Immunohematology Cases from the Crypt

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Senior Director, American Rare Donor Program

The need is constant.
The gratification is instant.
Give blood.™

American Red Cross

Disclosures

Grifols Advisory Board 2018

Speaker for Griofols 2018 TSEC Series

Neither pertinent to this presentation

The Tarim mummies are a series of mummies discovered in the Tarim Basin in Xinjiang, China, which date from 1900 BC to 200 AD. In addition to being very well-preserved finds, controversy flows around them as DNA tests seem to show that they are the result of Asian and Caucasian mating thousands of years before it's commonly thought that the two peoples intermingled.

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World Mysteries Blog

Objectives

- Identify the various tools used in Immunohematology to resolve a complex patient
- Discuss case critical observations and testing results for immunohematologic evaluations
- Understand the need to fully characterize for RHD and RHCE the patients and reagent red cells

Underneath St Peter's Basilica are the mysterious Scavi, or excavations. Also known as the Vatican Necropolis, Tomb of the Dead, or St. Peter's Tomb, the site is a burial ground dating back to the fourth century.

Case #1: Is it Compatible?

Case history:

- 32 y.o. AA female
- Sickle Cell Disease
- 7 weeks pregnant and in painful crisis

Case #1: Previous Antibody History

- Anti-S
- Warm auto-antibody
- Cold auto-antibody
- RBCs type weak D positive

The Good News:

- We have an HEA Beadchip result!
- The negative antigens are

  C, E, K, Jk^b, Fy^a, Fy^b, S

  and also the low prevalence antigens

  Kp^a, Js^a, Lu^a, Di^a, Co^b, LW^b, Sc:2
- Patient has not been transfused in past 5 years!

The Bad News is:

- HEA notes that the patient:
  - —Is predicted to be VS+ V+
  - —And has the GATA mutation
- The patient is at risk for:
  - — Anti-S (already identified)
  - — Anti- C, -E, -K, -Jk^b, -Fy^a
  - — And…..anti- Fy3 or Fy5
  - — And .....

Palermo's most famous citizens are very, very old. Underneath Sicily's capital city, known for mafioso and stately Baroque churches, preserved corpses fill five subterranean limestone corridors and have been attracting visitors with a morbid curiosity for centuries.

The Results of the *RHD* and *RHCE* Variant Allele Testing are in....

- Probable *RHD* genotype:
  - —*RHD* DAR1/RHD*DAR1*

    At risk for allo-anti-D
- Probable *RHCE* genotype:
  - — *RHCE*ceEK/RHCE*ce48C, 697C, 712G, 733G, 916A*

    Both alleles code for a predicted phenotype of hr^S-

    And at risk for: anti-c, -e, $-hr^S$, -f

Oh boy, what else will come crawling out of the crypt?

New Serology on Current Sample:

																							LISS			
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IS	37	IgG
1	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	1+	+	3+
2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	1+	+	3+
3	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	0	+	1+	0	2+
4	0	W	0	+	+	+	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+	1+	0	1+
5	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	1+	0	2+
6	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	0	+	1+	0	2+
AC																								1+	0	0√

All RBCs S-

All RBCs and autocontrol positive at RT

E+ RBCs positive at 37C (cells 1,2)

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New Serology on Current Sample:

																								LISS				
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		IS	37	IgG		
1	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+		1+	1+	3+		
2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+		1+	1+	3+		
3	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	0	+		1+	0	2+		
4	0	W	0	+	+	+	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+		1+	0	1+		
5	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+		1+	0	2+		
6	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	0	+		1+	0	2+		
AC																									1+	0	0√		

All RBCs S-

All RBCs and autocontrol positive at RT

E+ RBCs positive at 37C (cells 1,2)

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New Serology on Current Sample:

- DAT negative
- Antibodies detected:
  - Anti-E at 37C definitely suspected
  - Anti-S not assessed (previous)
  - Presumed cold autoantibodies reactive at RT only *Caught a little break here!*
  - Plus another antibody…….
- Wonder if the previous “warm autoantibody” was anti-E and anti-hr^S? having these two would yield reactivity with all normal RBCs tested

Case #1 - Allogeneic Adsorption using Papain Treated Adsorbing RBCs

																									R1 Ads	R2 Ads	rr Ads
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		PEG IgG	PEG IgG	PEG IgG
1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	0		0√	2+	2+
2	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+		3+	2+	3+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+		3+	0√	3+
4	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+		0√	2+	0√
5	+	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√	2+	0√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+		0√	2+	0√
AC																									0√	0√	0√

Adsorbing RBCs:
R1 D+ C+ E- c- e+ K- Jk(a-) S-
R2 D+ C- E+ c+ e- K- Jk(b-) s-
rr D- C- E- c+ e+ K-

Note: In NRLBGS, selection of adsorbing RBCs includes S and s typed RBCs to eliminate any concerns with ambiguity of enzyme treatment to eliminate reactivity to S and s antigens

Case #1 - Allogeneic Adsorption using Papain Treated Adsorbing RBCs

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#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	PEG IgG	PEG IgG	PEG IgG
1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	0	0√	2+	2+
2	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+	3+	2+	3+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	3+	0√	3+
4	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0√	2+	0√
5	+	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√	2+	0√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0√	2+	0√
AC																								0√	0√	0√

Adsorbing RBCs:
R1 D+ C+ E- c- e+ K- Jk(a-) S-
R2 D+ C- E+ c+ e- K- Jk(b-) s-
rr D- C- E- c+ e+ K-

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1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	0	+	0√	2+	2+
2	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+	3+	2+	3+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	3+	0√	3+
4	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0√	2+	0√
5	+	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√	2+	0√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0√	2+	0√
AC																								0√	0√	0√

Adsorbing RBCs:
R1 D+ C+ E- c- e+ K- Jk(a-) S-
R2 D+ C- E+ c+ e- K- Jk(b-) s-
rr D- C- E- c+ e+ K-

Note: In NRLBGS, selection of adsorbing RBCs includes S and s typed RBCs to eliminate any concerns with ambiguity of enzyme treatment to eliminate reactivity to S and s antigens

New Serology on Current Sample:

- Adsorption studies showed:
  - Anti-E at 37C and AHG in R1 and rr adsorption
  - Anti-C at AHG in R2 and rr adsorption
  - Anti-e at AHG in R2 adsorption (suspect anti-hr^S)
  - Anti-Hr not assessed, would be adsorbed to allogeneic RBCs
  - Anti-S not assessed

What is hr^S and Hr?

- hr^S- RBCs are also Hr-
- Some of the other alleles associated with being hr^S and Hr- are below, but e expression may be different from allele to allele:
  - *ceAR*
  - *ceEK*
  - *ceMO*
- Other RBCs also negative for hr^S and Hr
  - D - -
  - Rh_{null}

What to do?.....

Multiple choice question for you:

- A. Request allele selected blood from ARDP
- B. Monocyte Monolayer Test
- C. Least incompatible red cells by crossmatch
- D. Tell the Doctor there is no blood available
- E. Test siblings
- F. Autologous donation

