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: 6.2/16.5
- Sample 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-

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Initial IRL Testing

- ABO/Rh: O Positive
- DAT: Negative
- Initial IRL Panel:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^a	Lu^b	TESTING	
																							IS	PEG/IAT
1	R1wR1	+	+	O	O	+	O	O	+	+	O	+	+	O	+	O	+	O	+	+	O	+	0	2+
2	R1R1	+	+	O	O	+	O	+	+	O	+	O	+	+	+	O	+	O	+	+	O	+	0	2+
3	R2R2	+	O	+	+	O	O	O	+	O	+	O	+	+	+	O	+	O	O	+	O	+	0	2+
4	Ror	+	O	O	+	+	+	O	+	O	O	+	+	O	+	O	+	O	O	+	O	+	0	2+
5	r'r	O	+	0	+	+	+	O	+	+	+	+	+	+	O	+	O	O	+	+	O	+	0	1+
6	r"r	O	O	+	+	+	+	O	+	+	+	+	+	+	+	O	+	+	O	O	O	+	0	1+
7	rr	O	O	O	+	+	+	+	+	O	+	+	O	+	+	O	+	O	+	O	O	+	0	1+
8	rr	O	O	O	+	+	+	+	+	+	O	+	+	O	+	+	+	+	O	+	O	+	0	1+
9	rr	O	O	O	+	+	+	O	+	O	+	O	+	+	O	+	+	O	+	+	O	+	0	1+
10	rr	O	O	O	+	+	+	O	+	+	+	+	O	+	+	+	O	O	+	+	O	+	0	1+
11	R1R1	+	+	O	O	+	O	O	+	O	+	O	+	+	+	+	+	+	O	O	+	+	0	2+
Patient auto control																							0	0✓

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Next Steps

- DTT-treated rule out screen:

Cell	Rh-hr	D	C	E	c	e	f	K	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	NEAT		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-) P_1+

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- Phenotypically similar cells:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^a	Lu^b	Other	PEG IAT
1	rr	0	0	0	+	+	+	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+		**w+**
2	rr	0	0	0	+	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	0	+	Js(a+b+)	**w+**
3	rr	0	0	0	+	+	+	0	+	0	0	+	0	+	+	0	+	0	0	+	+	+		**w+**
Pt Phenotype		**+**	**0**	**0**	**+**	**+**		**0**		**0**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**0**	**+**				

- Cold Screen:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^a	Lu^b	15 min RT	15 min 4C
1	**rr**	**0**	**0**	**0**	**+**	**+**	**+**	**0**	**+**	**0**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**0**
2	**rr**	**0**	**0**	**0**	**+**	**+**	**+**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**+**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**0**
3	**rr**	**0**	**0**	**0**	**+**	**+**	**+**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**0**	**+**	**+**	**+**	**0**	**0**
Pt Phenotype		**+**	**0**	**0**	**+**	**+**		**0**		**0**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**0**	**+**			**0**	**1+**

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- Trypsin-treated rule out screen:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^a	Lu^b	Other	Trypsin IAT
1	r'r	0	+	0	+	+	+	+	+	0	+	+	0	+	0	+	0	0	+	+	0	+		0/0✓
2	r"r	0	0	+	+	+	+	0	+	+	+	+	0	+	+	+	+	+	0	+	0	+		0/0✓
3	rr	0	0	0	+	+	+	0	+	+	0	0	+	+	0	0	+	0	+	+	0	+		0/0✓
4	R1wR1	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	C^w+	w+

- Trypsin-treated phenotypically similar cells:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^a	Lu^b	Other	Trypsin IAT
1	rr	0	0	0	+	+	+	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+		0/0✓
2	rr	0	0	0	+	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	0	+	Js(a+b+)	0/0✓
3	rr	0	0	0	+	+	+	0	+	0	0	+	0	+	+	0	+	0	0	+	+	+		0/0✓
Pt Phenotype		**+**	**0**	**0**	**+**	**+**		**0**		**0**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**0**	**+**				

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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:

Titration performed at 60 min 37C IgG												
Titration dilution	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**
	0/0✓	**0/0✓**	**0/0✓**	**0/0✓**	**0/0✓**	**0/0✓**	**0/0✓**	**0/0✓**	**0/0✓**	**0/0✓**	**0/0✓**	**0/0✓**

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- Thawed Lu(a-b-) cells:

Rh-hr	D	C	E	c	e	f	K	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	PEG IAT
rr	0	0	0	+	+	+	0	0	+	+	0	+	0	0	+	0	+	0	w+
rr	0	0	0	+	+	+	0	0	+	+	0	+	0	0	+	0	+	0	w+

