

Molecular Testing REALLY does make a difference

Overview and Objective

Molecular testing has become an essential laboratory tool. The Immunohematology Reference Lab (IRL) relies on molecular testing to aid in antibody identification, help determine clinical significance of an antibody, and differentiate between auto- and allo-antibodies.

These case studies will highlight the usefulness of molecular testing in our routine testing.



Case 1

• Age: 20

Gender: Female

Race: Unknown/other
Diagnosis: Sickle cell anemia
Transfusions: 1 unit, February 2022

Hgb/Hct: 6.2/16.5Sample Date: 08/23/2022

Referring facility's results: O Pos, history of anti-D

Hospital is requesting 1 unit ASAP, Sickle cell protocol C-E-K-HgbS-



3

Initial IRL Testing

ABO/Rh: O PositiveDAT: Negative

Initial IRL Panel:

Cell	Rh-hr	D	С	Е	С	е	f	K	k	Fyª	Fy⁵	Jka	Jk ^b	М	N	S	s	Lea	Le ^b	P ₁	Lua	Lu ^b	TEST	ΓING
																							IS	PEG/IAT
1	R1wR1	+	+	0	0	+	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0	2+
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	+	0	+	+	0	+	0	2+
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	0	+	0	2+
4	Ror	+	0	0	+	+	+	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+	0	2+
5	r'r	0	+	0	+	+	+	0	+	+	+	+	+	+	0	+	0	0	+	+	0	+	0	1+
6	r"r	0	0	+	+	+	+	0	+	+	+	+	+	+	+	0	+	+	0	0	0	+	0	1+
7	rr	0	0	0	+	+	+	+	+	0	+	+	0	+	+	0	+	0	+	0	0	+	0	1+
8	rr	0	0	0	+	+	+	+	+	+	0	+	+	0	+	+	+	+	0	+	0	+	0	1+
9	rr	0	0	0	+	+	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	1+
10	rr	0	0	0	+	+	+	0	+	+	+	+	0	+	+	+	0	0	+	+	0	+	0	1+
11	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	0	2+
										Patient a	auto con	trol											0	0✓



Next Steps

DTT-treated rule out screen:

Cell	Rh-hr	D	С	Е	С	е	f	К	Fyª	Fy ^b	Jkª	Jk ^b	М	N	s	s	Leª	Le ^b	P ₁	N	EAT	DTT TREATED
																				IS	PEG IAT	PEG/IAT
1	R1R1	+	+	0	0	+	0	0	0	+	0	+	+	0	+	0	0	+	0	0	2+	2+
2	R2R2	+	0	+	+	0	0	0	+	0	0	+	+	0	0	+	0	+	+	0	2+	2+
3	rr	0	0	0	+	+	+	0	+	+	+	0	0	+	0	+	0	+	0	0	1+	w+

Patient phenotype performed:

C- E- c+ e+ K- Fy(a-b-) Jk(a+b-) M- N+ S- s+ Le(a-b-) P₁+

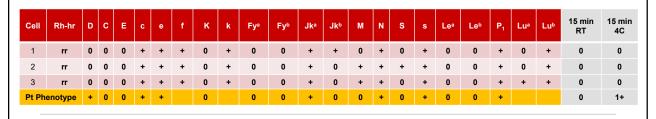


5

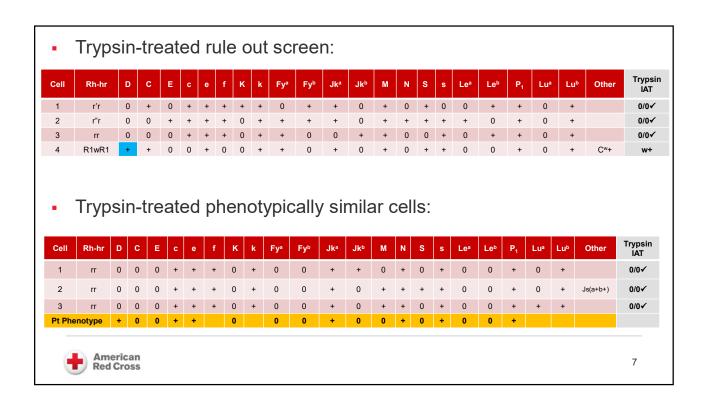
Phenotypically similar cells:



Cold Screen:

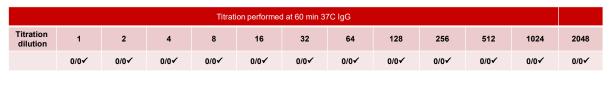




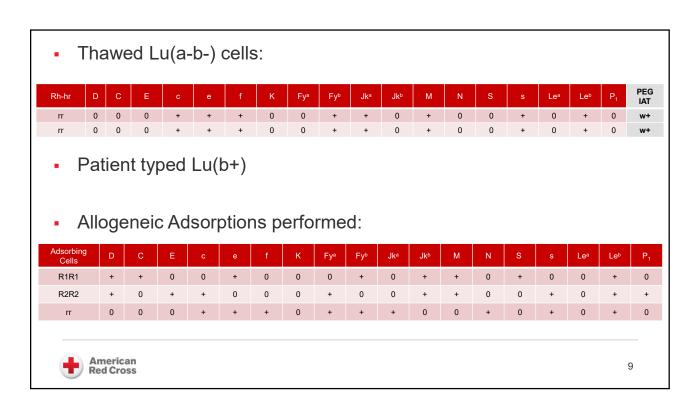


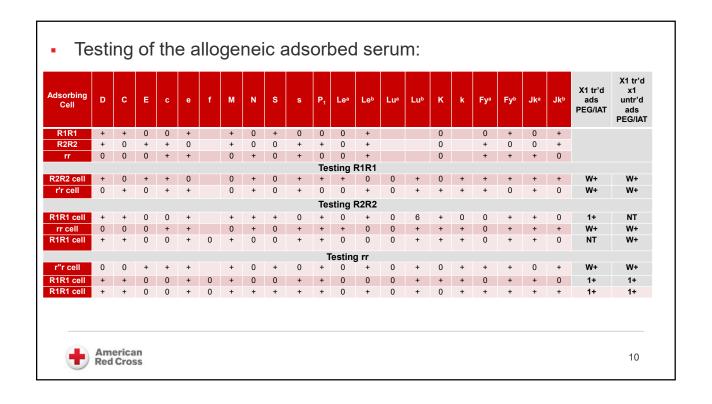
What we know at this point:

- Patient has history of anti-D.
- PEG/IAT is positive, negative auto control
- Cold screen is negative
- Trypsin/IAT is negative, most alloantibodies ruled out
- Phenotypically similar cell titer:

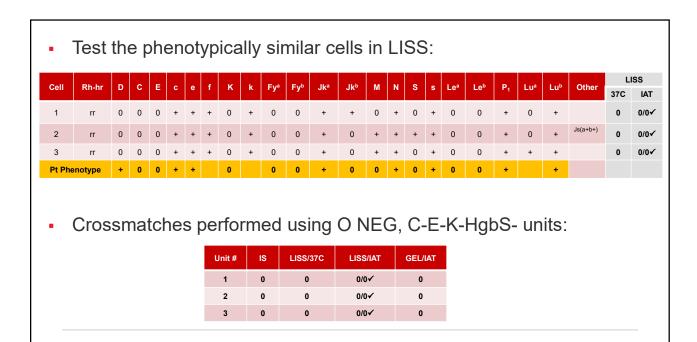




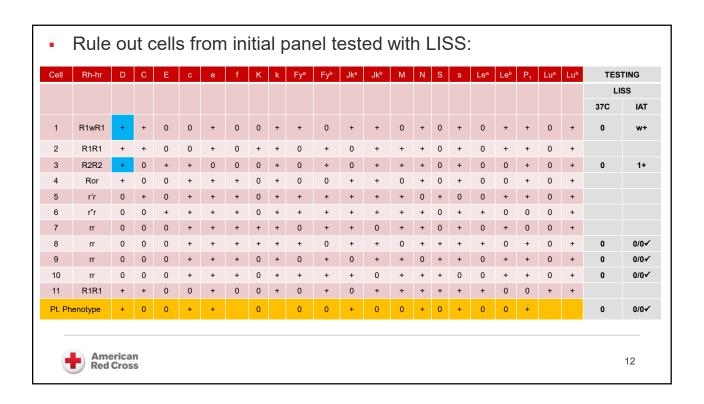




11



American Red Cross



Case 1 Conclusions:

- Anti-D detected at LISS/IAT, Trypsin/IAT, DTT/IAT.
- All other alloantibodies have been ruled out.
- Recommend sending sample RHD genotyping to the NML
- Broad nonspecific reactivity noted using PEG.
- Crossmatch compatible O NEG, C-E-K-HgbS- (per sickle cell protocol)



13

Genotype for RHD variant report for CASE 1

TESTING REQUESTED: Genotype for RHD variants

Т	ESTING PERFORM	MED	RESULT			
RHD Variants	Method	Analyte: Nucleotide (Amino Acid)	Nucleotide(s) Detected			
		186G>T (L62F)	T			
wRHD	DUD Amoust	410C>T (A137V)	T			
BEADCHIP™	RHD Array*	455A>C (N152T)	С			
		1048G>C (D350H)	С			

Only nucleotides which differ from consensus sequence are listed.

Probable RHD Genotype: RHD*04.01(RHD*DIVa) (hemizygous or homozygous)

Predicted phenotype: Partial D+ Go(a+)

COMMENTS: The patient has a partial RHD*DIVa which is associated with production of allo anti-D and expression of the low incidence Go(a+) antigen. Females of child-bearing potential with partial D should be considered D negative for transfusion.

The RHD*DIVa allele is often coinherited with the RHCE*ceTI allele that encodes partial e antigen and may be associated with C typing discrepancies. Please submit a service request for RHCE variant testing if this additional testing is desired. Please reference the ML# listed above (next to the patient's name) in your request to avoid testing delays.



Case 2

Age: 47Gender: Female

Race: African American

Diagnosis: Iron deficiency anemia due to chronic blood loss

Menometrorrhagia (excessive vaginal bleeding)

• Transfusions: 2 units on 06/17/2022, 2 units on 07/27/2022

Pregnancy history: 1 child, not currently pregnant

Hgb/Hct: 7.0 / 22.2

• Referring facility's results: A Neg; on 09/09/2022 the antibody screen was positive. The hospital performed testing and identified an antibody with anti-e specificity. The auto control was negative and the DAT was negative at that time. Hospital could not rule out anti-C and no sample was submitted to the IRL.

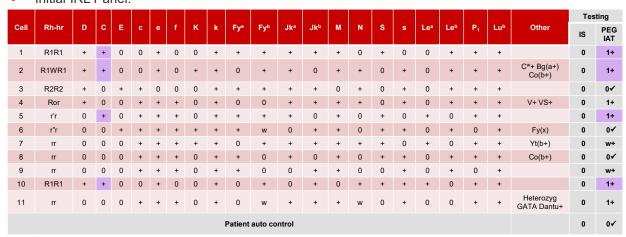
Current sample was submitted to the IRL for antibody ID and red cell phenotyping. The hospital had obtained
 2 C-e- units from another supplier to hopefully transfuse the patient once the workup was complete.