RH Allele Selection

The patient's HYPOTHETICAL *RHCE* alleles: *ce X / ceY*

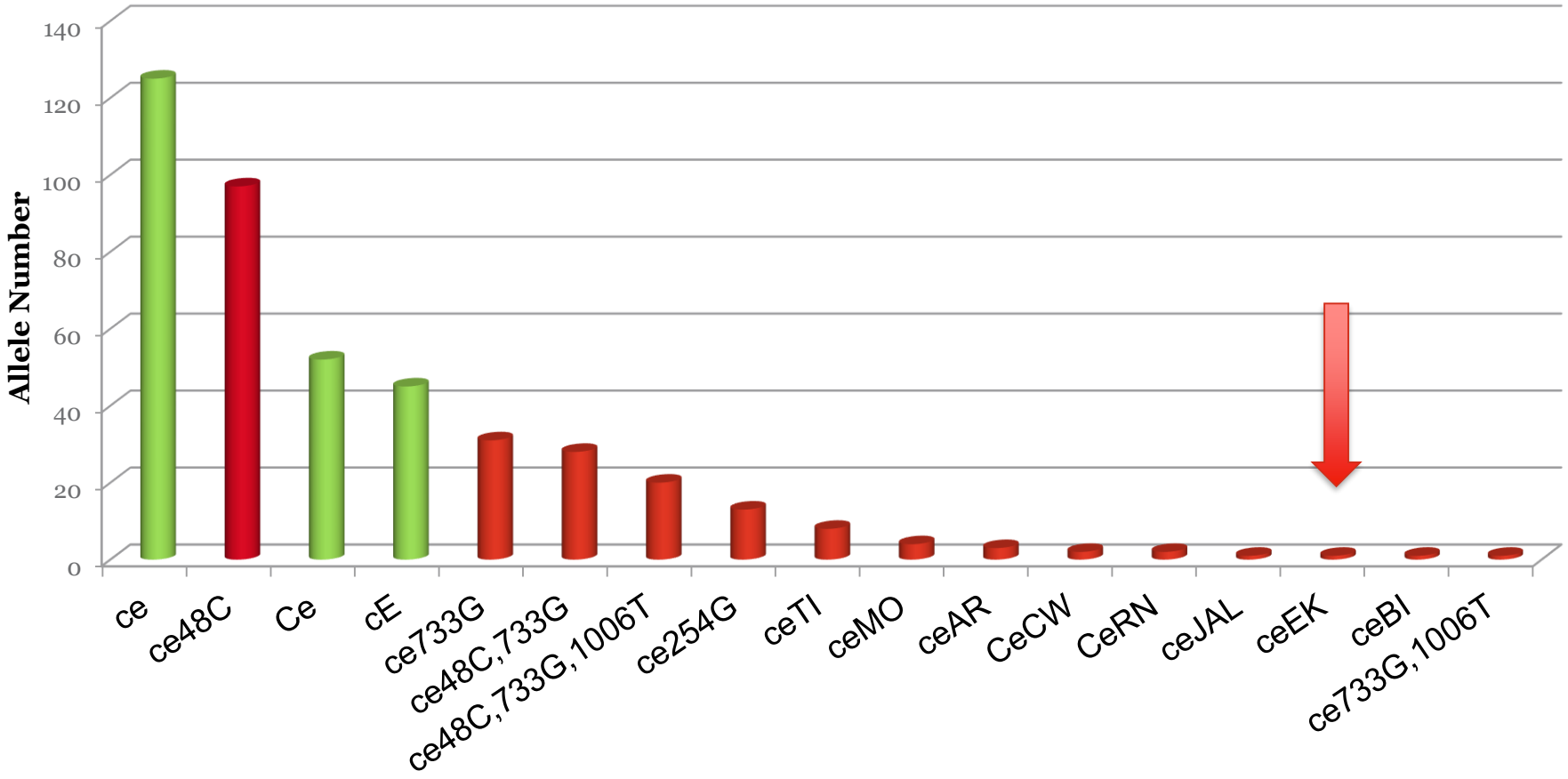
	Allele 1		
Allele 2	***ce X***	***ce Y***	***ce Z***
ce X	**Tier 2**	**Tier 1**	**Tier 3**
ce Y		**Tier 2**	**Tier 3**
ce Z			**Tier 3**

Tier 1 match has exactly the alleles of the patient
Tier 2 has no allele that the patient does not
Tier 3 has similar but not identical allele(s)

Then repeat the process with *RHD* alleles

From Dr Margaret Keller, National Molecular and Genomics Laboratory

RHCE Alleles in African American Donors



Keller MA et al. 2013. *Transfusion* 53(2S):28A

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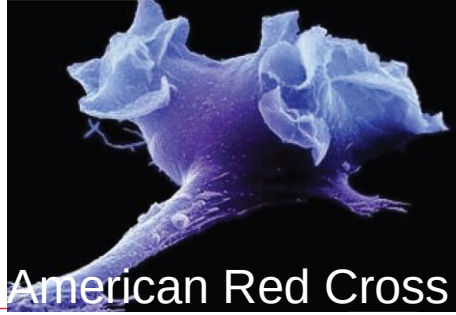
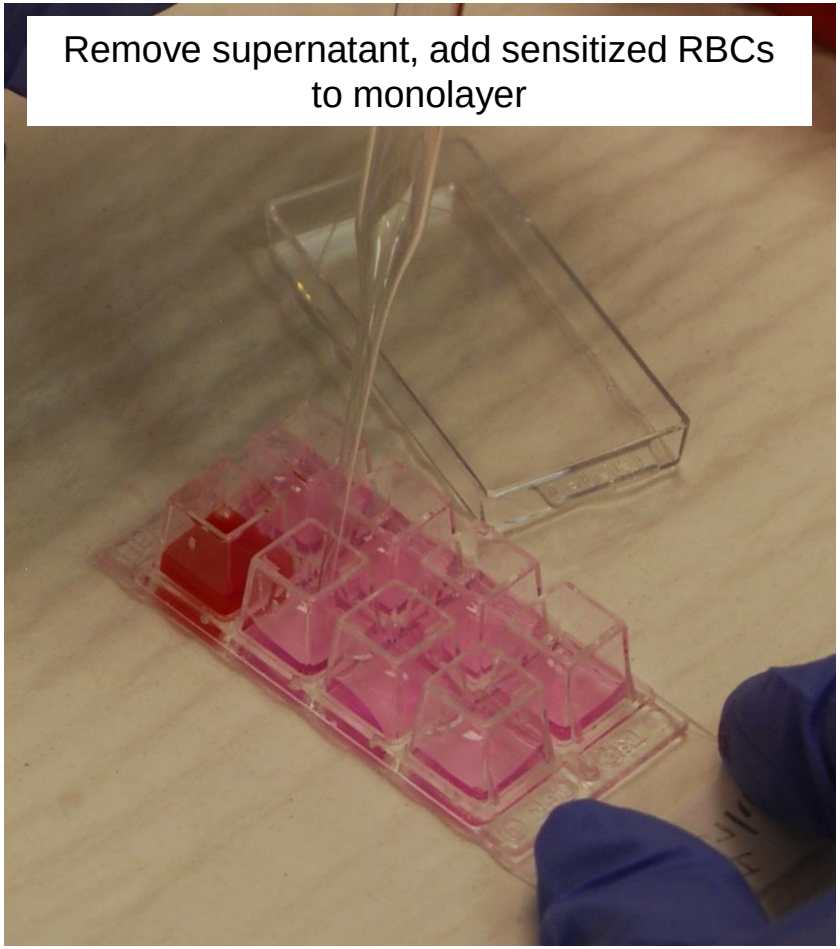
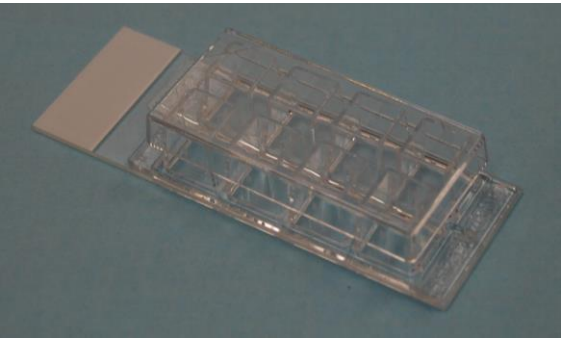
Monocyte Monolayer Assay



- Monolayer of monocytes prepared
- Reagent red cells sensitized in vitro with patient's antibody
- Sensitized reagent red cells added to prepared monolayer of monocytes



