Antibody Identification Using All the Tools in the Toolbox

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Conflicts of Interest

The presenters have no conflicts to declare.

Antibody Identification Using All the Tools in the Toolbox - Serology

- Effective use of test methods
- Experience of the technologist and supervisor
- Critical evaluation of the case history
- Critical evaluation of serologic and molecular test results

Antibody Identification Using All the Tools in the Toolbox – A Case

- What would happen in your facility if you saw this case?
- Presentation:
  - —45 y.o. female with Sickle Cell Disease (SCD) presents to ER in crisis
  - —She says she was previously pregnant and was transfused last 6 years ago following a D&C
  - —And that she had been transfused over the years before that

Routine Serologic Testing

- Serologic testing:
  - —Type B+, DAT negative with PS AHG
  - —Initial screen – all three RBCs positive in Gel
  - —Panel – all RBCs positive, autocontrol negative

Initial Panel

																									Gel	
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			IgG	
1	+	+	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	+			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	+			2+	
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0			2+	
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+			2+	
AC																										0√	

AC=Autocontrol - Patient's RBCs and Patient's serum

Immunohematology Serologic Testing

- Initial panel indicates antibody reactive to the same strength with all cells tested, autocontrol negative
- Possibilities:
  - —Antibody to high prevalence antigen
  - —Multiple antibodies to common antigens
  - —Medication caused reactivity (anti-CD38, or others)
- Next Steps
  - —Get pheno or genotype
  - —Test serum with phenosimilar cells
  - —Perform adsorptions with allogeneic RBCs
  - —Get medication, other clinical history

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Adsorption Studies: To Rule Out ABY to Common Antigens

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

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Adsorption Studies: To Rule Out ABY to Common Antigens

#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	R1 Ads IgG	R2 Ads IgG	rr Ads IgG
1	+	+	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	+	0√	2+	2+
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√	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	+	2+	0√	0√
7	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-
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1	+	+	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	+	0√	2+	2+
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√	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	+	2+	0√	0√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0√	0√	0√
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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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Immunohematology Serologic Testing

- Serologic testing:
  - —Adsorption removed the pan-reactivity
  - —Anti-S and anti-C identified in adsorbed sera
- Next steps – identify the reactivity in neat serum (potential antibody to high prevalence antigen)
  - —Phenotype or Genotype
  - —If phenotyping, concentrate on typing for "common" high prevalence antigens seen in patients of African ancestry
  - —Test serum with "modified" RBCs (C- S-)

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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[a+TS]; Ge2, Ge4
Neg	Pos	Neg	Pos	'N'; Fy[a], Fy[3]
Variable	Pos	Neg	Pos	S, s
Variable	Pos	Neg	Weak or Neg	Yt
Neg	Pos	Pos	Pos	En[a+]FS
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[a+]FR, U; PP1P[k]; RH; Lewis; Fy3; Kidd; 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* 2017, in press

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Immunohematology Serologic Testing

- Send for Genotyping
- Meanwhile set up serologic tests simultaneously

RBCs	Ficin	Trypsin	αChymotrypsin	DTT
Phenosimilar	2+	2+	2+	2+
Autologous	0√	0√	0√	0√

- **Well, Rats! All positive**

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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
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14 Courtesy of Christine Lomas-Francis *Immunohematology* 2017, in press

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Variable	Pos	Neg	Pos	S, s
Variable	Pos	Neg	Weak or Neg	Yt
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Immunohematology Serologic Testing

- Genotype
  - Often already known in patients with SCD
- Type for "common" AA high prevalence antigens:
  - Js^b, U, Fy3 (Fy^a and Fy^b) At^a, Jo^a, Hy, Sl^a
- Patient types are in:
  - S-s-U- by serology
  - Fy(a-b-) by serology, but C- S- Fy(a-b-) RBC on initial panel was positive
  - S-s-U- by genotype, not $U+^{VAR}$
  - Pos for other high prevalence antigens tested
- Serum non-reactive with frozen and liquid library S- s- U- RBCs

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Patient Clinical Events

- Physicians ordered exchange transfusion, 6 units needed ASAP
- Facility had 2 S- s- U- (by serology)units in house
- 4 units requested through American Rare Donor Program
- Patient's condition worsened, 2 units were transfused
- Patient stabilized, exchange transfusion (and ARDP order) cancelled
- Eleven days later, Hct dropped 4% below pre-transfusion levels, patient critical

