

A large, semi-transparent American Red Cross flag is visible in the background, featuring a white field with a prominent red cross. The flag is slightly out of focus, creating a soft, ethereal effect.

# Reference Lab Challenges

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# Objectives

- Demonstrate challenges in Reference Laboratory work
- Outline strategies for performing Reference Testing
- Review the importance of complete and accurate history for referred samples
- List ways referring hospitals can help improve the process

# Case 1

- 40 year old female admitted in sickle cell crisis
  - STAT order for 2 units - Hgb 6.2
  - No transfusion history provided by facility
  - 2 previous pregnancies
  - No reported alloantibodies
- Initial testing
  - O, D positive
  - DAT negative
  - Antibody Screen negative at IS and PeG-IAT

# Do we stop here?

- Request additional information from referring facility
  - By what technique did they find reactivity?
    - Gel
  - What cells did they test and which cells were positive?
    - Only tested screen; all cells positive
  - Any other pertinent information?
    - Patient had previously been treated in Mississippi
- Additional Ref Lab testing
  - Phenotype – DAT negative; no mixed field noted
  - Test in solid phase

# Solid phase panel

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		SP
1	+	+	0	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	0		2+
2	+	+	0	0	+	0	0	+	0	0	+	0	0	+	+	0	+	+	0		2+
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	+	+	0	+		0
4	+	0	0	+	+	0	0	+	+	0	+	+	0	0	+	+	0	0	+		2+
5	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+		2+
6	0	0	+	+	+	0	0	+	+	0	0	+	0	+	0	0	+	0	+		2+
7	0	0	0	+	+	0	0	+	+	+	+	+	+	0	+	0	+	+	+		2+
8	0	0	0	+	+	0	0	+	+	+	+	+	0	+	+	+	0	0	+		2+
9	0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+		2+
10	+	0	+	+	0	0	0	+	+	0	0	+	+	0	+	0	+	0	+		0
Auto	+	0	+	+	0	0	0		0	+	+	0						0	+		0

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	SP
1	+	+	0	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	0	2+
2	+	+	0	0	+	0	0	+	0	0	+	0	0	+	+	0	+	+	0	2+
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	+	+	0	+	0
4	+	0	0	+	+	0	0	+	+	0	+	+	0	0	+	+	0	0	+	2+
5	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	2+
6	0	0	+	+	+	0	0	+	+	0	0	+	0	+	0	0	+	0	+	2+
7	0	0	0	+	+	0	0	+	+	+	+	+	+	0	+	0	+	+	+	2+
8	0	0	0	+	+	0	0	+	+	+	+	+	0	+	+	+	0	0	+	2+
9	0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	2+
10	+	0	+	+	0	0	0	+	+	0	0	+	+	0	+	0	+	0	+	0
Auto	+	0	+	+	0		0		0	+	+	0						0	+	0

- Additional testing in SP confirms anti-e and excludes other common alloantibodies.
- Two e negative units found compatible.

# Are we finished now?

- Locate hospital in Mississippi where patient was previously treated.
  - Patient transfused in early January
  - Previously identified by another Reference Laboratory
    - Anti-C
    - Anti-e
    - Anti-K
    - Anti-Jk<sup>b</sup>
    - Anti-S

# Now, what must we do?

- Repeat phenotype with separated reticulocytes
  - Patient found to also be  $Fy^b$  negative
- Must find appropriate donor units negative for all known alloantibodies
  - C, e negative – 2%
  - K negative – 91%
  - $Jk^b$  negative – 30%
  - S negative – 55%



# How many donors to screen?

- $0.02 \times 0.91 \times 0.3 \times 0.55 = 0.003$  or 0.3%
  - % of random population negative for all antigens
  - 3 out of 1000 donors
- To find 2 units:
  - # of units needed / % of negative units
  - $2 / 0.003\% = 667$
- Strategy for screening
  - Start with C and e
  - Then move to next least frequent (Jk<sup>b</sup>, then S)
  - Finally K

# Are we lucky today??

- Our inventory is good!
- We have 12 R2R2, K- units
  - We type all of these units for Jk<sup>b</sup>
    - **4 are negative!**
  - We type these 4 for S
    - **2 are negative!**
- We have the 2 units for this order!



# Case 2

- 55 year old African-American male
  - Diagnosis of “anemia”
  - Transfused 4 RBC in 2001 following an auto accident
  - Initial Testing
    - A, D positive
    - Antibody Screen results

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IS	PeG IgG
1	+	+	0	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0	2+
2	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	+	+	0	2+
3	0	0	0	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	0	2+

# Case 2

- DAT
  - Polyspecific 3+
  - Anti-IgG 3+
  - Anti-C3 1+
- Patient phenotype
  - 2 RBC transfused on 1/15/09
  - Cell separation to harvest reticulocytes
  - EGA treat to remove bound IgG
    - DAT negative after EGA treatment of retics

<b>C</b> mono	<b>E</b> mono	<b>c</b> mono	<b>e</b> mono	<b>K</b> mono	<b>Fya</b>	<b>Fyb</b>	<b>Jka</b>	<b>Jkb</b>	<b>S</b>	<b>s</b>
+	0	0	+	0	0√	0√	0√	+	+	0√

# Next steps...

- Test serum with selected panel cells
  - Include phenotype-similar cells (PS)
- Prepare and test an eluate
  - Include last wash control tested in parallel
  - Test against the PS cells above