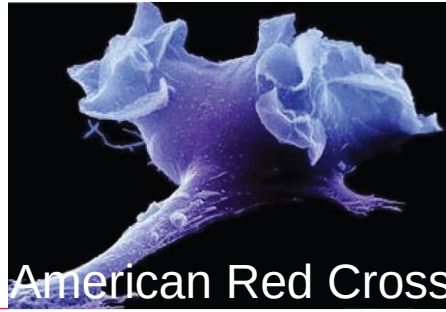


Tissue Culture Chamber Slide

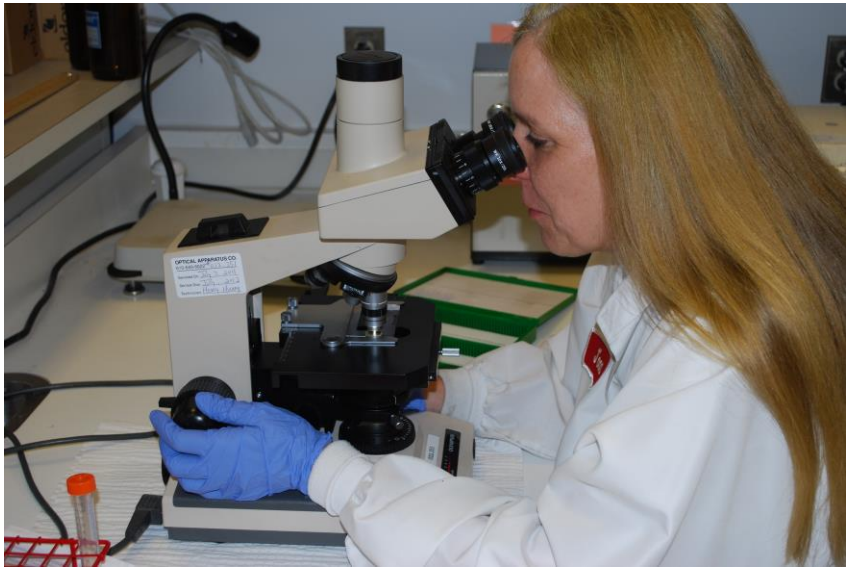
Monolayer of monocytes

American Red Cross

Monocyte Monolayer Assay

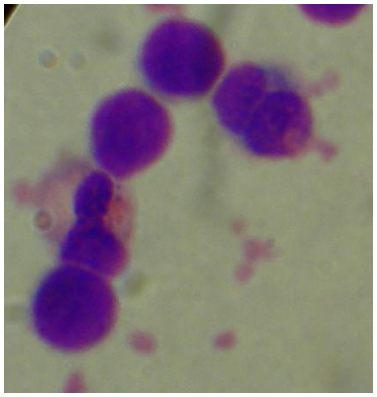


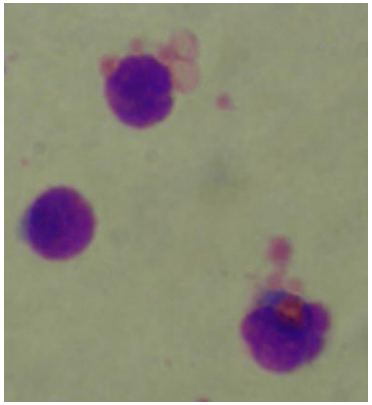
Slide stained and analyzed for adherent and phagocytosed red cells



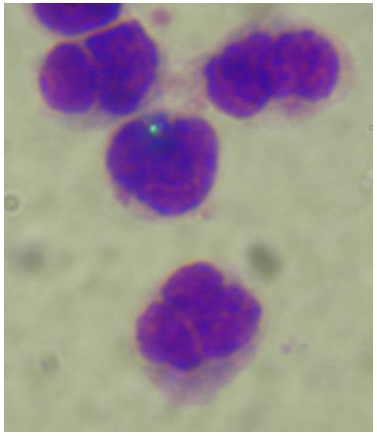
Joan Maurer, SBB reading MMA slides

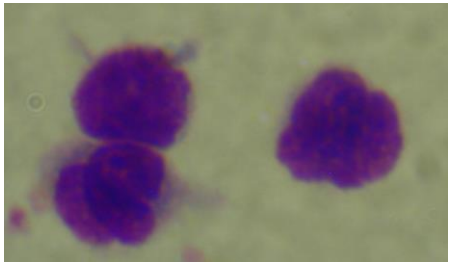
Positive MMA





Negative MMA





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Monocyte Monolayer Assay Data

NRLBGS 1995-2017

Anti-	TT	POS	NEG
AnWj	2	1	1
At^a	4	3	1
Au^a	1	0	1
Co^a	2	2	0
Cr^a	4	4	0
Di^b	11	8	3
Do^b	5	1	4
E	1	1	0
e	3	2	1
GE Sys	31	16	15
hr^B	3	2	1
hr^S	7	4	3
Hy	9	7	2
I	5	1	4
Jk3	1	0	1
Jo^a	10	4	6
Jr^a	15	9	6

Anti-	TT	POS	NEG
Js^b	1	1	0
Kp^b	6	2	4
Ku	1	1	0
Lan	11	7	4
LU Sys	21	19	2
Lu^b	14	12	2
Lw	3	2	1
M	11	5	6
N	2	1	1
$PP1P^k$	1	1	0
RH Sys	1	1	0
s	1	0	1
Sc1	1	1	0
Tc^a	2	1	1
U	4	2	2
Vel	13	10	3
Yt^a	195	119	76

Maurer J, Nance S, Nickle P. Monocyte Monolayer Assay (MMA) to Predict Clinical Significance of Alloantibodies: a 22 year Review. AABB Abstract 2018

American Red Cross

Least Incompatible Crossmatch?

- May be able to perform IS XM to check for ABO incompatibility
- May be able to detect incompatibility due to antibody to low prevalence antigen not detected in serologic work
- Will likely not make a difference in survival of transfused RBCs if antibody reactive with all RBCs tested
- May give a false sense of security about the potential for transfusion reaction

Tell Doctor No Blood is Available?

- Truthful if say no compatible blood available
- May be desired if patient needs blood now, to ensure that the physician is not holding off on transfusion for results of work-up

Roughly six million bodies were laid to rest in the Paris catacombs. The site became a popular burial ground during the 17th century, when Parisian cemeteries could no longer hold the city's dead.
Smithsonian

Test Siblings?

- Always a good idea!

The creepy Paris Catacombs is an underground labyrinth stocking the remains of about six million Parisians, removed from cemeteries at the end of the 18th century. Long tunnels are lined with neatly stacked bones, which will surprise but potentially gross out the whole family. Travel and Leisure

Autologous Donation?

- Always a good idea if possible
- Often can be arranged for planned surgeries
- Can do during pregnancy if mother healthy
  - —Freeze one unit (preferably the first) into aliquot
  - —Freeze one unit whole, 2 units if possible

Mutter Museum, Philadelphia:
wall of prisoner heads used in study of intellect related to head size!
No correlation was found.

What was Really Done and…… What Happened?