AND

- Anti-U detected in serum and eluate
- 6 C- S- U- units requested through ARDP

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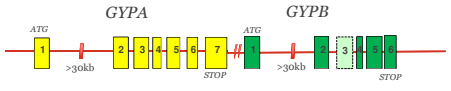
The Genetics of MNS

- The glycophorin gene family members that encode blood group antigens:
  - *GYPA* encodes GPA with M and N antigens
  - *GYPB* encodes GPB with S and s antigens
- Located on human chromosome 4q31-34
- >95% DNA sequence homology



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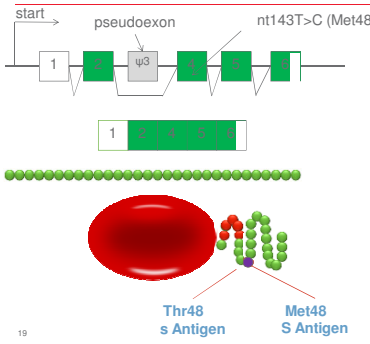
GYPB: Gene Schematic



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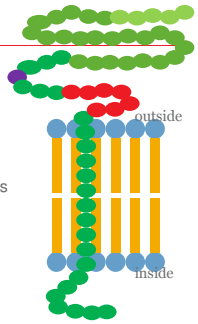
U, the universal antigen

- In the 1960s, it was noted that **S- s-** RBCs were associated with U- status
- ~1% of African Americans are S- s- U-
- The U- phenotype is common (37%) in West Africa and rare (0.001%) in Caucasians
- **U epitope** was mapped to GYPB amino acids 33-38 (near the membrane)
- In 1987, the genetic basis of S-s-U- was found to be a large deletion within the *GYPB* gene



Greenwalt *et al. PNAS* 1954 40:1126-1129.
Francis and Hatcher. *Vox Sang* 1966 11:213-216.
Storry and Reid *Transfusion* 1996; 36: 512-516.
Reid, ME *et al., Immunohematology*, 1997, 13:111-114.
Reid, ME. Immunohematology, 1999, 15:5-9.

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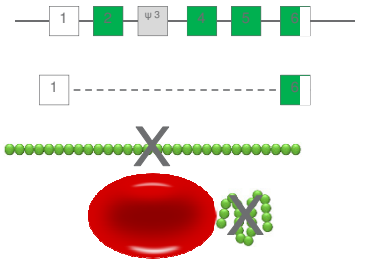
Genetic Determinant of S-s-U-



Huang CH *et al. Blood* 1987 70(6):1830-5.

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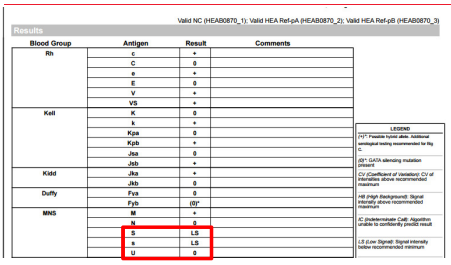
Predicted Phenotypes: MNS



Blood Group	Antigen	Result	Comments
Rh	C	+	
	c	0	
	e	+	
	E	0	
	V	+	
	VS	+	
Kell	K	0	
	k	+	
	Kpa	0	
	Kpb	+	
	Jsa	0	
	Jsb	+	
Kidd	Jka	+	
	Jkb	0	
Duffy	Fya	0	
	Fyb	(0)*	
MNS	M	+	
	N	0	
	S	LS	
	s	LS	
	U	0	

INTERPRETATION: The sample carries the *GYPB* deletion.