# Selected cell panel

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Is	PeG IgG	Eluate
1	+	+	0	0	+	+	0	+	0	0	0	+	+	0	+	+	0	+	0	0	2+	2+
2	+	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	+	+	0	0	2+	2+
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	+	+	0	+	0	2+	2+
4	+	0	0	+	+	0	0	+	+	0	+	+	0	0	+	+	0	0	+	0	2+	2+
5	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	2+	2+
6	0	0	+	+	+	0	0	+	+	+	+	+	0	+	+	+	0	0	+	0	2+	2+
7	0	0	0	+	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+	0	2+	
8	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	2+	
Auto																				0	2+	

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	is	PeG	IgG	Eluate
1	+	+	0	0	+	+	0	+	0	0	0	+	+	0	+	+	0	+	0	0	2+	2+	
2	+	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	+	+	0	0	2+	2+	
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	+	+	0	+	0	2+	2+	
4	+	0	0	+	+	0	0	+	+	0	+	+	0	0	+	+	0	0	+	0	2+	2+	
5	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	2+	2+	
6	0	0	+	+	+	0	0	+	+	+	+	+	0	+	+	+	0	0	+	0	2+	2+	
7	0	0	0	+	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+	0	2+		
8	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	2+		
Auto																				0	2+		



# What's next?

- Allogeneic adsorption
  - X2 with R1R1, K neg, Jkb neg, papain-treated cells
  - Using a PeG adsorption technique

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	x <sub>1</sub> sp <sub>2</sub>	
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	✓
4	+	0	0	+	+	0	0	+	+	0	+	+	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	+	0	+	+	0	+	0	✓
8	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	✓

# Transfusion recommendations

- Patient has apparent warm-reactive autoantibody.
- Patient has no underlying alloantibodies.
- ABO, Rh compatible random donor units are appropriate for transfusion.

BUT...

Referring hospital requests 4 units of phenotype-matched RBCs.

– Now what must we do??

# Patient's phenotype...

- Negative for c, E, K, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, s
- Available antigen typed units
  - All group O and A, R1R1, K negative – 30 units
    - 12 are Fy(a-b-)
      - 4 are Jk(a-), not tested for s
      - 3 are s-, not tested for Jk<sup>a</sup>
    - 6 are Jk(a-), not tested for Fy<sup>a</sup>, Fy<sup>b</sup> or s
    - 4 are s-, not tested for Jk<sup>a</sup>, Fy<sup>a</sup>, or Fy<sup>b</sup>
    - 10 have not been tested for Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup> or s
- What are the chances that we will find the four units we need for this order?

# The chances...

- Out of ABO compatible units -considering the appropriate population
  - ~12% of our donors are African American; Of those:
    - ~2% are R1R1 (c- and E-)
    - ~67% are Fy(a-b-)
    - ~98% are K-
    - ~8% are Jk(a-)
    - ~6% are s-
- To find 4 units:
  - $0.12 \times 0.02 \times 0.67 \times 0.98 \times 0.08 \times 0.06 = 0.000007$
  - 7 out of 1,000,000 donors!!
  - We only need to find 500,000 donors!

# Where do we start?

- We have 4 R1R1, K-, Fy(a-b-), Jk(a-) units
  - Test for s
    - **2 are negative!! 😊**
- We have 3 R1R1, K-, Fy(a-b-), s- units
  - Test for Jk<sup>a</sup>
    - **1 is negative!! 😊**
- We have 5 R1R1, K-, Fy(a-b-) units
  - Test for Jk<sup>a</sup> and s
    - All are either Jk<sup>a</sup> or s positive ☹
- We have 6 R1R1, K-, Jk(a-) units
  - Test for Fy<sup>a</sup> and Fy<sup>b</sup>
    - **2 are negative for both 😊**
  - Test for s
    - **1 is negative! 😊**
- We have the 4 requested units ready to go!!

# But wait...

- The hospital calls back to say they have also typed the patient and found him to be M negative.
  - And now the order is STAT!!
- Order is updated to add M to the list of antigens requested.
  - ~25% of African American donors are M negative
- Anti-M is not considered to be clinically significant unless shown to be reactive at 37C or AHG.
  - The patient does not have anti-M.

# Back to our order...

- Of the 4 R1R1, K-, Fy(a-b-), Jk(a-), s-units, only 1 is M negative.
  - We still need 3 more units.
- We still have 4 R1R1, K-, s- units, not tested for Jk<sup>a</sup>, Fy<sup>a</sup>, or Fy<sup>b</sup>
  - Test for Fy<sup>a</sup> and Fy<sup>b</sup> first
    - Only 1 is negative for both
  - Test for Jk<sup>a</sup>
    - This unit is Jk<sup>a</sup> positive

# Now what??

- We still need 3 units -
  - We have the remaining 10 O and A, R1R1, K- units that had not been tested for the other antigens.
  - None of these units match the needed phenotype.
- OR...
  - We contact our Medical Director to consult with the hospital physician regarding the order for M negative units.



# Take home messages...

- Case 1 illustrates the importance of providing complete and accurate patient history and testing results to the Reference Laboratory.
- Case 2 illustrates the impact of a 'simple' change to an order.
- Both cases illustrate the amount of testing involved in providing antigen-negative units for patient orders.

# What you can do to help...

- Ensure that request forms contain complete and accurate information.
- Ensure that sample labeling is correct and legible.
- Contact the Reference Lab with any additional pertinent information as needed.
- Attempt to obtain info when requested by Ref Lab.
- By helping us to help you, we can provide excellent patient care!!

# Questions??