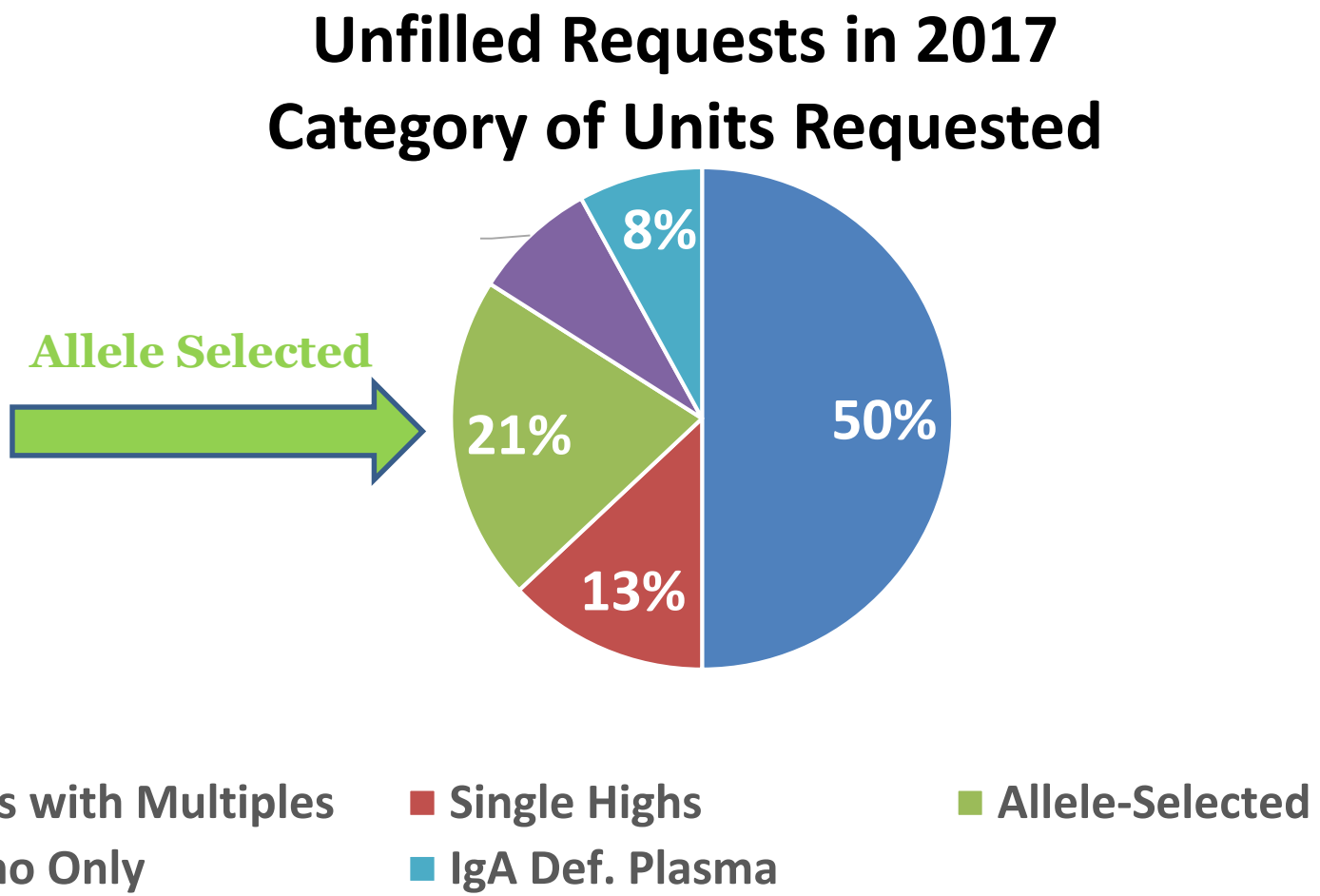
MMA Results

- *RHCE*ceAR/RHCE*ceAR* resembles *ceEK*
  - —Negative in IAT, 0.2% MMA
- *RHCE*ceMO/RHCE*/ceAR* resembles *ceEK*
  - —2+ in IAT, 2.2% MMA
- *RHCE*ceEK/RHCE*ceEK-like* lacking 48C
  - —1+ in IAT, 8.8% MMA
- rr
  - —3+ in IAT, 43% MMA

RBC sample with Exact allele match not available for testing

Patient: *RHCE*ceEK/RHCE*ce48C, 697C, 712G, 733G, 916A*

ARDP 2017 Unfilled Requests



AABB Poster 2018. Fludd D, Maurer M, Facey, D, Keller, M, Nance S et al. Defining the Rarest of the Rare Blood Requests to the American Rare Donor Program: Unfilled Requests from 2017, BBC 87

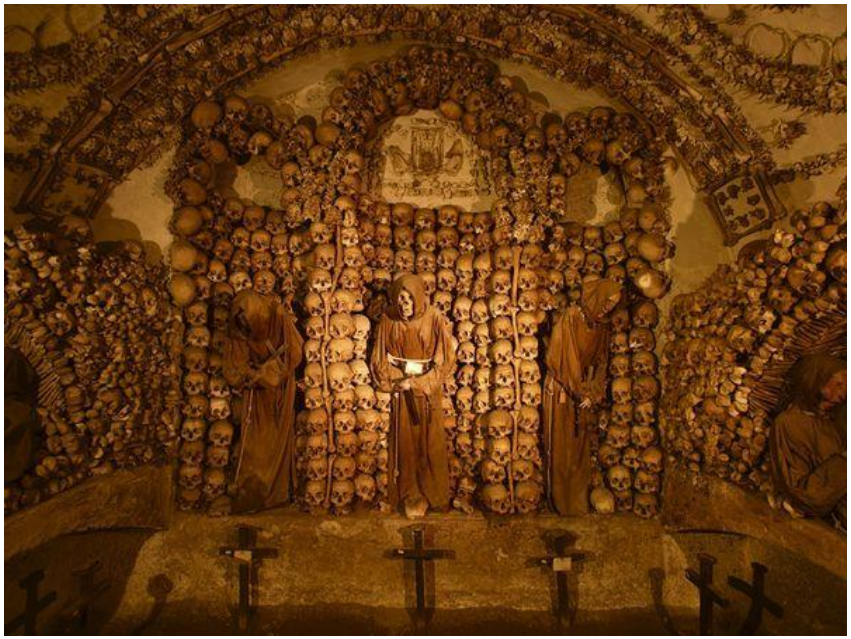
ARDP 2017 Unfilled Requests

Unfilled Antigen Negative Requests	
Lu(a-b-) E- c- M- S- Kp(a-)	hr^{B}- C- E- K- Fy(a-b-) N- s- Js(a-) (allele selected)
U- C-	hr^{S}- D- C- E- K- Fy(a-)(allele selected)
At(a-) Jk(a-) S-	hr^{S}-D- C- E- (allele selected)
At(a-) E-	hr^{B-} C- E- K- Fy(a-b-) N- s- Js(a-)(allele selected)
U- D- C- E- K- Fy(a-) V-	hr^{S}- C- E- K- S- (allele selected)
Jo(a-) E- Fy(a-b-) Jk(b-) Js(a-) Do(b-)	hr^{B}- C- E- K-F y(a-) S- (allele selected)
Jk(a-b-) D-	Jk(a-b-)
Kp(b-) C- e- K-	Jk(a-b-)
Jk(a-b-) K-	Ge:-2, -3
E- c- K- Fy(a-b-)N- s-	IgA Deficient Plasma
C- E- K- Fy(a-b-) Jk(b-) M- S- V- Js(a-) Go(a-)	IgA deficient Plasma
D- C -E- K- Fy(a-b-) Jk(b-) S- Js(a-)	
C- E- K- Fy(a-) Jk(a-) S-	

AABB Poster 2018. Fludd D, Maurer M, Facey, D, Keller, M, Nance S et al. Defining the Rarest of the Rare Blood Requests to the American Rare Donor Program: Unfilled Requests from 2017, BBC 87

This Crypt Case had Many Scary Creaks!

- Anti-hr^{S} with multiple other alloantibodies
- Has potential to make anti-D if do not match for *DAR1* allele – YIKES!
- Has potential to make anti- Fy3/5 – YIKES!



An elaborate homage to the dead—and a reminder of mortality to the living—adorns a crypt under Santa Maria della Concezione church in Rome. These macabre ornamentations are constructed from the bones of deceased Capuchin friars. Pinterest

American Red Cross

Case #2: What the Heck is in There?