Predicted phenotype: S-s-U-

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Clinical Significance of anti-U

- Transfusion
  - Mild to severe transfusion reactions, with a fatality reported
  - Associated with decreased survival of transfused U+ RBCs
- Hemolytic Disease of the Fetus and Newborn (HDFN)
  - Mostly mild, with one case reported that required intrauterine transfusion

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U+^{VAR}

- The U antigen can exist in a variant form
  - S-s- RBCs that were reactive with anti-U were called U variant (U+^{VAR})
  - U+^{VAR} is expressed very weakly, and is often not detected
- S-s-U- individuals can make anti-U-like antibodies to U+^{VAR} cells
- S-s-U+^{VAR} individuals can make anti-U
- Historically, U+^{VAR} was detected using serology
  - adsorption/elution with anti-U
  - PEG enhancement
  - limited by the anti-U specificity (anti-U vs. anti-U/GPB)
- The genetic bases of U+^{VAR} was elucidated in 2003

Issitt PD. *Vox Sang* 1990;58:70-71.
Reid ME et al. Immunohematology 1997;13:111-4.
Peyrard T *et al. Transfusion* 2012

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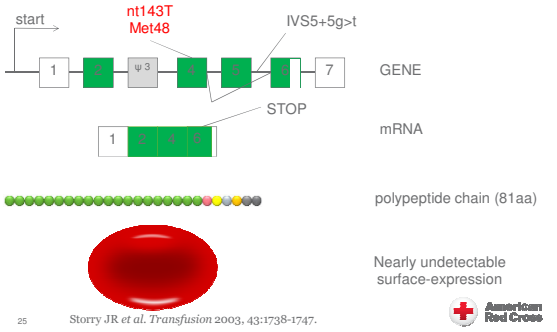
Genetic Determinant of U+^{VAR}, Part I

GYPB.P2 or GYPB IVS5



25 Storry JR *et al. Transfusion* 2003, 43:1738-1747.

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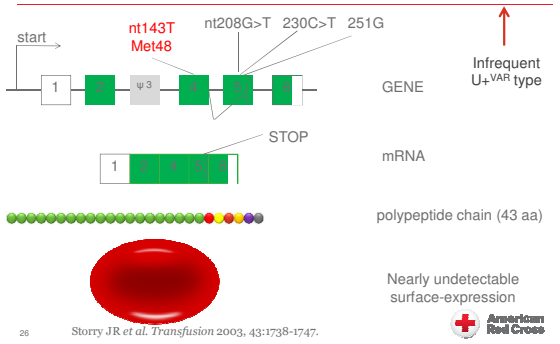
Genetic Determinant of U+^{VAR}, Part II

GYPB.NY or GYPB 230



26 Storry JR *et al. Transfusion* 2003, 43:1738-1747.

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Antibody Studies with Rare RBCs

																									PEG Serum	ELUATE
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	U Mol type	IgG	IgG
1	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	0	0	U+ VAR	1+	2+
2	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	0	U+ VAR	1+	2+
3	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	0	U-	0√	0√
4	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	U-	0√	0√
AC																									0√	0√

If the anti-U is anti-U/GPB, then PEG method may enhance reactivity and react with U+^{VAR} RBCs

Reid ME, Storry JR, Maurer J, Nance S. Practical Method for Determination of the U Status of S-s- erythrocytes. *Immunohematology* 1997; 13:111-114.

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Antibody Identification Using All the Tools in the Toolbox – Rare Red Cells

- 6 units requested from ARDP, C- S- known to be U negative by molecular testing, no U+^{VAR} or U+^{VAR} unknown
- ARDP Current SOP – partial copy of unit request form

RARE UNIT REQUEST FORM

Product Request (to be completed by requesting ARDP member))

Date/time/completed by: _____ | Patient ABO/Rh _____ | Transfusion date _____ On hold until _____ (date)

Product requested: RBCs ___ IgA def plasma* ___ Other _____ # units requested _____

*If IgA def plasma requested, was anti-IgA detected? Yes ___ No___ Date/place of testing _____

*If IgA def plasma requested, is need: emergency _____ non-emergency _____

Phenotype needed: (Circle antigens for which RBC units should be negative)