- Patient typed Lu(b+)

- Allogeneic Adsorptions performed:

Adsorbing Cells	D	C	E	c	e	f	K	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1
R1R1	+	+	0	0	+	0	0	0	+	0	+	+	0	+	0	0	+	0
R2R2	+	0	+	+	0	0	0	+	0	0	+	+	0	0	+	0	+	+
rr	0	0	0	+	+	+	0	+	+	+	0	0	+	0	+	0	+	0

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- Testing of the allogeneic adsorbed serum:

Adsorbing Cell	D	C	E	c	e	f	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	X1 tr'd ads PEG/IAT	X1 tr'd x1 untr'd ads PEG/IAT
R1R1	+	+	0	0	+		+	0	+	0	0	0	+			0		0	+	0	+		
R2R2	+	0	+	+	0		+	0	0	+	+	0	+			0		+	0	0	+		
rr	0	0	0	+	+		0	+	0	+	0	0	+			0		+	+	+	0		
Testing R1R1																							
R2R2 cell	+	0	+	+	0		0	+	0	+	+	+	0	0	+	0	+	+	+	+	+	W+	W+
r'r cell	0	+	0	+	+		0	+	0	+	0	0	+	0	+	+	+	+	0	+	0	W+	W+
Testing R2R2																							
R1R1 cell	+	+	0	0	+		+	+	+	0	+	0	+	0	6	+	0	0	+	+	0	1+	NT
rr cell	0	0	0	+	+		0	+	0	+	+	+	0	0	+	+	+	0	+	+	+	W+	W+
R1R1 cell	+	+	0	0	+	0	+	0	0	+	+	0	0	0	+	+	+	0	+	+	0	NT	W+
Testing rr																							
r''r cell	0	0	+	+	+		+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	W+	W+
R1R1 cell	+	+	0	0	+	0	+	0	0	+	+	0	0	0	+	+	+	0	+	+	0	1+	1+
R1R1 cell	+	+	0	0	+	0	+	+	+	+	+	0	+	0	+	0	+	+	+	+	+	1+	1+

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- Test the phenotypically similar cells in LISS:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^a	Lu^b	Other	LISS	
																								37C	IAT
1	rr	0	0	0	+	+	+	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+		0	0/0✓
2	rr	0	0	0	+	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	0	+	Js(a+b+)	0	0/0✓
3	rr	0	0	0	+	+	+	0	+	0	0	+	0	+	+	0	+	0	0	+	+	+		0	0/0✓
Pt Phenotype		**+**	**0**	**0**	**+**	**+**		**0**		**0**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**0**	**+**		**+**			

- Crossmatches performed using O NEG, C-E-K-HgbS- units:

Unit #	IS	LISS/37C	LISS/IAT	GEL/IAT
1	0	0	0/0✓	0
2	0	0	0/0✓	0
3	0	0	0/0✓	0

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- Rule out cells from initial panel tested with LISS:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^a	Lu^b	TESTING	
																							LISS	
																							37C	IAT
1	R1wR1	+	+	0	0	+	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0	w+
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	+	0	+	+	0	+		
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	0	+	0	1+
4	Ror	+	0	0	+	+	+	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+		
5	r'r	0	+	0	+	+	+	0	+	+	+	+	+	+	0	+	0	0	+	+	0	+		
6	r"r	0	0	+	+	+	+	0	+	+	+	+	+	+	+	0	+	+	0	0	0	+		
7	rr	0	0	0	+	+	+	+	+	0	+	+	0	+	+	0	+	0	+	0	0	+		
8	rr	0	0	0	+	+	+	+	+	+	0	+	+	0	+	+	+	+	0	+	0	+	0	0/0✓
9	rr	0	0	0	+	+	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	0/0✓
10	rr	0	0	0	+	+	+	0	+	+	+	+	0	+	+	+	0	0	+	+	0	+	0	0/0✓
11	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+		
Pt. Phenotype		+	0	0	+	+		0		0	0	+	0	0	+	0	+	0	0	+			0	0/0✓

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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)

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Genotype for RHD variant report for CASE 1

TESTING REQUESTED: Genotype for *RHD* variants

TESTING PERFORMED			RESULT
***RHD* Variants**	**Method**	**Analyte: Nucleotide (Amino Acid)**	**Nucleotide(s) Detected**
w*RHD* BEADCHIP™	*RHD* Array*	186G>T (L62F)	T
		410C>T (A137V)	T
		455A>C (N152T)	C
		1048G>C (D350H)	C

*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.

