Case Study:

Suspected Hemolytic Transfusion Reaction with a Negative Antibody Screen

American Red Cross

Transfusion Reaction Workup

Patient O.L.D.

- 77 year old male
- Diagnosis of MDS, GERD, and gastritis

The patient presented to the ER with a three day history of weakness and lightheadedness with the addition of nausea/vomiting the previous day.

The patient has had multiple blood transfusions due to chronic anemia, averaging about 3 transfusions per month over the past year.

Two Unit Transfusion Ordered

The patient's Hgb was 5.2 g/dl upon admission and a two unit transfusion was ordered.

The first unit was started at 17:35 and completed at 19:00.

Prior to starting the second unit, the patient complained of back pain and chills. A transfusion reaction work-up was ordered. Blood and urine samples and the blood bags were sent to the Blood Bank for work-up.

Transfusion Reaction Workup

Test		Pre-Transfusion	Post-Transfusion
Temperature		99°F	101°F
Hgb		5.2 g/dl	6.3 g/dl (decreasing to 6.0 g/dl)
Urinalysis:	Color	Clear	Clear
	Blood	Negative	Small
	RBC	None	<1
Serum Tests:	Bilirubin	1.08	3.67
	Creatinine	0.75	0.78
	Haptoglobin	<40	<40
	LDH	<209	265

Blood Bank Work-up

Test	Pre-Transfusion	Post-Transfusion
ABO/Rh	O Positive	O Positive
Antibody Screen	Negative	Negative
DAT	Negative	W+ IgG; Negative C3
Elution	NT	Negative
AHG Crossmatch	Positive	Positive
Gram Stain on Unit	Negative	

The conclusion of Hemolytic Transfusion Reaction was made based on the clinical and laboratory data. Pre- and Post-transfusion samples were sent to the IRL for additional testing.

IRL Testing Pre-Transfusion Sample

IMMUNOHEMATOLOGY STUDIES

Name: O.L.D.	Ref #: 2496-14	Date of Specimen: 08172014 ☐ Plasma	☒ Pretransfusion Sample ☐ Post Transfusion Sample	
ID Number:	Facility:			
Last Transfused on: 07142014	Date Started: 08182014	Tech: CB	WID: 1 (2) 3 4 5 6 7 (8) ☐ 011.1642 ☐ 011.1414 ☐ 011.6233	

	Cell Typings												Serum Grouping					DAT				
	A	B	A,B	H	D	Rh Ct	C	E	c	e	Ct*	Weak D: D	Weak D: Rh Ct	O	Auto	A_1	A_2	B		Poly	IgG	c'	Sal Ct
IS	0	0	0		3+	0										3+	2+	4+	IS	0		NA	
5' RT																			5' RT	0	NA		