Five years into the Sicily Mummy Project, six macabre collections are offering scientists a fresh look at life and death on the Mediterranean island from the late 16th century to the mid-20th.
National Geographic

American Red Cross

Case #2: Patient Presentation Information

- 63 year old male
- ABO/Rh: O+
- Diagnosis: anemia
- Race: Unknown
- Previous antibodies: Not known
- Transfusions:
  - One single donor platelet 4 months previous
  - RBCs transfused over 5 years ago
- Order for transfusion

Case #2 Current Serology

- Gel A/S: Cell 1: 2+

  Cell 2: 1+

- Gel panel:

  All Reagent RBCs positive: 1-2+ including autocontrol

- DAT:

Polyspecific:	2+
Anti-IgG:	2+
Anti-C3:	Not tested
Control:	Not tested

IRL Testing

- DAT:

  Polyspecific: 3+

  Anti-IgG: 3+

  Anti-C3: $+^{w}$

  Control: Negative

Mummies displayed along the walls of the Capuchin Catacombs of Palermo, Italy (pictures: Sterflinger)

Case #2: Initial Panel

																								PEG			
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			IS	PEG Anti-IgG		
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	2+		
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	2+		
5	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+			0	1+		
6	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+			0	1+		
AC																										0	3+		

Case #2: Initial and Autoadsorption Panel

																								PEG	PEG
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IS	PEG Anti-IgG	Auto Ads* Anti-IgG
1	+	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	0	3+	3+
2	+	+	0	0	+	0	0	+	0	+	+	0	0	+	+	0	+	+	+	+	+	0	+	0	3+	3+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+	0	3+	3+
4	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	0	0	3+	3+
5	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	3+	3+
6	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	0	3+	3+
AC																								0	3+	

*Autoadsorption with ZZAP treated RBCs (DTT + Papain)

Case #2: Autoadsorbed Serum with DTT Treated RBCs

																								DTT Rx			
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s				PEG Auto Ads* Anti-IgG		
1	+	+	0	+	+	0	0	+	0	+	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	+	0	+	0	+				2+		
4	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	0				2+		
5	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+				1+		
6	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+				1+		

Autoadsorption with ZZAP treated RBCs (DTT + Papain)

Reactivity decreased by one grade with DTT Treated RBCs

Allo - Adsorbed Sera Panel

																							R1 Ads		R2 ads		rr Ads	
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	UN Rx		Un Rx		Un Rx	
1	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	0	2+		2+		2+	
2	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	2+		2+		2+	
3	0	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	2+		2+		2+	
4	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	2+		2+		2+	
5	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	2+		2+		2+	
AC																													

R1 D+ C+ E- c- e+ K- Fy(b-) Jk(b-) N- s-
R2 D+ C- E+ c+ e- K-Fy(b-) Jk(a-) M- S-
rr D- C- E- c+ e+ K- Fy(a-) M- S-

Finally, Previous History…..

- Anti-LW
  - —non-reactive with DTT treated RBCs and auto adsorbed with no reactivity remaining
- No history of RhIg, IVIg or WinRho treatment

- Now what? Some choices….
  - —DTT treat RBCs and test with Adsorbed Sera?
  - —PEG adsorption?

Allo - Adsorbed Sera Panel: Neat and DTT Treated RBCs

																						R1 Ads		R2 ads		rr Ads	
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	UNRx	DTTRx	UnRx	DTTRx	UnRx	DTTRx
1	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	0	2+	0√	2+	0√	2+	2+
2	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	2+	0√	2+	0√	2+	2+
3	0	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	2+	0√	2+	0√	2+	0√
4	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	2+	0√	2+	0√	2+	0√
5	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	2+	0√	2+	0√	2+	0√
AC																													

R1 D+ C+ E- c- e+ K- Fy(b-) Jk(b-) N- s-
R2 D+ C- E+ c+ e- K-Fy(b-) Jk(a-) M- S-
rr D- C- E- c+ e+ K- Fy(a-) M- S-

DTT Sensitive Reactivity and
Anti- D??? WOW – did not see that coming!

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Case #2: Further Testing

																										PEG		
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			rr Ads	rr Ads DTT PEG IgG AHG		Eluate
1	+	+	0	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0			2+	1+		1+
2	+	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	0	+			2+	1+		1+
3	+	0	+	+	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+			2+	1+		1+
4	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	0			2+	0√		0√
5	0	0	0	+	+	+	+	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+			2+	0√		0√
6	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+			2+	0√		0√

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PEG Adsorptions: Advantages

- Shorter incubation times
- Fewer adsorptions needed

PEG Adsorptions: Concerns

- Loss of alloantibody activity
- Untreated RBCs do not pack as efficiently which may result in dilution of serum
- Correct serum to PEG to red blood cell ratio is needed to prevent precipitation and PEG concentrations differ between manufacturers

PEG Adsorption Summary

	Number allo antibodies (ABY) studied	Number ABY missed in PEG adsorption	Number ABY missed in other adsorption	Significant loss of ABY reactivity	Same ABY reactivity	Comment
Leger (1)	10	0	0	0	10	
Cheng (2)	1	0	0	Not Mentioned		
Barron (3)	14	2	0	4	13	
Judd (4)	11	1	0	4		Titers of 6 aby markedly weaker in PEG ads
Combs (5)	107	9	0	15	54	
Champagne(6)	2	2 (with 2 drops)	0	1 (with 4 drops)	0	
Das (8)	7	0	0	0	7	
Total	152	14 (9%)	0	24(16%)	84 (55%)	

References

1. Leger RM, Garratty G. Evaluation of methods for detecting alloantibodies underlying warm autoantibodies. Transfusion 1999;39:11-6.

2. Cheng CK, Wong ML, Lee AW. PEG adsorption of autoantibodies and detection of alloantibodies in warm autoimmune hemolytic anemia. Transfusion 2001;41:13-

3. Barron CL, Brown MB. The use of polyethylene glycol (PEG) to enhance the adsorption of autoantibodies. Immunohematology 1997;13:119-22.

4. Judd WJ, Dake L. PEG adsorption of autoantibodies causes loss of concomitant alloantibody. Immunohematology2001;17:82-5.

5. Combs MR, Eveland D, Jewet-Keefe B, Telen MJ. The use of polyethylene glycol in adsorptions: more evidence that antibodies may be missed. Paper presented at: AABB Annual Meeting 2001. San Antonio, TX; Transfusion 2001;41(Suppl):30S.

6. Champagne K, Moulds MK. Autoadsorptions for the detection of alloantibodies—should polyethylene glycol be used? Transfusion 1996;36:384.

7.Leger RM, Ciesielski D, Garratty G. Effect of storage on antibody reactivity after adsorption in the presence of polyethylene glycol. Transfusion 1999;39:1272-3.

8. Das SS, Chaudhary R. Utility of adsorption techniques in serological evaluation of warm autoimmune hemolytic anemia. Blood Transfus. 2009 Oct; 7(4):300-4.

What RBCs to Recommend for Transfusion

- D negative
- Unknown if allo or auto anti-D

Michelangelo's Tomb at Basilica of Santa Croce. Florence, Italy This tomb is ornamented with lamenting angels and a bust of Michelangelo.

American Red Cross

Summary of Crypt Case #2

- Should have been easy, given history of anti-LW
  - —And would have been terrific to know in advance
- BUT, reactivity was different in sample
- Since autoadsorption did not remove, (although did not have enough autologous RBCs to do PEG autoadsorption): there is a risk of adsorption of antibody to high prevalence antigen
- Recommend molecular testing for *RHD* variants

Crypt Case #3

When you think of Pompeii, you think of the massive eruption in 79 CE and subsequent mass killing of the Romans who inhabited Pompeii and Herculaneum. You may picture the eerie casts that lay around the streets of Pompeii and the faces of those who perished in the fires of Vesuvius. National Geographic

Patient History

- 64 year old male
- Coronary artery disease
- Possible Cath Lab procedure- awaiting surgery Italian descent

National Geographic cast from Pompeii

American Red Cross

Laboratory Findings

- O+
- Hgb: 11.7 gm/dL
- Panagglutinin on screen and panel
- Negative autocontrol noted
- Called floor for transfusion history
  - Received plasma 10 years ago
  - No transfusions within 3 months, but unable to get clear history of red cell transfusion

Medications

Allopurinol (zyloprim)	Magnesium oxide
Aspirin	Metroprolol (Lopressor)
Atorvastatin (Lipitor)	Morphine
Famotidine (Pepcid)	Oxycodone (OxyContin)
Heparin	Torsemide (Demedex)
Insulin	Docusate sodium (Colace)
Isosorbide mononitrate	Oxycodone/APAP (Percocet)
KCL	

IRL Testing – First IRL's Initial Assessment

ABO/Rh: O+

RH typing: C+ E- c+ e+

C typing +w/4+ AND c typing 1+/4+

DAT: Negative with polyspecific, anti-IgG, and anti-C3, control negative

Antibody Panel: AHG phase only with Anti-IgG:

1+ in Alb, 2+ in PEG, 2+ in ficin, 1+ in Gel, 1-2+ in LISS with reagent RBCs

negative autocontrol

First IRL Tested:

Serum with the following RBCs:	Patient's RBCs with antisera to the following antigens:
k-, Lu(a+b-), Js(a+b-), Vel-, Co(a-b+), Yt(a-), Cs(a-), Yk(a-), Lu(a-b-), Di(b-), LAN-, Ge:-2 -3, Kp(a+b-), Rg-, AnWj-, Ko	At^a, Sc1, Er^a, Cr^a

Antibody reacted to a dilution of 128
All antibodies to common antigens ruled out with alloadsorbed serum

NRLBGS Testing

- Reviewed the testing worksheets from referring IRL
- Confirmed negative DAT
- Rule out allo-antibodies first

Adsorbed Sera Panel: Critical to rule out antibodies to common antigens first

																							R1 Ads	R2 ads	rr Ads
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	PEG IgG	PEG IgG	PEG IgG
1	+	+	+	+	0	0	0	+	0	+	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	0	+	+	0	+	0	+			0√
4	+	0	+	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+			
5	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+			
AC																										

Anti-E, -c, -Fy^{b}, - M ruled out -RBC#1, R1 Ads
Anti-S ruled out RBC#1, R2 Ads
Anti-D ruled out on RBC#1 - rr Ads
Anti- s ruled out – RBC#2, R1 Ads
Anti-C ruled out RBC#3, rr Ads

R1 D+ C+ E- c- e+ K- Fy(b-) Jk(b-) N- s-
R2 D+ C- E+ c+ e- K-Fy(b-) Jk(a-) M- S-
rr D- C- E- c+ e+ K- Fy(a-) M- S-

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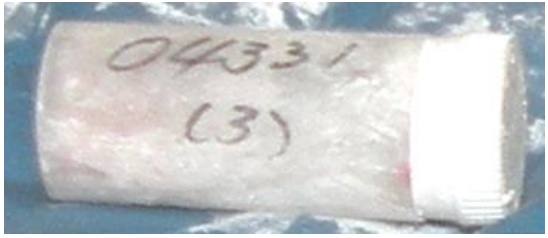
What is it?
RBC Library Time



Liquid Nitrogen storage of very small aliquots of rare RBCs

Joan Maurer, SBB, Lead, American Rare Donor Program

Case #3: RBC Library Time

																								PEG			
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			PEG IgG AHG		
1	+	0	0	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0			1+		
2	+	0	0	+	+	0	0	+	0	+	+	0	0	0	+	0	0	+	+	+	+	0	+			1+		
3	+	0	0	+	+	0	0	+	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+			1+		
4	+	0	0	+	+	0	0	+	0	+	0	+	0	0	0	0	0	0	+	+	+	0	0			Jr(a-)	0√		
5	0	0	0	+	+	0	0	+	0	+	0	+	0	0	+	0	0	+	+	0	+	0	+			Jr(a-)	0√		
6	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	0	+	0	+	+	+	+			Jr(a-)	0√		
AC																										0√		

Anti- Jr^{a}!, Pt RBCs Jr(a-)

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Monocyte Monolayer Assay Data

NRLBGS 1995-2017

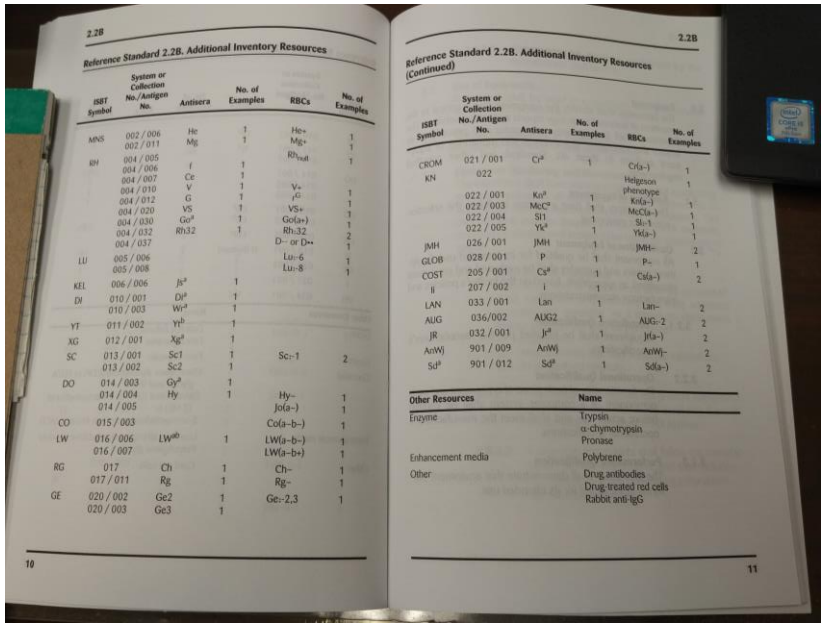
Anti-	TT	POS	NEG
AnWj	2	1	1
At^a	4	3	1
Au^a	1	0	1
Co^a	2	2	0
Cr^a	4	4	0
Di^b	11	8	3
Do^b	5	1	4
E	1	1	0
e	3	2	1
GE Sys	31	16	15
hr^B	3	2	1
hr^S	7	4	3
Hy	9	7	2
I	5	1	4
Jk3	1	0	1
Jo^a	10	4	6
Jr^a	15	9	6

Anti-	TT	POS	NEG
Js^b	1	1	0
Kp^b	6	2	4
Ku	1	1	0
Lan	11	7	4
LU Sys	21	19	2
Lu^b	14	12	2
Lw	3	2	1
M	11	5	6
N	2	1	1
$PP1P^k$	1	1	0
RH Sys	1	1	0
s	1	0	1
Sc1	1	1	0
Tc^a	2	1	1
U	4	2	2
Vel	13	10	3
Yt^a	195	119	76

Maurer J, Nance S, Nickle P. Monocyte Monolayer Assay (MMA) to Predict Clinical Significance of Alloantibodies: a 22 year Review. AABB Abstract 2018

What is the Scary Thing Here?

- Need to be able to have the library of RBCS and antisera to test in order to find the specificity
- AABB IRLs have a required inventory of rare RBCs and antisera to be accredited



2.2B

Reference Standard 2.2B. Additional Inventory Resources

ISBT Symbol	System or Collection No./Antigen No.	Antisera	No. of Examples	RBCs	No. of Examples
MNS	002 / 006	He	1	He+	1
	002 / 011	Mg	1	Mg+	1
RH	004 / 005			Rh_{null}	1
	004 / 006	f	1		
	004 / 007	Ce	1		
	004 / 010	V	1	V+	1
	004 / 012	G	1	$^{-G}$	1
	004 / 020	VS	1	VS+	1
	004 / 030	Go^a	1	Go(a+)	1
	004 / 032	Rh32	1	Rh:32	2
	004 / 037			D– or D++	1
LU	005 / 006			Lu:-6	1
	005 / 008			Lu:-8	1
KEL	006 / 006	Js^a	1		
DI	010 / 001	DI^a	1		
	010 / 003	Wr^a	1		
YT	011 / 002	Yt^b	1		
XG	012 / 001	Xg^a	1		
SC	013 / 001	Sc1	1	Sc:-1	2
	013 / 002	Sc2	1		
DO	014 / 003	Gy^a	1		
	014 / 004	Hy	1	Hy–	1
	014 / 005			Jo(a–)	1
CO	015 / 003			Co(a–b–)	1
LW	016 / 006	LW^{ab}	1	LW(a–b–)	1
	016 / 007			LW(a–b+)	1
RG	017	Ch	1	Ch–	1
	017 / 011	Rg	1	Rg–	1
GE	020 / 002	Ge2	1	Ge:-2,3	1
	020 / 003	Ge3	1		

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2.2B

Reference Standard 2.2B. Additional Inventory Resources (Continued)

ISBT Symbol	System or Collection No./Antigen No.	Antisera	No. of Examples	RBCs	No. of Examples
CROM	021 / 001	Cr^a	1	Cr(a–)	1
KN	022			Helgeson phenotype	1
	022 / 001	Ku^a	1	Kn(a–)	1
	022 / 003	McC^a	1	McC(a–)	1
	022 / 004	SI1	1	SI:-1	1
	022 / 005	Yk^a	1	Yk(a–)	1
JMH	026 / 001	JMH	1	JMH–	2
GLOB	028 / 001	P	1	P–	1
COST	205 / 001	Cs^a	1	Cs(a–)	2
Ii	207 / 002	i	1		
LAN	033 / 001	Lan	1	Lan–	2
AUG	036/002	AUG2	1	AUG–2	2
JR	032 / 001	Jr^a	1	Jr(a–)	2
AnWj	901 / 009	AnWj	1	AnWj–	2
Sd^a	901 / 012	Sd^a	1	Sd(a–)	2

Other Resources	Name
Enzyme	Trypsin α-chymotrypsin Pronase
Enhancement media	Polybrene
Other	Drug antibodies Drug-treated red cells Rabbit anti-IgG

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Almost as scary as a crypt!!!