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Case 2

- Age: 47
- Gender: 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.

Initial IRL Testing

- ABO/RH: A Negative
- DAT: Negative
- Initial IRL Panel:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	Other	Testing	
																							IS	PEG IAT
1	R1R1	+	+	0	0	+	0	0	+	+	+	+	+	+	0	+	0	0	+	+	+		0	1+
2	R1WR1	+	+	0	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	+	+	C^w+ Bg(a+) Co(b+)	0	1+
3	R2R2	+	0	+	+	0	0	0	+	+	+	+	+	0	+	0	+	0	+	+	+		0	0✓
4	Ror	+	0	0	+	+	+	0	+	0	0	+	+	+	+	0	+	0	+	+	+	V+ VS+	0	1+
5	r'r	0	+	0	+	+	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+		0	1+
6	r"r	0	0	+	+	+	+	+	+	+	w	0	+	+	0	+	+	0	+	0	+	Fy(x)	0	0✓
7	rr	0	0	0	+	+	+	+	+	0	+	+	+	+	+	+	0	+	0	+	+	Yt(b+)	0	w+
8	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	0	+	+	+	Co(b+)	0	0✓
9	rr	0	0	0	+	+	+	0	+	+	0	0	+	+	0	0	+	0	+	0	+		0	w+
10	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	+		0	1+
11	rr	0	0	0	+	+	+	0	+	0	w	+	+	+	w	0	+	0	0	+	+	Heterozyg GATA Dantu+	0	1+
Patient auto control																							0	0✓

- Second IRL panel:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	Other	Testing	
																							IS	PEG IAT
1	rr	0	0	0	+	+	+	+	0	+	w	+	+	+	+	0	+	+	0	+	+		0	0✓
2	Ror	+	0	0	+	+	+	0	+	+	0	+	0	+	0	0	+	+	0	0	+		0	0✓
3	rr	0	0	0	+	+	+	0	+	0	+	0	+	+	0	+	+	+	0	0	+	Lu(a+)	0	0✓
4	Ror	+	0	0	+	+	+	0	+	0	0	+	0	+	0	+	0	0	+	+	+	V+ VS+	0	0✓
5	Ror	+	0	0	+	+	+	0	+	0	0	+	+	0	+	+	0	0	+	+	+		0	0✓
6	r"r	0	0	+	+	+	+	0	+	+	+	+	0	0	+	0	+	0	0	+	+		0	0✓

- Crossmatches:

Random A Neg units (Historically C-)	IS	GEL/IAT
Unit 1	**0**	**0**
Unit 2	**0**	**1+**
Unit 3	**0**	**1+**
Unit 4	**0**	**0**

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- GEL panel:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	Other	GEL IAT
1	R1wR1	+	+	0	0	+	0	0	+	0	+	+	0	+	+	+	+	+	0	+	+		2+
2	R1R1	+	+	0	0	+	0	+	+	+	0	+	+	+	+	0	+	+	0	+	+		2+
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+		0
4	Ror	+	0	0	+	+	+	0	+	0	+	+	+	+	0	+	0	0	0	+	+	V+	2+
5	r'r	0	+	0	+	+	+	0	+	+	+	+	0	+	0	+	+	0	+	+	+		2+
6	r"r	0	0	+	+	+	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	Kp(a+b+)	0
7	rr	0	0	0	+	+	+	+	+	0	+	0	+	+	+	0	+	0	+	0	+		2+
8	rr	0	0	0	+	+	+	0	+	+	+	+	0	0	+	0	+	+	0	0	+	Lu(a+)	2+
9	rr	0	0	0	+	+	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+		2+
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	0	0	+	+		2+
11	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+		2+
Patient auto control																							**0**

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- Additional GEL testing:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	Other	GEL IAT
1	Ror	+	0	0	+	+	+	0	+	+	0	+	0	+	0	0	+	+	0	0	+		**0**
2	R2R2	+	0	+	+	0	0	0	+	+	0	0	+	+	+	0	+	0	+	+	+		**0**
3	RzR1	+	+	+	0	+	0	0	+	+	0	+	+	+	+	0	+	0	+	+	+	Di(a+)	**2+**
4	R2R2	+	0	+	+	0	0	0	+	+	0	+	+	+	0	+	0	0	+	0	+		**0**
5	rr	0	0	0	+	+	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+		**2+**
6	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+		**0**

- Patient phenotype performed:

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

- Cold Screen:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	15 min RT	15 min 4C
1	R1R1	+	+	0	0	+	0	0	+	0	+	+	0	0	+	+	0	+	0	0	+	**0**	**2+**
2	R2R2	+	0	+	+	0	0	+	+	+	+	0	+	+	+	0	+	0	+	w+	+	**0**	**2+**
3	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	0	+	0	+	w+	+	**0**	**3+**
Patient auto control																						**0**	**2+**

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- Allogeneic adsorption using papain treated cells, x1 at 4C

Adsorbing Cell	D	C	E	c	e	f	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Other	PEG IAT
R1R1	+	+	0	0	+		+	0	+	0	+	0	0			0		+	0	+	0		
R2R2	+	0	+	+	0		+	0	0	+	+	0	+			0		0	+	+	0		
rr	0	0	0	+	+		0	+	0	+	+	+	0			0		+	+	0	+		
Testing R1R1																							
Ror cell	+	0	0	+	+	+	+	+	0	+	+	0	+	0	+	0	+	0	0	+	+	V+ Js(a+)	0 ✓
R2R2 cell	+	0	+	+	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+		0 ✓
R2R2 cell	+	0	+	+	0	0	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+		0 ✓
rr cell	0	0	0	+	+	+	+	+	0	+	+	+	0	0	+	+	0	+	w+	+	+		0 ✓
Testing R2R2																							
R1R1 cell	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	C^w+	W+
r'r cell	0	+	0	+	+	+	+	0	+	0	+	+	0	0	+	0	+	+	+	+	0		W+
rr cell	0	0	0	+	+	+	+	w+	0	+	+	0	0	0	+	0	+	0	w+	+	+		0 ✓
Ror cell	+	0	0	+	+	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	0		0 ✓
R1R1 cell	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+		W+
Testing rr																							
R1R1 cell	+	+	0	0	+	0	0	+	+	+	+	+	0	0	+	0	+	0	+	0	+		W+
Ror cell	+	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0		0 ✓

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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.

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HEA molecular report for CASE 2

Blood Group	Antigen	Result
Rh	c	+
	C	(+)*
	e	+
	E	0
	V	0
	VS	+
Kell	K	0
	k	+
	Kp^a	0
	Kp^b	+
	Js^a	0
	Js^b	+
Duffy	Fy^a	+
	Fy^b	(0)*
Kidd	Jk^a	+
	Jk^b	+
MNS	M	0
	N	+
	S	0
	s	+
	U	+
Lutheran	Lu^a	0
	Lu^b	+

Diego	Di^a	0
	Di^b	+
Colton	Co^a	+
	Co^b	0
Dombrock	Do^a	+
	Do^b	+
	Hy	+
	Jo^a	+
Landsteiner-Wiener	LW^a	+
	LW^b	0
Scianna	Sc1	+
	Sc2	0
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 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)ce^s 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.

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Genotype for RH variants for CASE 2

TESTING PERFORMED			RESULT
RHD Variants	Method	Analyte: Nucleotide (Amino Acid)	Nucleotide(s) Detected
wRHD BEADCHIP™	*RHD* Array*	186G>T (L62F)	T
		410C>T (A137V)	T
		455A>C (N152T)	C
		Int3-Exon7 markers	absent
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*	733C>G (L245V)	C/G**
		1006G>T (G336C)	G/T

*Only nucleotides which differ from consensus sequence are listed.
** Intermediate call was obtained for c.48C; this is not expected to affect the phenotype.

Probable *RHD* Genotype: *RHD*DIIIa-CE(4-7)-D* (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^a+

COMMENTS: The *RHD*DIIIa-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.1006T 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.

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 Positive
- DAT: Negative
- Previous IRL history: Nov 2020, anti-E, Rh phenotype C-E-c+e+
- Initial IRL Panel:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	TESTING	
																						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+
Patient auto control																						0	0✓

Additional selected cell testing

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	TESTING	
																						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-) P_1+

- Phenotypically similar cells:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	TESTING	
																						IS	PEG IAT
1	Ror	+	0	0	+	+	+	0	+	0	0	+	0	0	+	0	+	0	0	+	+	0	1+
2	Ror	+	0	0	+	+	+	0	+	0	0	+	0	+	+	0	+	0	0	+	+	0	1+
Patient auto control		+	0	0	+	+		0		0	0	+	0	+	+	0	+	0	0	+			

- More selected cells:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	TESTING	
																						IS	PEG IAT
1	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	0✓
2	R1R1	+	+	0	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	0	0✓
3	**R1R1**	**+**	**+**	**0**	**0**	**+**	**0**	**0**	**+**	**+**	**0**	**0**	**+**	**+**	**0**	**+**	**0**	**0**	**+**	**+**	**+**	**0**	**w+**
4	R1R1	+	+	0	0	+	0	0	+	+	+	+	0	+	0	+	0	0	+	+	+	0	w+
5	R1R1	+	+	0	0	+	0	0	+	0	+	+	+	0	+	0	+	0	0	S	+	0	w+
6	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	w+