American Red Cross

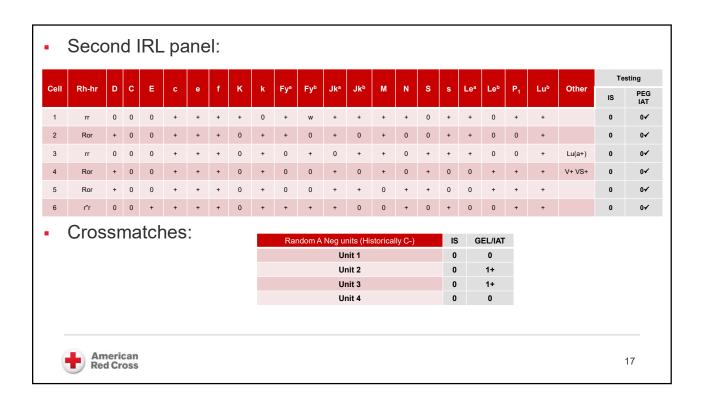
15

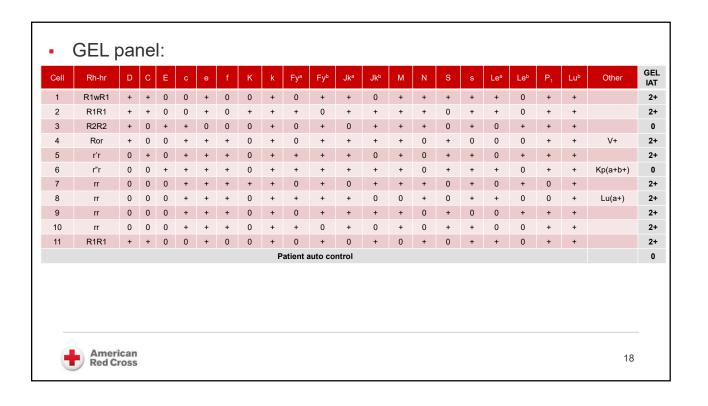
Initial IRL Testing

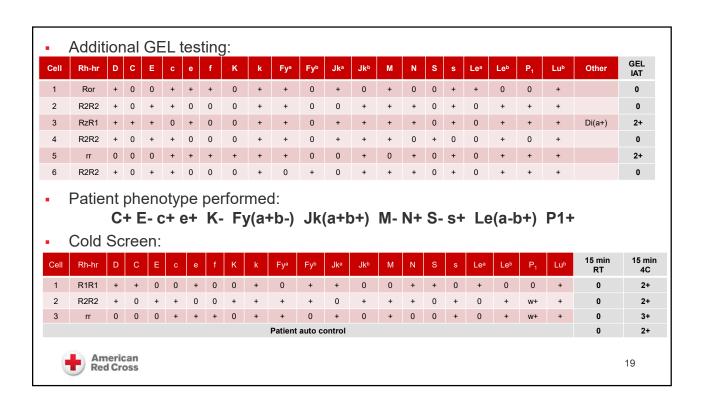
ABO/RH: A NegativeDAT: NegativeInitial IRL Panel:

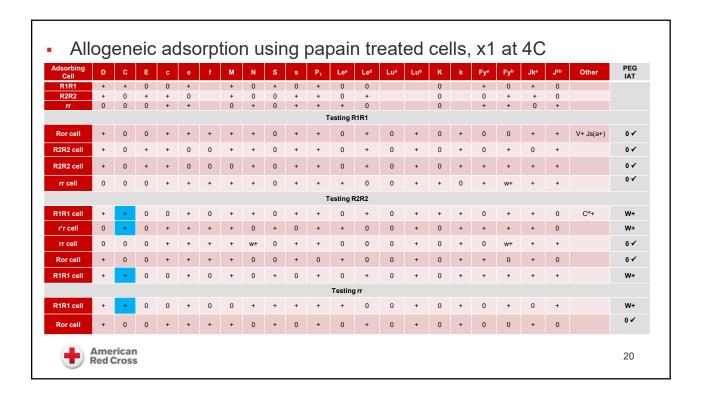


American Red Cross









What we know:

- DAT is Negative, Negative auto control at PEG/IAT and GEL/IAT
- Cold autoantibody at 4C, PEG/IAT, and GEL/IAT
- Anti-C detected at PEG/IAT following the 4C papain treated allogeneic adsorptions
- All additional alloantibodies have been ruled out.
- Recommend giving crossmatch compatible units (A Neg or O Neg) that are C Neg

Wait... the patient phenotype is: C+ E- c+ e+

Recommend sending a sample to the NML for RHD and RHCE genotyping.



21

HEA molecular report for CASE 2

Blood Group	Antigen	Result	Diego	Dia	0
Rh	С	+		Dib	+
	С	(+)*	Colton	Coa	+
	e	+		Cob	0
	E	0	Dombrock	Doa	+
	V	0		Dob	+
Kell	K	0	- 1	Ну	+
Kell	k	+		Joa	+
	Kpa	0	Landsteiner-	LWa	+
	Kpb	+	Wiener	1 W/b	0
	Jsa	0	Scianna	Sc1	+
	Jsb	+		Sc2	0
Duffy	Fya	+	Hemoglobin S	HbS	0
	Fyb	(0)*		1100	
Kidd	Jka	+			
	Jkb	+			
MNS	M	0			
	N	+			
	S	0			
	s	+			
	U	+			
Lutheran	Lua	0			
	Lub	+			

COMMENTS: The sample has the single nucleotide variant in the GATA-1 binding site in the promoter of the gene that encodes FY antigens. This variant disrupts binding of the erythroid transcription factor GATA-1 resulting in loss of Fy^b expression in RBCs. Fy^b expression on tissue endothelium is not affected and these individuals would not be expected to make anti-Fy^b (Castilho 2007) but anti-Fy3 has been reported (Castilho 2009).