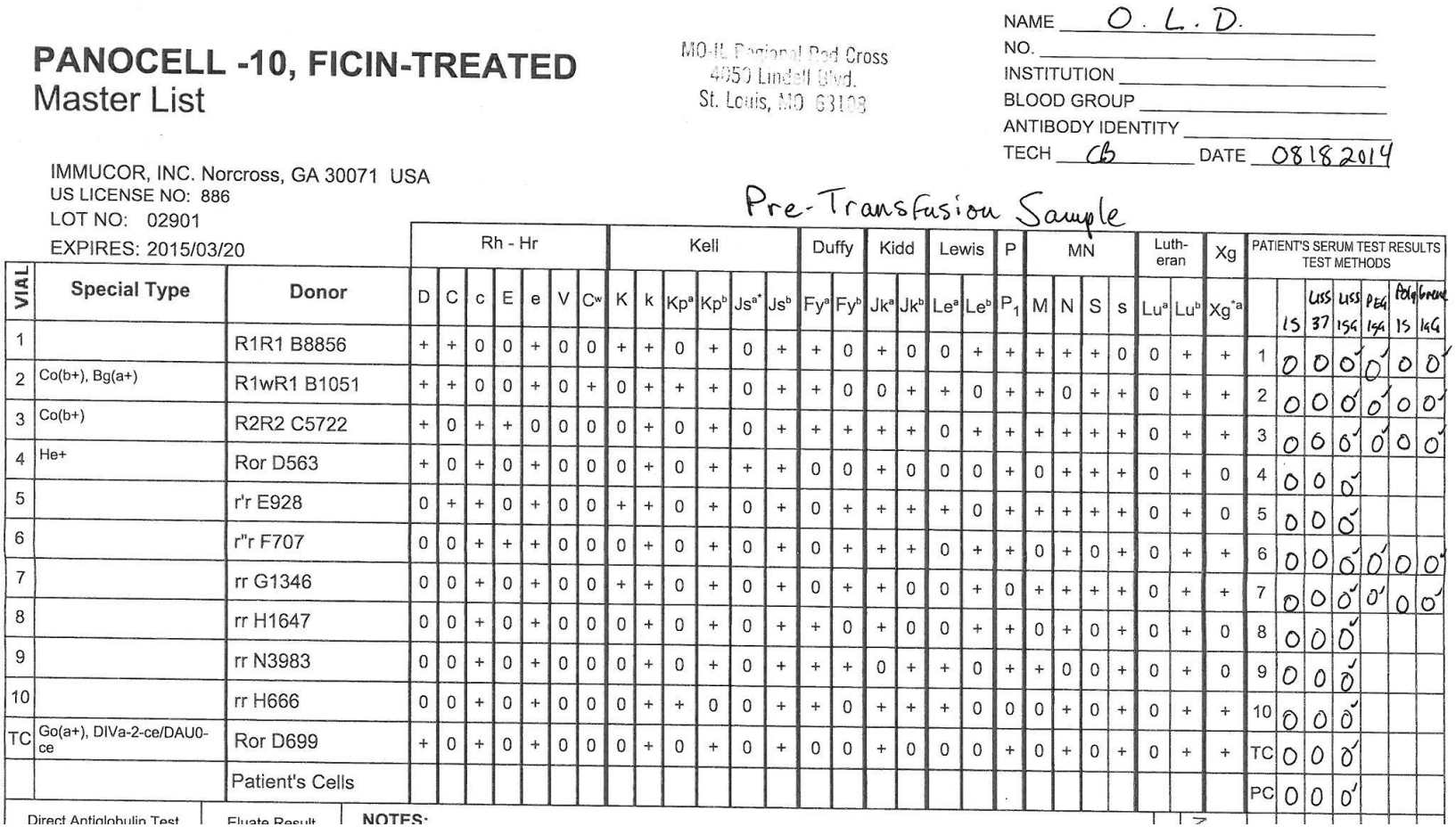
** Ct = Rh phenotype control, use only when required by manufacturer's instructions* — *Cell Treatments: EGA = EDTA Glycine-Acid; CDP=Chloroquine Diphosphate*

Other Antigens:																	
Result: Neat																	
Result: Cell Sep.																	
Cell treatment	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP
Manufacturer Lot Number Expiration																	

Manufacturers: I = Immucor; O = Ortho; A = American Red Cross (in house or Diagnostic Manufacturing Division); B = Biorad; Q = Alba Bioscience/Quotient

	Routine							Eluate		Last Wash		Crossmatching:					
	15' 4C	15' RT	IS	LISS 37C	LISS IgG	PEG IgG	GEL IgG	IgG	PEG IgG	IgG	PEG IgG		IS	LISS 37	LISS IgG		
I												W20111413408	0	0	1+		
II																	

IRL Testing Pre-Transfusion Sample



PANOCELL -10, FICIN-TREATED
Master List

MO-IL Regional Red Cross
4050 Lindell Blvd.
St. Louis, MO 63108

NAME O. L. D.
NO.
INSTITUTION
BLOOD GROUP
ANTIBODY IDENTITY
TECH CB DATE 08182014

IMMUCOR, INC. Norcross, GA 30071 USA
US LICENSE NO: 886
LOT NO: 02901
EXPIRES: 2015/03/20

Pre-Transfusion Sample

VIAL	Special Type	Donor	Rh - Hr							Kell						Duffy		Kidd		Lewis		P	MN				Luth-eran		Xg	PATIENT'S SERUM TEST RESULTS TEST METHODS						
			D	C	c	E	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	P_1	M	N	S	s	Lu^a	Lu^b	Xg^{*a}		IS	LISS 37	LISS IS/G	PEG IS/G	Polybrene IS	Polybrene IS/G
1		R1R1 B8856	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	0	0	+	+	1	0	0	0	0	0	0
2	Co(b+), Bg(a+)	R1wR1 B1051	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0	+	+	2	0	0	0	0	0	0
3	Co(b+)	R2R2 C5722	+	0	+	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	+	0	+	+	+	3	0	0	0	0	0	0
4	He+	Ror D563	+	0	+	0	+	0	0	0	+	0	+	+	+	0	0	+	0	0	0	+	0	+	+	+	0	+	+	0	4	0	0	0			
5		r'r E928	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	0	+	+	0	5	0	0	0			
6		r"r F707	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	6	0	0	0	0	0	0
7		rr G1346	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	0	+	+	+	7	0	0	0	0	0	0
8		rr H1647	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	+	0	8	0	0	0			
9		rr N3983	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	0	+	0	+	+	0	9	0	0	0			
10		rr H666	0	0	+	0	+	0	0	0	+	+	0	0	+	+	0	+	+	+	0	0	0	+	0	+	0	+	+	+	10	0	0	0			
TC	Go(a+), DIVa-2-ce/DAU0-ce	Ror D699	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	TC	0	0	0			
		Patient's Cells																											PC	0	0	0			