D C E c e K Fy^a Fy^b Jk^a Jk^b M N S s P1 Le^a Le^b Lu^a Lu^b

C^w V VS k U Vel hr^B hr^S Kp^a Kp^b Js^a Js^b Jk3 Jr^a At^a Co^a Co^b Di^a Di^b

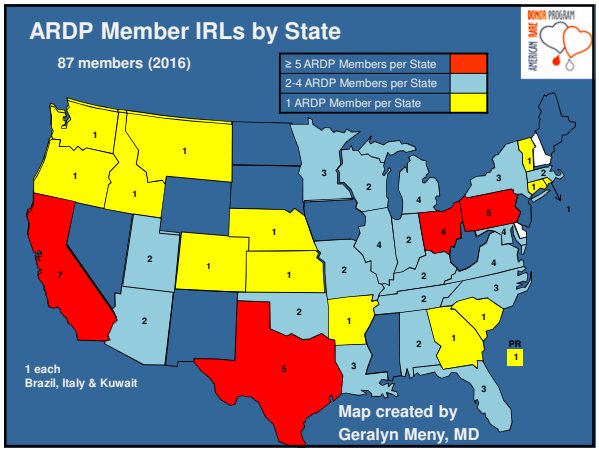
Do^a Do^b Ge2 Ge3 Gy^a Hy I Lan P P^k Sc1 Yt^a Other _____

If requesting U neg or Vel neg, will accept variants? Yes ___ No ___

If no, will accept U neg units not molecularly tested? Yes ___ No ___

ARDP Member IRLs by State

87 members (2016)



Antibody Identification Using All the Tools in the Toolbox – ARDP SOP 2017

- Donor submission criteria for American Rare Donor Program Members:

(e) Antigen confirmation is routinely based on serologic test pending antisera with exception of:
(1) Molecular testing is required for submission as:
hr^B-, hr^S-, V-, VS-, U-, Do(a-), Do(b-), Jo(a-), Hy-

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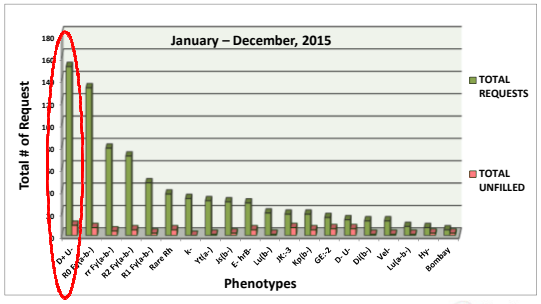
Requests and # Unfilled Requests:
Top 20 ARDP Phenotypes



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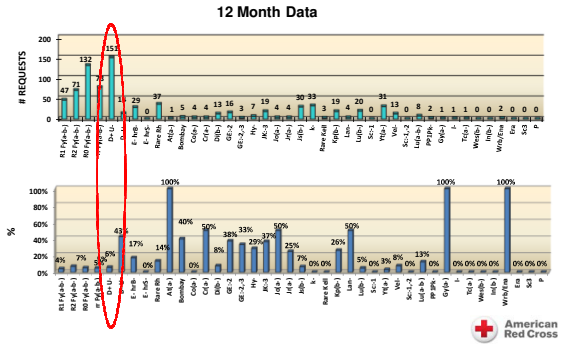
American Rare Donor Program –
Unfilled Requests / % Unfilled Requests



American Red Cross

The Case: Genotyping for U status

- Patient's RBCs were U- (*GYPB* deleted)
- Transfused Unit Investigation
  - —One donor confirmed U- (*GYPB* deleted)
  - —One donor was S-s-U- serologically
    - subsequent molecular testing showed donor was U+^{VAR}
- Records of previously transfused donor units were evaluated by National Molecular Laboratory in Philadelphia
  - —6 U+^{VAR} units transfused
- Increases in the patient's anti-U reactivity occurred following receipt of U+^{VAR} donor blood

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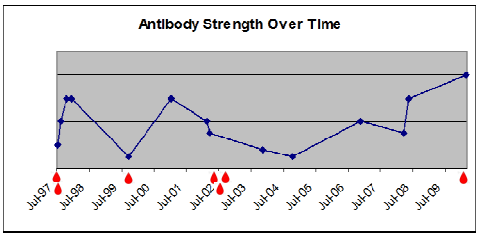
American Red Cross

Another Look at the Timeline



♦ Transfusion of U+^{VAR} Unit

NC Johnson, SB Learn, S Nance. *Transfusion* 2011:51;155A (Suppl)

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American Red Cross

How do Blood Centers handle U?

- American Red Cross Greater Chesapeake and Potomac region developed a survey in 2011 to assess how IRLs characterize S- s- donors as well as how they handle U- patients.

