

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

LeeAnn Prihoda, MEd, MT(ASCP)SBB
Manager, Reference Laboratory
ARC – Southern Region



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^b
 - 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^b, 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^b
 - **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

C mono	E mono	c mono	e mono	K mono	Fya	Fyb	Jka	Jkb	S	s
+	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 ₁ sp ₂	
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^a, Fy^b, Jk^a, 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^a
 - 6 are Jk(a-), not tested for Fy^a, Fy^b or s
 - 4 are s-, not tested for Jk^a, Fy^a, or Fy^b
 - 10 have not been tested for Fy^a, Fy^b, Jk^a 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^a
 - **1 is negative!!** 😊
- We have 5 R1R1, K-, Fy(a-b-) units
 - Test for Jk^a and s
 - All are either Jk^a or s positive 😞
- We have 6 R1R1, K-, Jk(a-) units
 - Test for Fy^a and Fy^b
 - **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^a, Fy^a, or Fy^b
 - Test for Fy^a and Fy^b first
 - Only 1 is negative for both
 - Test for Jk^a
 - This unit is Jk^a 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??