Direct Antiglobulin Test | Eluate Result | NOTES:

IRL Testing Post-Transfusion Sample

IMMUNOHEMATOLOGY STUDIES

Name	Ref #	Date of Specimen ☐ Plasma	☐ Pretransfusion Sample ☒ Post Transfusion Sample
O.L.D.	2496.14	08172014	
ID Number		Facility	
Last Transfused on: 08172014	Date Started: 08182014	Tech: CB	WID 1 (2) 3 4 5 6 7 (8) ☐ 011.1642 ☐ 011.1414 ☐ 011.6233

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IS	0	0	0		3+	0										3+	2+	4+	IS	W+	W+	NA	0
5' RT																			5' RT	W+	NA	0✓	0

* Ct = Rh phenotype control, use only when required by manufacturer's instructions Cell Treatments: EGA = EDTA Glycine-Acid; CDP=Chloroquine Diphosphate

Other Antigens:																		
Result: Neat																		
Result: Cell Sep.																		
Cell treatment	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP	☐ EGA ☐ CDP
Manufacturer Lot Number Expiration																		

Manufacturers: I = Immucor; O = Ortho; A = American Red Cross (in house or Diagnostic Manufacturing Division); B = Biorad; Q = Alba Bioscience/Quotient

	Routine							Eluate		Last Wash		Crossmatching:					
	15' 4C	15' RT	IS	LISS 37C	LISS IgG	PEG IgG	GEL IgG	IgG	PEG IgG	IgG	PEG IgG		IS	LISS 37	LISS IgG		
I												W20111411340 8	0	0	1+		
II																	
III																	
Auto																	
DAT Neg Auto																	

IRL Testing Post-Transfusion Sample

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Post-Transfusion Sample

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			D	C	c	E	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	P_1	M	N	S	s	Lu^a	Lu^b	Xg^{*a}		IS	PEG IgG	Eluate IgG	Eluate PEG IgG		
1		R1R1 B8856	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	0	0	+	+	1	0	0✓	0	0✓		
2	Co(b+), Bg(a+)	R1wR1 B1051	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0	+	+	2	0	0✓	0	0✓		
3	Co(b+)	R2R2 C5722	+	0	+	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	+	0	+	+	3	0	0✓	0	0✓		
4	He+	Ror D563	+	0	+	0	+	0	0	0	+	0	+	+	+	0	0	+	0	0	0	+	0	+	+	+	0	+	0	4	0	0✓				
5		r'r E928	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	0	+	0	5	0	0✓				
6		r''r F707	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	6	0	0✓	0	0✓		
7		rr G1346	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	0	+	+	7	0	0✓	0	0✓		
8		rr H1647	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	0	8	0	0✓				
9		rr N3983	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	0	+	0	+	0	9	0	0✓				
10		rr H666	0	0	+	0	+	0	0	0	+	+	0	0	+	+	0	+	+	+	0	0	0	+	0	+	0	+	+	10	0	0✓				
TC	Go(a+), DIVa-2-ce/DAU0-ce	Ror D699	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	TC	0	0✓				
		Patient's Cells																											PC	0	0✓				

Direct Antiglobulin Test | Eluate Result | NOTES:

Next Steps

Options for Identifying an Antibody to a Low Prevalence Antigen

- Run a panel of cells known to be positive for various low prevalence antigens with the patient's serum.
- Treat the incompatible unit with enzymes or chemicals to help determine the specificity.
- Type the red cells from the incompatible unit with known examples of antibodies directed against low prevalence antigens.