Reference Standard 2.2B. Additional Inventory Resources

ISBT Symbol	System or Collection No./Antigen No.	Antisera	No. of Examples	RBCs	No. of Examples
MNS	002 / 006	Hil	1	Hil-	1
	002 / 011	Mg	1	Mg+	1
RH	004 / 005			Rh_{null}	1
	004 / 006	f	1		
	004 / 007	Ce	1		
	004 / 010	V	1	V+	
	004 / 012	G	1	r^G	1
	004 / 020	VS	1	VS+	1
	004 / 030	Go^a	1	Go(a+)	1
	004 / 032	Rh32	1	Rh32	1
	004 / 037			D- or D+	2
LU	005 / 006			Lu-6	1
	005 / 008			Lu-8	1
KEL	006 / 006	Jk^a	1		
DI	010 / 001	Di^a	1		
	010 / 003	Wr^a	1		
YT	011 / 002	Yt^b	1		
XG	012 / 001	Xg^a	1		
SC	013 / 001	Sc1	1	Sc:-1	2
	013 / 002	Sc2	1		
DO	014 / 003	Gy^a	1		
	014 / 004	Hy	1	Hy-	1
	014 / 005			Jo(a-)	1
CO	015 / 003			Co(a-b-)	1
LW	016 / 006	LW^{ab}	1	LW(a-b-)	1
	016 / 007			LW(a-b+)	1
RG	017	Ch	1	Ch-	1
	017 / 011	Rg	1	Rg-	1
GE	020 / 002	Ge2	1	Ge:-2,3	1
	020 / 003	Ge3	1		

Reference Standard 2.2B. Additional Inventory Resources (Continued)

ISBT Symbol	System or Collection No./Antigen No.	Antisera	No. of Examples	RBCs	No. of Examples
CROM	021 / 001	Cr^a	1	Cr(a-)	1
KN	022			Helgeson phenotype	1
	022 / 001	Kn^a	1	Kn(a-)	1
	022 / 003	McC^a	1	McC(a-)	1
	022 / 004	Sl1	1	Sl-1	1
	022 / 005	Yk^a	1	Yk(a-)	1
JMH	026 / 001	JMH	1	JMH-	2
GLOB	028 / 001	P	1	P-	1
COST	205 / 001	Cs^a	1	Cs(a-)	2
k	207 / 002	k	1		
LAN	033 / 001	Lan	1	Lan-	2
[illegible]	036/002	AUG2	1	AUG-2	2
JR	032 / 001	Jr^a	1	Jr(a-)	2
AnWj	[illegible]	AnWj	1	[illegible]	2
Sd^a	901 / 012	Sd^a	1	Sd(a-)	2

Other Resources	Name
Enzyme	Trypsin α-chymotrypsin Pronase
Enhancement media	Polybrene
Other	Drug antibodies Drug-treated red cells Rabbit anti-IgG

Effect of Enzymes and DTT (Dithiothreitol) on Antigens in Antibody Identification

Possible antibody specificity is based on *general* patterns of reactions against enzyme and DTT-treated (200mM) RBCs (assuming no anti-enzyme is present or an eluate is used).

Ficin/Papain	*Trypsin*	*α-chymotrypsin*	*DTT (200mM)*	*Possible specificity*
Neg	Neg	Neg	Pos	Bp^a; Ch/Rg; Xg
Neg	Neg	Neg	Neg	Indian; JMH
Neg	Neg	Pos	Pos	M, N, En^aTS; Ge2, Ge4
Neg	Pos	Neg	Pos	'N'; Fy^a, Fy^b
Variable	Pos	Neg	Pos	S, s
Variable	Pos	Neg	Weak or Neg	Yt
Neg	Pos	Pos	Pos	En^aFS
Pos	Neg	Neg	Weak or Neg	Lutheran; MER2
Pos - Papain Weak or neg - Ficin	Neg	Neg	Neg	Knops
Pos	Neg	Weak	Neg	Dombrock
Pos	Pos	Neg	Weak	Cromer
Pos	Pos	Neg	Pos	Some Diego (on 3rd loop)
Pos	Pos	Pos/Weak	Neg	LW
Pos	Pos/Weak	Pos/Weak	Pos	Scianna
Pos	Pos	Pos	Neg	Kell (but KALT & KYOR are trypsin sensitive)
Pos	Pos	Pos	Enhanced	Kx
Pos	Pos	Pos	Pos	ABO; En^aFR, U; $PP1P^k$; RH; Lewis; Fy3, Jk; most Diego; Colton; H; Ge3; OK; I/i; P, FORS; JR; LAN; Cs^a; ER; LKE, PX2; VEL; At^a; Emm; AnWj; Sd^a; PEL; MAM; ABTI

Courtesy of Christine Lomas-Francis
Immunohematology 2018, in press

Case #4: Amazing Serological Work

National Geographic

King Tutankhamun, like all prominent ancient Egyptians, hoped that people would remember him forever, calling out his name into eternity.
But even in his wildest fantasies, the teenage ruler could never have imagined that he would become the rock star of the pharaohs.
Since British archaeologist Howard Carter discovered his tomb in 1922, countless thousands of tourists have come to visit, descending a flight of stairs and a sharply sloping corridor to arrive at the painted burial chamber.

Case #4 Clinical History

- 59 year old Caucasian male
- Admitted through Emergency Department
- Symptoms included:
- Shortness of breath
- Fatigue

- No previous history of hospitalization
- Patient states no transfusions, ever

Case #4 – Laboratory Values

Laboratory Parameter	Patient's Value	Normal Range
Hemoglobin	5.4 g/dL	11.1- 15.9 g/dL
Reticulocyte Count	5%	0.5-2.5%
Bilirubin (Total)	2.5 mg/dL	0-1.2 mg/dL

American Red Cross

Case #4 – Hospital Testing

Automated Gel testing:

- Antibody Screen:
  - 1+ with all three antibody screening RBCs
- Gel antibody identification panel:
  - 3 RBCs negative
  - 8 RBCs positive: wk+ to 1+
  - Autocontrol: negative
- 2nd Gel antibody identification panel:
  - 2 RBCs negative
  - 9 RBCs positive: wk+ to 1+
  - Autocontrol: negative

Case #4 – Hospital Testing Interpretations

ABO/Rh: A positive

DAT: negative with anti-IgG in Gel DAT

Antibody Screen Method: Gel Test

Antibody Screen Results: Positive

Antibody Identification Method: Gel Test

Antibody Identification initial testing:

No specificity identified by reactivity

Case #4 – Challenges with Current Testing at Hospital

The hospital has automated Gel testing only, and performs antibody screen and has purchased 2 panels for Gel testing only

The hospital has no other identification methods or panels, and refers the sample to their blood center IRL

The physicians in charge of the patient manage the patient medically, awaiting antibody identification for compatible blood

Case #4– IRL Initial Testing

ABO/Rh: A+

DAT: negative with polyspecific AHG, anti-IgG, anti-C3 and control

Rh Phenotype: D+ C+ E- c+ e+

noted that C typing 1+ at Immediate Spin

and 4+ after incubation

Case #4 – Further Work in IRL

IRL Technologist evaluates the hospital information, sees no discernable specificity in Gel panels, decides to perform tube testing in PEG (similar sensitivity*)

	D	C	c	E	e	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	P1	Le^a	Le^b	M	N	S	s		PEG	
1	+	+	0	0	+	0	+	0	+	+	+	+	+	0	0	+	0	+	+	0	+	0		1+	
2	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+	0	+	0		2+	
3	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+		2+	
4	0	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	+		2+	
5	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+		2+	
6	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+		2+	
7	0	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	0	+	0	+		2+	
8	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0		2+	
9	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	+		2+	
10	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+		2+	
A/C																								2+	

*PEG – Polyethylene Glycol at antiglobulin phase with Anti-IgG

Case #4 – Evaluate IRL Panel

IRL Technologist performs tube test to determine the reactivity in other methods

- To establish that testing can be in PEG, since Gel testing requires specially prepared panel RBCs
- PEG panel showed slightly stronger reactivity (2+)
- One panel cell slightly weaker at 1+
- **Autocontrol positive in PEG - 2+ at AHG**
  - Shows importance of performing autocontrol with each method tested

Case #4 – Further Work – Notable Points

#1 RBC was 1+ vs. rest of panel 2+, may or may not be significant as was not a difference of 2 grades. Autocontrol 2+, and DAT was negative!

