Immunohematology Case Studies - 12

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Clinical History

49 year old Sickle Cell male patient admitted for surgery

Patient was last transfused a month ago before his current admission

Serologic History

No known history of Red Blood Cell antibody(ies)

Current Sample Presentation Data

ABO/D type	O RhD pos
DAT	Negative
Antibody Screen Method	LISS-IAT CAT (Bio-Rad)
Antibody Screen Results	4+ with all Cells (3-cell panel)
Antibody Identification Method	LISS-IAT CAT (Bio-Rad)
Antibody Identification Preliminary Results	4+ with all Cells (11-cell panel)

Challenge with the Current Presentation

This antibody(ies) reacted at the same strength with all panel red cells

Panel Sample

IAT-LISS CAT

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		Gel
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+		4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0		4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+		4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+		4+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+		4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0		4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+		4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+		4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0		4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0		4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+		4+

Autocontrol not tested due to recent transfusion

Panel Sample

CAT-Enzyme Method

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Gel Enzyme
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	4+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	4+

Autocontrol not tested due to recent transfusion

Interim Antibody Identification
Possible Answers and Next Steps

The serological work-up suggests the presence of antibody to high prevalence antigen(s)

Further testing

Saline phase

Washed Panel test (to avoid intervention of RBC preservative-specific antibodies which have been seen in the past)

Panel Sample

Saline CAT (washed Panel test)

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Saline
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	4+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	4+

Autocontrol not tested due to recent transfusion

Panel Sample

IAT-LISS CAT (washed Panel test)

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IAT
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	4+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	4+

Autocontrol not tested due to recent transfusion

Interpretation Suggested further testing

This antibody reacted both in Saline and in IAT phase in DiaMed Gel Card using washed cell-panel

Further testing included RBC phenotyping

Phenotyping Results*

R_1r

K+ k-

Fy(a-b+)

Jk(a-b+)

Le(a-b+)

P1+

M- N+ S+ s+

Lu(a-b+)

*Patient was transfused 1 month previously, phenotype performed for investigative purposes

Further suggested testing

Probability of anti-k !!, since patient is k negative

Testing against k negative cells would be ideal to confirm the k specificity

Unfortunately, there was no prompt access to rare units of red cells that are k negative at that point

Further testing

Tube Method

Since we were unable to validate the use of DTT in the Bio-Rad Gel in our lab, tube method was used instead, to test treated plasma and RBCs with 0.01M and 0.2M DTT, respectively. Indeed, antigens in the Kell system are known to be destroyed by 0.2M DTT

The red cell panel was washed in all testing stages to avoid intervention of RBC preservative-specific antibodies

Panel Sample

Saline-Tube Method (washed panel cells)

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IAT Saline
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	4+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	4+

Autocontrol not tested due to recent transfusion

Panel Sample

IAT-LISS Tube Method (washed panel cells)

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		IAT
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+		4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0		4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+		4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+		4+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+		4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0		4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+		4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+		4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0		4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0		4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+		4+

Autocontrol not tested due to recent transfusion

Panel Sample

Two-Stage enzyme tube method (washed panel cells)

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IAT Enzyme
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	4+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	4+

Autocontrol not tested due to recent transfusion

Panel Sample

IAT-LISS Tube- 0.01M DTT treated plasma

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Plasma treated with 0.01M DTT
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	4+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	4+

Interpretation Suggested Further Testing

Again, this antibody(ies) reacted both in Saline and in IAT phase in Tube Method using washed cell-panel

It suggested that this is only an IgG antibody since its reactivity in saline phase was not destroyed after treating the plasma with 0.01M DTT, but IgM antibody reactivity could also be present in addition to an IgG antibody

Further testing included treating panel cells with 0.2M DTT reagent

Panel Sample

IAT-LISS Tube – using 0.2M DTT treated red cells

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	RBCs treated with 0.2M DTT
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	1+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	1+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	2+
5	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	1+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	1+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	2+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	0
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	2+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	1+

Well, it makes the difference here. Anti-M is detected and anti-k very likely present

Further testing

This patient has developed anti-M with possible anti-k

This time, 3 examples of k-negative cells were obtained locally from our rare donor registry for use in testing in our laboratory

One of these cells was k- M-

Panel Sample

IAT-LISS Tube: k negative mini panel

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	LISS IAT
1	+	0	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0
2	0	+	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	0	0	1+
3	+	+	+	0	+	0	+	0	0	+	0	+	0	+	+	+	+	+	0	1+

IAT Enzyme Tube: k negative mini panel

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IAT Enzyme
1	+	0	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0
2	0	+	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	0	0	0
3	+	+	+	0	+	0	+	0	0	+	0	+	0	+	+	+	+	+	0	0

Conclusions

This patient has:

- Developed IgG form of anti-k reactive in the saline phase
- Developed anti-M

Summary of Case Challenges

- Identification of anti-k, an antibody to a high prevalence antigen, in a laboratory with limited access to rare RBC inventory to investigate reactivity or to provide rare RBCs for transfusion

- Very uncommon for a person of African descent to be k negative

- Going back to tube method in a lab that completely converted to new CAT technology

Lessons Learned by the Case

- Never rely on one method, especially with those known to be associated with false negative or positive results or not appropriately validated
- Consistency in testing is the key of solving problems
- Remember, the tube method can be very valuable in investigating cases referred to IRLs