- 57 blood centers queried
- 35 Red Cross regions
- 17 non-Red Cross blood centers (US, Canada, Sweden, New Zealand)

- 34 blood centers (60%) responded
- 25 Red Cross regions
- 8 non Red Cross blood centers

35 Johnson *et al. Transfusion* 51(9): SP297 2011

American Red Cross

Survey Responses, 2011

Q3: When U- units are requested, is molecular confirmation of antigen status of the patient requested?	
YES	NO
26%	74%

Q4: When U- units are supplied, is molecular confirmation of antigen status of the donor provided?	
YES	NO
58%	42%

How are we doing now?

36 Johnson *et al. Transfusion* 51(9): SP297 2011

American Red Cross

Red Cross and U

- American Red Cross uses molecular methods to characterize S- s- donors
  - Donors who type S-s- or who are listed in the donor database as U- based on serology alone are being genotyped
    - Nearly half have been found to be U+^{VAR}

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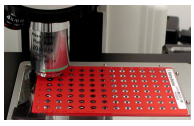
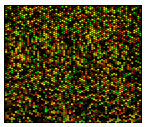
Red Cross and U

We routinely screen donors for U status using genotyping

The American Red Cross National Molecular Lab has screened more than 150,000 since 2011

- 54 new U- and 73 U+^{VAR} donors In 2012
- 55 new U- and 56 U+^{VAR} donors In 2013
- 58 new U- and 88 U+^{VAR} donors In 2014
- 81 new U- and 111 U+^{VAR} donors in 2015
- 145 new U- and 160 U+^{VAR} donors in 2016





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U: Take Home Messages

- Mostly all U- RBCs are S-s- but not all S-s- RBCs are U-
- Molecular methods can differentiate U negative from U+^{VAR}
- S-s- blood donors (new and historic) should be genotyped to determine if they are U- or U+^{VAR}
- Patients with anti-U should be genotyped to determine if they are U- or U+^{VAR}
- U- patients with anti-U should be given only U- blood

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Antibody Identification Using All the Tools in the Toolbox- Clinical- Case 2

- National Reference Laboratory for Blood Group Serology receives stat request for antibody identification from an AABB Accredited IRL
- What do we know about the case:
  - 18 y.o. female with Sickle Cell Disease
  - A negative (!)
  - Hgb 8.2, Hct 22.9
  - Not transfused in last 3 months
  - Transfused 2 units 7 years ago and 1 unit 9 years ago
  - No history of pregnancies, current pregnancy test negative (whew!)
  - Medications: Azithromycin, folic acid, Decadron, Senna-Plus, Hydroxyurea, Claritin, Prilosec, Protonix, Tylenol, Toradol

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Additional Information Case 2

- Physician note on the request is to use A's only*
- 2 units requested
  - Phenotype specific and
  - Antigen negative
- Previous/current antibodies listed as
  - Anti-e Anti-K Anti-Fy^{a} Anti-Jk^{b}
- Physician orders one unit to be sent now
- Noted on form that 2nd unit is requested to be sent and that patient is stable

*Refaai M, Henrichs K, Cahill C, Kirkley SA, et al, Transfusion of ABO Non-identical Red Cells and Mortality in Patients Undergoing Massive Transfusion. AABB Abstract Orals 2016

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Serology at NRLBGS

- First discovery – Patient's D type
  - Negative first read
  - Positive at AHG phase
  - Possible partial D?
- DAT Negative with polyspecific AHG
- Set up a panel of Ag negative reagent RBCs
- Evaluate *RHD*, *RHCE* variant testing
  - suspect D and e variants

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RBC Genotyping Panel

Results

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	0	
	e	+	Anti-e
	E	0	
	V	0	
	VS	0	
Kell	K	0	Anti-K
	k	+	
	Kpa	0	
	Kpb	+	
	Jsa	0	
	Jsb	+	
Kidd	Jka	+	
	Jkb	0	Anti-Jk^b
Duffy	Fya	0	Anti-Fy^a
	Fyb	(0)*	
MNS	M	+	
	N	+	
	S	0	
	s	+	
	U	+	

RHD Genotyping: Medium Resolution

TESTING PERFORMED			RESULT
RHD Variants	Method	Analyte: Nucleotide (Amino Acid)	Nucleotide(s) Detected
RHD Exon 8	RFLP	1136C>T (T379M)	C
wRHD BEADCHIP™	RHD Array*	602C>G (T201R)	G
		667T>G (F223V)	G**
		1025 T>C (I342T)	C

*Only nucleotides which differ from consensus sequence are listed.
**Low signal at *RHD* c.676G>C marker was observed and is a known limitation of *RHD* BeadChip™ in samples homozygous for c.667G.