COMMENTS: Samples with (+)* may have a hybrid RHD-CE-D encoding altered C antigen. As the C status for this sample is reported to be positive by serology, this is consistent with the presence of an r-S i.e. (C)ceS haplotype. Further RH characterization is required to assess the risk of allo-anti-C production.

COMMENTS: Testing for *RHCE* and *RHD* variants is in progress and results will follow in a separate report.



Genotype for RH variants for CASE 2

T	ESTING PERFORM	IED	RESULT
RHD Variants	Method	Analyte: Nucleotide (Amino Acid)	Nucleotide(s) Detected
		186G>T (L62F)	T
wRHD	RHD Array*	410C>T (A137V)	T
BEADCHIP™	And Allay	455A>C (N152T)	С
		Int3-Exon7 markers	absent
RHCE Common	Method	Analyte	Product present/absent
RHCE gene	RHCE Array	С	absent
MICE gene	ATIOE ATIAY	С	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)	С
wRHCE	RHCE Array*	733C>G (L245V)	C/G**
BEADCHIP™	KHOE Allay	1006G>T (G336C)	G/T

Probable RHD Genotype: <u>RHD*DIIIa-CE(4-7)-D</u> (hemizygous or homozygous)

Probable RHCE Genotype: RHCE*ce/RHCE*ce48C,733G,1006T

Predicted phenotype: D- altered C+ E- c+ e+ VS+ V- hr^S+ hr^B+

COMMENTS: The RHD*DIlla-CE(4-7)-D allele does not encode RhD but instead encodes an altered C antigen. Though the RHCE c.48C variant yielded an indeterminate call, it is typically coinherited with c.733G and c.100fT which were both found in this patient. This testing confirms that the patient does carry the r's haplotype that encodes an altered C antigen.

Based on this testing, the patient is predicted to be at risk for allo-anti-D, -C, -E, and -V.



23

Case 3

• Age: 75

Gender: Female

Race: African American

Diagnosis: Anemia

Transfusions: 2 units on 08/31/2022

Hgb/Hct: 7.2 / 22.1

- The hospital did not provide copies of their testing but did indicate that they suspected multiple antibodies to be present. They report the patient's blood type as O Positive
- Request is for 2 units of antigen negative units for transfusion at completion of the workup
- Sample was submitted to IRL for antibody ID



Initial IRL Testing

ABO/Rh: O PositiveDAT: Negative

Previous IRL history: Nov 2020, anti-E, Rh phenotype C-E-c+e+

Initial IRL Panel:

Cell	Rh-hr	D	С	Е	С	е	f	K	k	Fya	Fyb	Jka	Jkb	M	N	s	s	Lea	Le ^b	P ₁	Lub	TE	STING
																						IS	PEG IAT
1	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	0	0✓
2	rr	0	0	0	+	+	+	+	0	0	+	+	+	+	+	0	+	0	+	0	+	0	W+
3	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	0	+	+	+	0	W+
4	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	1+
									Pat	tient au	to con	trol										0	0✓