	D	C	c	E	e	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	P1	Le^a	Le^b	M	N	S	s		PEG	
1	+	+	0	0	+	0	+	0	+	+	+	+	+	0	0	+	0	+	+	0	+	0		1+	
2	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+	0	+	0		2+	
3	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+		2+	
4	0	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	+		2+	
5	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+		2+	
6	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+		2+	
7	0	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	0	+	0	+		2+	
8	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0		2+	
9	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	+		2+	
10	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+		2+	
A/C																								2+	

*PEG – Polyethylene Glycol at antiglobulin phase with Anti-IgG

Case #4 - Is this Antibody to High Prevalence Antigen or Autoantibody? Further Testing:

- Sent sample for molecular testing for predicted phenotype of common and some high prevalence antigens
- What if it is an antibody to High Prevalence antigen and DAT result is correct?
  - Allogeneic adsorption - to rule out underlying antibodies to common antigens which could complicate interpretation of testing with rare reagent RBCs, if present

Case #4 - Allogeneic Adsorption-
No antibodies to common antigens!

																							R1 Ads	R2 Ads	rr Ads
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	PEG IgG	PEG IgG	PEG IgG
1	+	+	0	0	+	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√
5	+	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√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0√	0√	0√
AC																								0√	0√	0√

Adsorbing RBCs:
R1 D+ C+ E- c- e+ K- Jk(a-) S-
R2 D+ C- E+ c+ e- K- Jk(b-) s-
rr D- C- E- c+ e+ K-

Note: In NRLBGS, selection of adsorbing RBCs includes S and s typed RBCs to eliminate any concerns with ambiguity of enzyme treatment to eliminate reactivity to S and s antigens

Case #4 - Is this Antibody to High Prevalence Antigen or Autoantibody? Further Testing:

- Allogeneic adsorption – no antibodies to common antigens detected in adsorbed sera - good, now we can proceed
- Subsequent testing considered:
  - Patient's serum with chemically modified RBCs to assist in determining antibody specificity
  - Patient's serum with RBCs negative for high prevalence antigens
  - Patient's RBCs with antisera to high prevalence antigens

Case #4 - Further Work – Test Other Methods to help in Identification of Antibody

Patient's Serum +	Antibody Screen Cell #1	Antibody Screen Cell #2	Autocontrol
Ficin Treated RBCs	$+^{W}$	$+^{W}$	$+^{W}$
DTT Treated RBCs	0√	0√	0√
Albumin Method	0√	0√	0√
Gel Method	1+	1+	1+

Notable results:

1. IRL Gel tests positive, including autocontrol, different from hospital testing (RBCs for Gel test manually prepared) which uses a different solution
2. DTT Treated RBCs non-reactive, ficin weaker, - not typical for autoantibody
   Most likely Blood System implicated: KEL
   Other antigens DTT sensitive and Ficin and Trypsin Resistant: LW
   Variable reactivity antigen to consider: Cr
3. Albumin testing also negative, due to technique or strength of antibody?

Case #4 – Further Antibody Investigation

High prevalence antigen negative RBCs tested:

RBC	Phenotype	PEG AHG Result
1	k-	2+
2	Kp(b-)	2+
3	Js(b-)	0√
4	Lu(a-b-)	2+
5	Yt(a-)	2+

Js(b-) RBCs negative!!!

Case #4 - Further Work – Genotype Results are In

Patient's genotype is in!

Predicted RBC Phenotype is:

D+ C+ c+ E- e+, Fy(a-b+), Jk(a+b+), M+ N+ S- s+ U+, K- k+ Kp(a-b+) Js(a-b+), Do(a+b-) Hy+, Sc:1.-2, Di(a-b+), LW(a+b-), Co(a+b-), Lu(a-b+), Yt(a+b-)

No negative results for high prevalence antigens, and in particular, predicted to be Js(b+)!

Case #4 - Updated Clinical Information

Hospital confirmed:

- Patient was not ever transfused
- Patient is Caucasian

Case #4 - Is this Autoantibody or Antibody to KEL System High Prevalence Antibody?

IRL decided to do autoadsorption since patient not transfused and autocontrol was positive in some techniques (though DAT negative) rather than thawing rare KEL System high prevalence antigen negative RBCS

Autoadsorption with papain treated patient's RBCs
Tests performed in PEG and read at AHG with anti-IgG, while 3 times adsorbed serum was still positive, four times autoadsorbed patient serum was negative!!!

	D	C	c	E	e	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	P1	Le^a	Le^b	M	N	S	s		X3		X4
I	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	+		$+^W$		0√
II	+	0	+	+	0	+	+	0	+	0	+	+	+	0	+	0	0	+	+	0	+	0		$+^W$		0√
III	0	0	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	+	+	+	+	+		$+^W$		0√

Case #4 – Antibody Identification

- Anti-Js^b specificity
- Patient's RBCs typed serologically Js(a-b+)
- No underlying alloantibodies to common antigens detected
- Confounding tests:
  - Negative DAT in tube and Gel tests
  - Positive autocontrol with anti-IgG in:
    - PEG test 2+
    - Ficin test $+^w$
    - IRL Gel test 1+

Case #4 – Testing Interpretations

- Autoadsorption removed anti-Js^b reactivity after four adsorptions
  - IRL considered that the antibody could have been diluted by the 4^{th} adsorption
  - IRL SOP allows up to six adsorptions
  - Therefore, concluded the antibody was adsorbed and was auto-specificity
- Patient's RBCs tested Js(b+)
- Patient's genotype predicted the phenotype as Js(b+)

Case #4 - Testing Not Performed but in Retrospect, of Academic Interest

- Did not test other rare KEL system high prevalence antigen negative RBCs
  - Maybe Js^b antigen is weaker on some rare RBCs and unknown
- Did not perform sequencing to look for variant Js^b
- Did not prepare and test an eluate from the patient's RBCs – even though DAT negative, might have been interesting
- Did not prepare and test and eluate from the adsorbing RBCs – to confirm Js^b specificity

Case #4 - Conclusions

- Patient's report concluded that this antibody was auto anti-Js^{b}
- Blood needs met by random units the same day
- Patient closely monitored with no laboratory or clinical signs of transfusion reaction
- Patient discharged and has not returned to the hospital

Tomb of Frederic Chopin Pere Lachaise cemetery in Paris Dreamstime

Heart of Chopin Warsaw, Poland Medical Express

American Red Cross

Case #4 – Lessons Learned from the Case

- The DAT does not always match the autocontrol
- Important to test autocontrol with each different serologic test method, the results could be different
- It helps to assess the serologic reactivity with different test methods
- Always attempt to phenotype the patient for the antigen you are assigning an antibody specificity to, it could be an autoantibody
- Genotyping is essential in complex cases

Immunohematology Cases from the Crypt

HAPPY HALLOWEEN – HOPE YOU HAD FUN AND LEARNED SOMETHING ALONG THE WAY

Sandra.Nance@redcross.org

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