Antigen Typing of the Incompatible Product for Various Low Prevalence Antigens

American Red Cross
Washington, D.C. 20006

Preliminary Donor Antigen Testing Worksheet

American Red Cross

Batch Information

Batch #	081814.2010.4	Pages in batch:	Page 2 of 2

Position #	DIN/WBN Preliminary Type	Anti-Kp^a	Anti-Js^a	Anti-V	Anti-Cw	Anti-Wr^a	Anti-Di^a	Anti-Co^b	Anti-y+b	Anti-Lu^a	Anti-
		Test Results									
1	W2011 14 113408	0✓	0✓	0✓	0	2+	0✓	0✓	0✓	0✓	
		FE	FE	FE	FE	FE	FE	FE	FE	FE	FE
		SE	SE	SE	SE	SE	SE	SE	SE	SE	SE
		FE	FE	FE	FE	FE	FE	FE	FE	FE	FE
		SE	SE	SE	SE	SE	SE	SE	SE	SE	SE

Confirmation of Anti-Wr^a

Additional cells confirm the presence of anti-Wr^a in the pre-transfusion serum and the post-transfusion serum and eluate.

Patient's Name O.L.D
Patient's Number
Date: Collected Date: Tested 08182014

SELECTED PANEL 1:58 pm, 3/9/2015
American Red Cross, Missouri-Illinois Region
4050 Lindell, St. Louis, MO 63108
Technologist: CB

	Supplier Lot #	Donor/ Vial#	RhHr								MN				P	Lew		Lut		Kell						Duf		Kid		X	Additional Antigens	PRE Serum		POST Serum		POST Elwash		LW
			D	C	E	c	e	f	V	Cw	M	N	S	s	P1	Lea	Leb	Lua	Lub	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga		LISS	15g	LISS	15g	15g	15g	
1 OOD	Imm-10F 42727	B2849 TC	+	+	0	0	+		0	0	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	Wr(a+)	1+		1+		2+	0	
2 OOD	Immucor 38680	B5269 2	+	+	0	0	+		0	+	+	+	+	+	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	Wr(a+)	1+		1+		2+	0	
3 OOD	Imm-16 35638	H1484 8	0	0	0	+	+		0	0	+	0	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	Wr(a+)	1+		1+		1+	0	

Discussion

- AABB Standards for Blood Banks and Transfusion Services state “If no clinically significant antibodies were detected…and there is no record of previous detection of such antibodies, at a minimum, detection of ABO incompatibility shall be performed.” 5.15.1.1
  - Tests for ABO incompatibility could include an Immediate Spin crossmatch (as was performed in this case) or a computer crossmatch.
  - These techniques will not detect an incompatible crossmatch causes by an antibody to a low prevalence antigen.

The Wr^a Antigen

- The Wr^a antigen was identified in 1953 as the cause of HDFN in the Wright family.
- The antigen was assigned to the Diego blood group system in 1995.
- Production of the Wr^a antigen is controlled by a gene on chromosome 17.
- The occurrence of the antigen is less than 0.01%
- The antithetical antigen is Wr^b, which has an incidence of 100% (only three accounts of patients with Wr(b-) cells have been described).
- The antigen is resistant to chemical treatment including enzymes (Ficin, Papain, Trypsin), DTT, and acid.

Anti-Wr^a

- Anti-Wr^a can be IgM or IgG.
  - The IgM antibody reacts optimally at room temperature, while the IgG antibody reacts optimally at IAT.
- There have been documented reports of mild to severe transfusion reaction associated with anti-Wr^a. These reactions ranged from immediate to delayed, and some were hemolytic.
- The antibody is known to cause mild to severe Hemolytic Disease of the Fetus and Newborn.

Anti-Wr^a

- Anti-Wr^a may be a naturally-occurring antibody and is often found in the serum of persons with no known exposure.
- The antibody is frequently found in serum with multiple antibody specificities.
- Anti-Wr^a is a common specificity associated with patients with AIHA.
- The incidence of anti-Wr^a increases in patient populations with active immune systems.

Incidence of Anti-Wr^a

Normal Blood Donors	Post-partum Women	Post-partum Women who have developed Rh Antibodies	AIHA Patients
1 in 100-200	1 in 50	1 in 14	1 in 2-3

The occurrence of anti-Wr^a is much higher than one would expect based on the prevalence of the antigen. One theory is that patients with active immune systems make anti-Wr^a when exposed to antigens on protein band 3, which carries the Diego blood group antigens.

Managing Patients with Antibodies to Low Prevalence Antigens

- If clinically significant, compatibility testing must be performed at the antiglobulin phase.
- If the antibody is detected and reacting at a strength of 1+ or greater, we would recommend giving crossmatch compatible units.
- If the antibody is not detected, weakly reactive, or we are unable to assess whether the antibody is still reactive, we would recommend giving antigen negative units, provided that the appropriate rare antisera is available.

Questions