a-b-),Jk(b-),S- units were transfused
    - Prevalence:
      - In Caucasian population: 0.15%
      - In African American population: 13.6%

## Objectives

- Describe the tests used to resolve the most complex serologic workups
- Interpret a variety of test results in order to direct the course of a workup and identify complex antibodies
- Select appropriate units for transfusion



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