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Next steps

- Test the initial rule out screen in LISS:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	TESTING	
																						LISS	
																						37C	IAT
1	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	0	0/0✓
2	rr	0	0	0	+	+	+	+	0	0	+	+	+	+	+	0	+	0	+	0	+	0	0/0✓
3	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	0	+	+	+	0	0/0✓
4	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0/0✓
Patient auto control																						0	0/0✓

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- Cold 4C testing:

Cell	Rh-hr	D	C	E	c	e	f	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1	Lu^b	TESTING	
																						15 min RT	15 min 4C
1	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	**0**	**0**
2	rr	0	0	0	+	+	+	+	0	0	+	+	+	+	+	0	+	0	+	0	+	**0**	**0**
3	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	0	+	+	+	**0**	**0**
4	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	**0**	**0**
5	Ror	+	0	0	+	+	+	0	+	0	0	+	0	0	+	0	+	0	0	+	+	**0**	**0**
6	Ror	+	0	0	+	+	+	0	+	0	0	+	0	+	+	0	+	0	0	+	+	**0**	**w+**
7	R1R1	+	+	0	0	+	+	0	+	+	0	0	+	+	0	+	0	0	+	+	+	**0**	**0**
8	R2R2	+	0	+	+	0	0	0	+	0	+	+	0	0	+	+	+	0	+	+	+	**w+**	**1+**
9	rr	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	+	+	0	+	+	**0**	**w+**
Patient auto control																						**0**	**1+**

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- Allogeneic adsorptions using papain treated cells at 4C:

Adsorbing Cell	D	C	E	c	e	f	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	TESTING	
																						X1 ads PEG/IAT	X2 ads PEG/IAT
R1R1	+	+	0	0	+	0	+	0	+	0	+	0	0			0		+	0	+	0		
R2R2	+	0	+	+	0	0	+	0	0	+	+	0	+			0		0	+	+	0		
rr	0	0	0	+	+	+	0	+	0	+	+	+	0			0		+	+	0	+		
Testing R1R1																							
R1R1 cell	+	+	0	0	+	0	0	+	0	+	0	0	+	0	+	+	+	0	+	+	0	**0✓**	
rr cell	0	0	0	+	+	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	**w+**	**w+**
rr cell	0	0	0	+	+	+	+	0	0	+	+	+	0	0	+	0	+	+	0	+	0	**w+**	**w+**
R1R1 cell	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+		**0✓**
Testing R2R2																							
R1R1 cell	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	**0✓**	
Ror cell	+	0	0	+	+	+	0	+	0	+	+	0	0	0	+	0	+	0	0	+	0	**0✓**	
Testing rr																							
R1R1 cell	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	**0✓**	
rr cell	0	0	0	+	+	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	**0✓**	

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

Testing done with the x2 4C R1R1 adsorbed serum
PEG/IAT: 0✓

- Crossmatches with O POS E-c- units:

Unit #	TESTING		
	IS	GEL/IAT	30 min Prewarm/IAT
1	0	0	NT
2	0	1+	0/0✓

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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.

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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.

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.

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HEA Molecular Report CASE 3

Blood Group	Antigen	Result
Rh	c	+
	C	0
	e	+
	E	0
	V	+
	VS	+
Kell	K	0
	k	+
	Kp^a	0
	Kp^b	+
	Js^a	0
	Js^b	+
Duffy	Fy^a	0
	Fy^b	(0)*
Kidd	Jk^a	+
	Jk^b	0
MNS	M	+
	N	+
	S	0
	s	+
	U	+
Lutheran	Lu^a	0
	Lu^b	+
Diego	Di^a	0
	Di^b	+
Colton	Co^a	+
	Co^b	0
Dombrock	Do^a	0
	Do^b	+
	Hy	+
	Jo^a	+
Landsteiner-Wiener	LW^a	+
	LW^b	0
Scianna	Sc1	+
	Sc2	0
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 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: Genotype results predict that the sample expresses a partial e antigen and may be hr^B-. The hr^B status cannot be assigned solely based on PreciseType HEA Molecular BeadChip genotype information. Further *RH* characterization may aid in predicting hr^B 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.

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Genotype for RHCE variants Report CASE 3

TESTING REQUESTED: Genotype for RHCE variants

TESTING PERFORMED			RESULT
***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*	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}.

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Comments/Questions

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