25

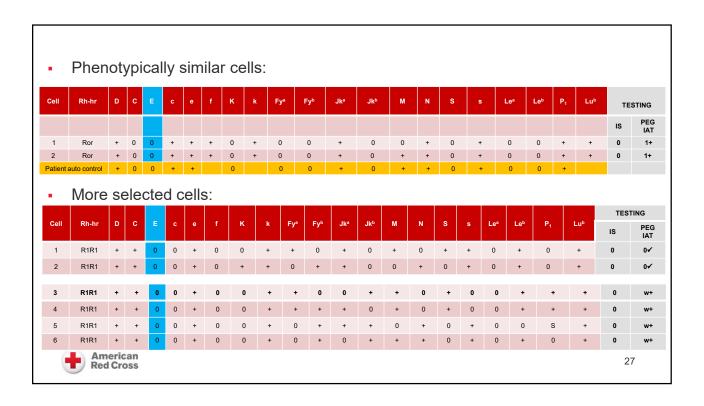
Additional selected cell testing

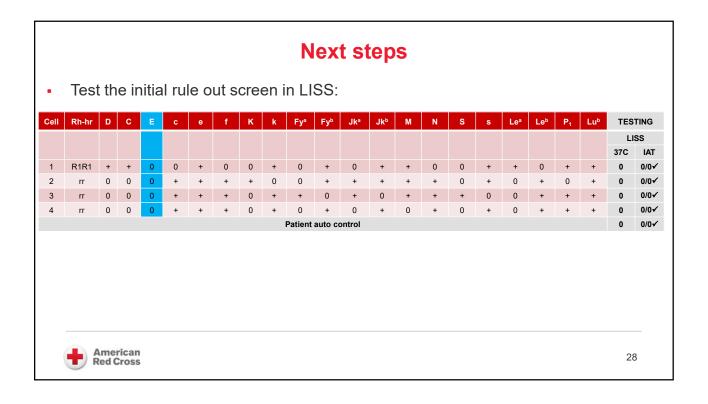
Cell	Rh-hr	D	С	Е	С	е	f	K	k	Fya	Fy⁵	Jka	Jkb	М	N	S	s	Lea	Le ^b	P ₁	Lub	TES	STING
																						IS	PEG IAT
1	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	0	+	+	0	+	+	0	1+
2	r'r	0	+	0	+	+	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+	0	W+
3	r'r	0	+	0	+	+	+	+	+	+	0	0	+	+	0	+	+	+	0	0	+	0	0✓

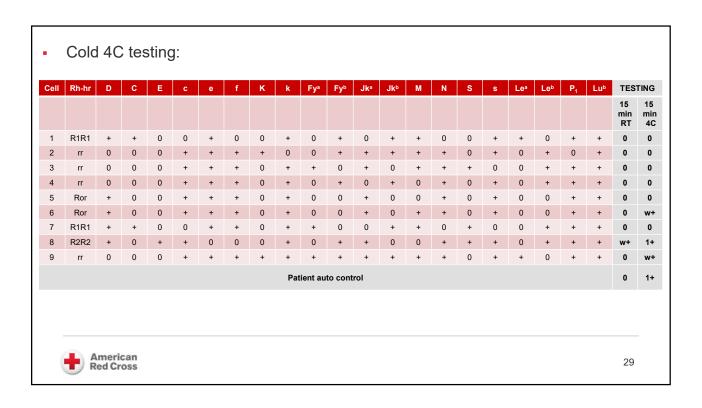
• Patient phenotype completed:

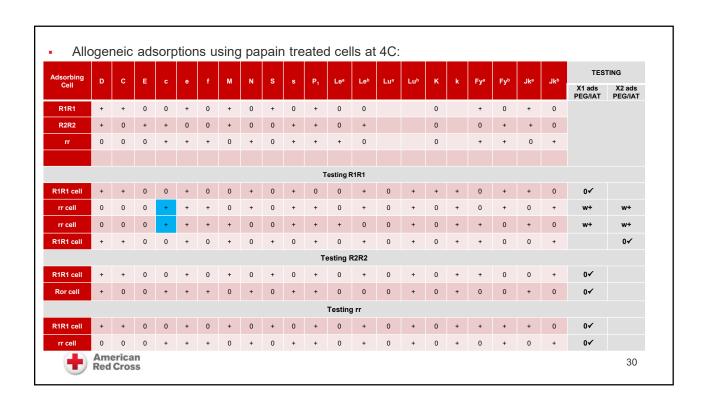
K- Fy(a-b-) Jk(a+b-) M+ N+ S- s+ Le(a-b-) P₁+







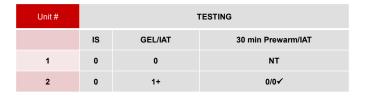




Test the DAT Neg Cell Sep with the R1R1 adsorbed serum:



Crossmatches with O POS E-c- units:





31

Conclusions:

- O Positive
- DAT Negative
- Cold autoantibody at 4C, PEG/IAT and GEL/IAT
- Previous anti-E not assessed, anti-c detected at PEG/IAT following 4C allogeneic adsorptions, possibly alloantibody in nature.
- Additional alloantibodies were ruled out at IS, LISS/37, LISS/IAT, PEG/IAT (neat serum and following allogeneic adsorption)
- Give crossmatch compatible units E-c-, use prewarm technique as needed
- Recommend molecular testing to determine if the patient expresses RH variant antigens which would put them at risk for alloimmunization, especially c variant testing.