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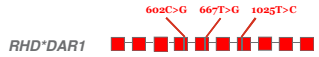
American Red Cross

RHD Genotyping: Medium Resolution



Probable Genotype: *RHD*DAR1* (hemizygous or homozygous)

Predicted Phenotype: Partial D+

The patient may be at risk for production of allo-anti-D

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American Red Cross

RHCE Genotyping: Medium Resolution

RHCE Common	Method	Analyte	Product present/absent
RHCE gene	RHCE Array	C	absent
		c	present
		Analyte: Nucleotide (Amino Acid)	Nucleotide(s) Detected
RHCE Exon 5	RHCE Array	676G>C (A226P)	G
RHCE Variants	Method	Analyte: Nucleotide (Amino Acid)	Nucleotide(s) Detected
RHCE Exon 2	RFLP	254C>G (A85G)	C
wRHCE BEADCHIP™	RHCE Array*	48G>C (W16C)	G/C
		712A>G (M238V)	G

*Only nucleotides which differ from consensus sequence are listed.

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American Red Cross

RHCE Genotyping: Medium Resolution

Table 1: Genetic markers in the RHCE BeadChip Assay

Amino Acid	Nucleotide Polymorphism
W16C	48 G>C √
A36T	106 G>C
Q41R	122 A>G
P103S	307 C>T
*109Ins	109 bp intron 2 insert
R114W	340 C>T
L115R	344 T>G
S122L	365 C>T
T152N	455 C>A
R154T	461 G>C
M167K	500 T>A
G180R	538 G>C
R201T	602 G>C

Amino Acid	Nucleotide Polymorphism
V223F	667 G>T
A226P	676 G>C
Q233E	697 C>G
M238V	712 A>G √
L245V	733 C>G
V250M	748 G>A
dT744dC	744 T>C
A273V	818 C>T
I306V	916 A>G
G336C	1006 G>T
T342I	1025 C>T
Rh r^s	Cde^s 5'UTR

Known hr^S- Alleles	
*ceAR**	48C,712G, 733G, 787G, 800A, 916G
ceEK	48C,712G,787C, 800A
ceMO	48C,667T
ceBI	48C,712G,818T,1132G
ceSM	48C,712,818T

*with or without c.698G or 455A

Unexpected genotype result
Higher resolution testing is needed

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American Red Cross

RHCE Genotyping: High Resolution

TESTING PERFORMED			RESULT	
RHCE Variants	Method	Analyte: Nucleotide (Amino Acid)	Nucleotide(s) Detected	Amino Acid
RHCE Sequencing	cDNA seq*	*RHCE* PCR Product	48G/C 712G 787G 800A	W16C 238V 263G 267K
		RHCE plasmids (N=4)	712G 787G 800A	238V 263G 267K
		RHCE plasmids (N=1)	48C 712G 787G 800A	16C 238V 263G 267K

*Only nucleotides which differ from consensus sequence are listed.

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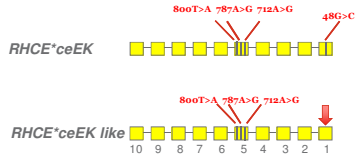
American Red Cross

RHCE Alleles



Probable Genotype: ***RHCE*ceEK / *RHCE*ceEK like***

Probable Phenotype: C-, E- partial c+ partial e+ hr^S-

The patient may be at risk for production of allo-anti-c, -e, -f, -hr^S

American Red Cross

Initial Panel: Let's See What We've Got

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

All reagent RBCs tested were e- K- Fy(a-) Jk(b-) and D+

American Red Cross

Adsorbed Sera Panel: Let's rule out the antibodies to common antigens

#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	R1 Ads IgG	R2 ads IgG	rr Ads 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-

American Red Cross

Serologic Summary

- History of anti-e, -K, -Fy^a, -Jk^b
- Anti- D, -C, -E, -Fy^b, -Jk^a, -M, -N, -S, -s ruled out
- What is the pan-reactive antibody seen on initial panel then?
  - An antibody to high prevalence Ag
  - What is the true specificity of the historic anti-e?