This is NOT the end to Case 3's story

- 3 weeks later, a new sample was submitted for Antibody ID.
- The patient has now been transfused with 3 units since the last workup.
- DAT is POSITIVE (1+ with IgG only); Eluate reacts 3+ with all cells tested. DAT neg Cell Sep tested with eluate shows reactivity, suggesting a warm autoantibody is present on the patient's cells.
- An initial rule out panel was tested at PEG/IAT (all cells positive w+ to 1+) but LISS/37 and LISS/IAT testing was negative; Auto control is 1+ at PEG/IAT and LISS/IAT.
- DAT neg Cell Sep tested with the neat serum shows 1+ reactivity, suggesting a warm autoantibody is present in the serum.



33

New sample on CASE 3

- Allogeneic adsorptions at 37C using papain treated cells is performed to see if any alloantibodies are underlying the warm autoantibody in the serum.
- Testing of the adsorbed serum shows no underlying alloantibodies.



Conclusions on new sample for CASE 3:

- Previous anti-E and anti-c were not assessed.
- Warm autoantibody detected in the serum with no new underlying allo-antibodies detected.
- DAT 1+ with IgG only. Eluate has a warm autoantibody, no need to adsorb it as no new allo-antibodies were detected in the serum and it has been >21 days since the last transfusion.
- Give crossmatch incompatible units negative for E and c.
- Sample submitted again to the NML for the HEA and RHCE variant testing.



35

Blood Group	Antigen	Result	$H \vdash \Delta$	Mole	cular	Report CASE 3
Rh	С	+		IVIOIC	Culai	Report CAGE 3
i	C	0	Diego	Dia	0	
	е	+		Dib	+	COMMENTS: The sample has the single nucleon
	E	0	Colton	Coa	+	promoter of the gene that encodes FY antigens. Th
	V	+			-	transcription factor GATA-1 resulting in loss of Fy
	VS	+		Cob	0	endothelium is not affected and these individuals v
Kell	K	0	Dombrock	Doa	0	2007) but anti-Fy3 has been reported (Castilho 200
i	k	+		Dob	+	COMMENTS: Genotype results predict that the s
	Kpa	0		Hy	+	The hr ^B status cannot be assigned solely based on I
	Kpb	+		-		information. Further RH characterization may aid i
	Jsa	0		Joa	+	desired, submit a service request indicating e variat
	Jsb	+	Landsteiner-	LWa	+	additional sample is needed.
Duffy	Fya	0	Wiener	LWb	0	The sample has two variant RHCE*ce alleles. If the
	Fyb	(0)*	Scianna	Sc1	+	consider further characterization. If such additional
Kidd	Jka	+		Sc2	0	indicating e variant testing and reference the ML# :
	Jkb	0				
MNS	M	+	Hemoglobin S	HbS	0	

COMMENTS: The sample has the single nucleotide variant in the GATA-1 binding site in the promoter of the gene that encodes FY antigens. This variant disrupts binding of the erythroid ranscription factor GATA-1 resulting in loss of Fyb expression in RBCs. Fyb expression on tissue endothelium is not affected and these individuals would not be expected to make anti-Fyb (Castilho

2007) but anti-Fy3 has been reported (Castilho 2009).

COMMENTS: Genotype results predict that the sample expresses a partial e antigen and may be hr^B-. The hrB status cannot be assigned solely based on PreciseType HEA Molecular BeadChip genotype information. Further RH characterization may aid in predicting hrB status. If this additional testing is desired, submit a service request indicating e variant testing and reference the ML# above. No additional sample is needed.

The sample has two variant RHCE*ce alleles. If the patient is likely to receive future transfusions, consider further characterization. If such additional testing is desired, submit a service request indicating e variant testing and reference the ML# above. No additional sample is needed.



s

U

Lua Lub 0

0

Genotype for RHCE variants Report CASE 3

TESTING REQUESTED: Genotype for RHCE variants

Ţ	ESTING PERFORM	ED	RESULT
RHCE Common	Method	Analyte	Product present/absent
RHCE gene	RHCE Array	С	absent
raroz gene	TOL Allay	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)	С
WRHCE BEADCHIP™	RHCE Array*	733C>G (L245V)	G

Probable RHCE Genotype: RHCE*ce733G/RHCE*ce733G

Predicted phenotype: C- E- partial c+ partial e+ VS+ V+ hr^S+ hr^B+vw/-

COMMENTS: The patient carries *RHCE*ce* alleles expressing partial antigens. Based on this testing, the patient is predicted to be at risk for allo-anti-c, -e, -f (ce), and possibly anti-hr^B.



37

Comments/Questions