American Red Cross

Let's go to the Library

RHD and *RHCE* Variant RBCs

RBCs					PEG IgG
	D	*RHD* variant	e	*RHCE* variant	
r"r"	+	NA	o	NA	3+
Ro 6109	+	*DAR*	+	*ceAR/ceAR*	o√
CTo269	o	NA	+	*ceEK/ceEK*	o√
GJ 1678	+	*DAR/DAR*	+	*ceEK/ceAR*	o√
KC 0104	+	*DAUO/DAR*	+	*ceMO/ceAR*	o√
CB 0030	+	*DAUO/DAUO*	+	*ceMO/ceMO*	o√

Neat Sera
Also ruled out:
Anti-Fy^b
Anti-Fy3
Anti-Hy
Anti-Js^b

Historic anti-e is now testing like anti-hr^S and likely anti-HR (check out reactivity with r"r")

American Red Cross

Let's go to the Library and Adsorbed Sera

RHD and *RHCE* Variant RBCs

RBCs					PEG IgG	ABY	R2 Ads Sera	
	D	*RHD* variant	e	*RHCE* variant		Anti-Hr/hr^S		Anti-Hr/hr^S
rr	o	NA	+	NA	3+	both	3+	hr^S
R2	+	NA	o	NA	3+	Hr	o√	NA
Ro 6109	+	*DAR*	+	*ceAR/ceAR*	o√	NA	o√	NA
ST3013	+	NA	+	*ceMO/cE*	3+	Hr	o√	NA
WH0598	+	NA	+	*ceEK/cE*	3+√	Hr	o√	NA

Reagent RBCs were also K- Fy(a-) Jk(b-)

Adsorbing RBC – D+ C- e- K- Fy(a-b-) Jk(a-)

American Red Cross

Clinical Relevance of anti-Hr and -hr^S

RBCs	e	*RHCE* variant	Saline IgG	MMA %	MMA Interpretation
rr	+	NA	3+	35.0%	Positive
R2	0	NA	2+	44.1%	Positive
Ro 6109	+	*ceAR/ceAR*	0√	0.2%	Negative
Ro 2710	+	*ceMO/ceMO*	0√	0.3%	Negative
Auto	+		0√	0.0%	Negative

MMA Reagent RBCs were also K- Fy(a-) Jk(b-)

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RHCE Genotype Matching Tiers

	Allele 1					
Allele 2	*ceEK*	*ceEK no 48C*	*ceAR*	*ceMO*	*ceSM*	*ceBI*
ceEK	2	1	3	3	3	3
ceEK no 48C		2	3	3	3	3
ceAR			3	3	3	3
ceMO				3	3	3
ceSM					3	3
ceBI						3

Tier 1	Perfect match on both alleles
Tier 2	Donor homozygous for one of the alleles of the patient
Tier 3	Donor has same phenotype (hrS) but different allele(s)

Beware! Not all hr^S- types are compatible!

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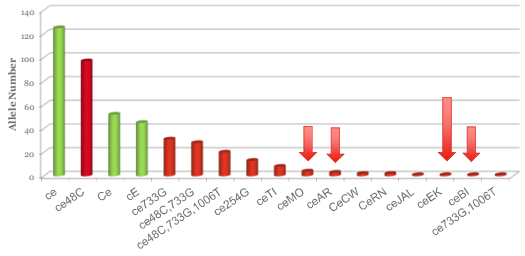
RHCE Alleles in African American Donors



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

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Conclusions

- Challenging antibody identification cases can be tackled using "all the tools in the toolbox"
- Molecular methods are helpful to identify variant antigens
- Molecular methods are efficient at identifying donors lacking high prevalence antigens or expressing variant antigens
- The American Rare Donor Program can assist in locating rare blood for patients with alloantibodies, including *RH* genotype-matched donors.

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Thank you for your attention!

Questions?

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