Eureka!

Laboratory Aha Moments

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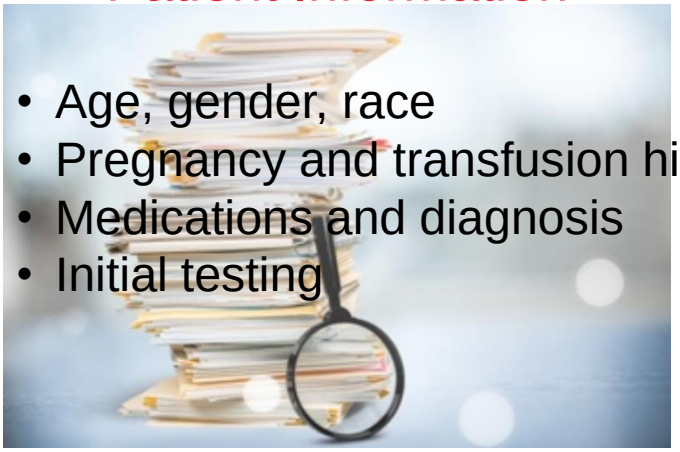
Immunohematology Reference Laboratory Technologist II

Learning Objectives

After participating in this course, you will be able to:

- recognize the unpredictable nature of antibody identification
- evaluate patient information for clues to antibody identification
- analyze initial serologic blood bank test results to make an educated prediction for the cause of antibody reactivity in a patient sample
- select further testing techniques to prove or disprove a suspected cause of reactivity in a serologic blood bank investigation

Antibody Identification Clues
Patient Information



- Age, gender, race
- Pregnancy and transfusion history
- Medications and diagnosis
- Initial testing

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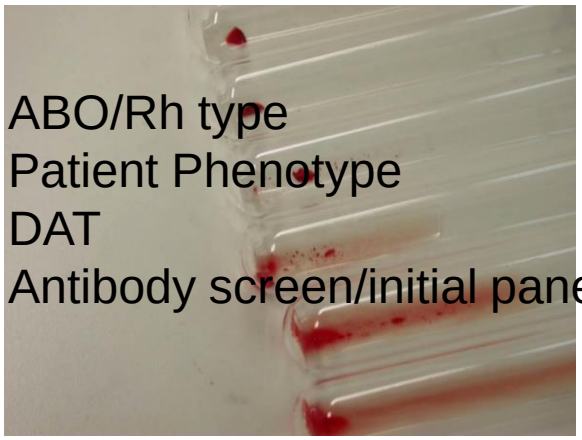
Antibody Identification Clues
Initial Test Results



- ABO/Rh type
- Patient Phenotype
- DAT
- Antibody screen/initial panel

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Antibody Identification Clues Additional Tests

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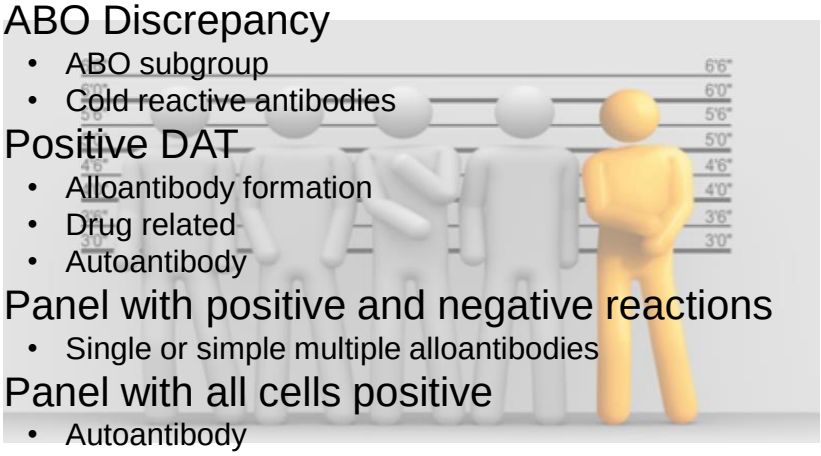
Antibody Identification - The Usual Suspects

- ABO Discrepancy
  - ABO subgroup
  - Cold reactive antibodies
- Positive DAT
  - Alloantibody formation
  - Drug related
  - Autoantibody
- Panel with positive and negative reactions
  - Single or simple multiple alloantibodies
- Panel with all cells positive
  - Autoantibody
  - Drug related/reagent dependent antibody



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Initial IRL Testing:

When a sample is referred to the Immunohematology Reference Laboratory we already know it is a problem! Initial IRL testing includes:

- ABO using anti-A, anti-B, anti-A,B, and A_1, A_2, and B cells
- Rh type using anti-D including weak D testing
- Rh phenotype testing the patient for C, E, c, and e
- DAT is performed using polyspecific antihuman globulin
  - If positive, we will test with Anti-IgG and anti-C3
- A reagent red cell panel and auto control in tube at IS and PEG/IAT
  - Testing in other methods such as LISS, enzymes, or Gel is usually part of the follow up testing

Case Study #1
Patient Information

- Age: 57
- Medications: Not Provided
- Gender: Female
- Race: Unknown
- Diagnosis: AMS, Fever
- Pregnancy History: Not Provided
- Transfusion History: Within the last 3 months
- Referring Facility's Results: Not Provided

Case Study #1
Initial Testing

ABO/Rh								
Cell Typing						Serum Grouping		
	A	B	A,B	D	Control	A1	A2	B
IS	0	4+	3+	4+	0	3+	2+	0

DAT				
	Poly	IgG	C3	Control
IS	w+	m+		0
5' RT	1+		1+	0

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Case Study #1
Initial Testing

	D	C	E	c	e	f	C^w	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	PEG IAT
1	0	0	0	+	+	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	2+
2	0	0	0	+	+	+	0	+	+	+	+	0	+	0	0	+	+	+	+	+	+	+	0	2+
3	0	+	0	+	+	+	0	+	+	0	+	W	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	+	W	+	0	0	+	0	+	+	+	+	0	0	2+
6	+	0	0	+	+	+	0	0	+	+	+	+	+	0	0	+	0	+	0	0	+	+	0	2+
7	+	+	0	0	+		0	+	0	+	0	W	+	0	+	+	0	+	0	+	0	+	0	2+
8	+	+	0	0	+		0	+	0	0	+	W	0	+	0	+	+	+	0	+	0	+	0	2+
9	+	+	0	0	+		+	+	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	2+
10	+	0	+	+	0		0	+	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	2+
11	+	+	+	0	+		0	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	2+
Auto																							0	2+

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Case Study #1

The Clues

What we learned from patient information and initial testing:

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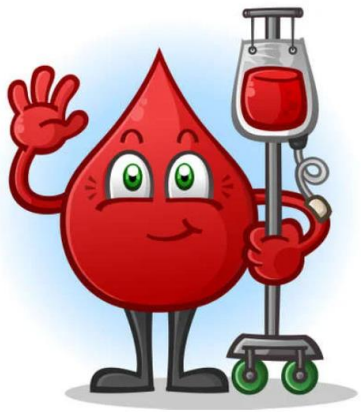
Case Study #1
The Clues



Clue
- DAT is positive for both IgG and C3

Prediction
- IgG antibodies are coating the red blood cells

Addtional Testing
- Perform an elution

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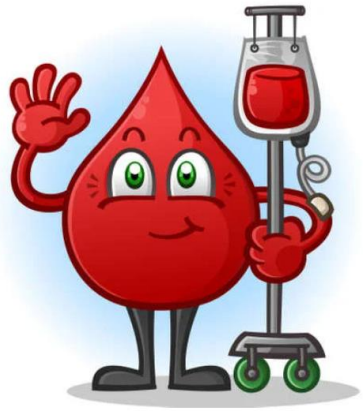
Case Study #1
The Clues



Clue
- Antibody panel is positive with all cells, including auto control

Prediction
- Positive auto control reacting at same strength as all panel cells indicates a potential warm autoantibody

Additional Testing
- Due to recent transfusion, perform alloadsorption.

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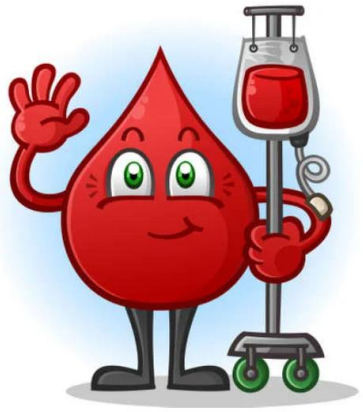
Case Study #1
The Clues



Clue
- Patient has been transfused within the last 3 months.

Prediction
- Patient has potential to produce alloantibodies and transfused cells are probably still present in the patient's circulation.

Additional Testing
- Cell separation will be required prior to antigen typing.

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Case Study #1
Course of Action

Hypothesis: Patient has a warm autoantibody.

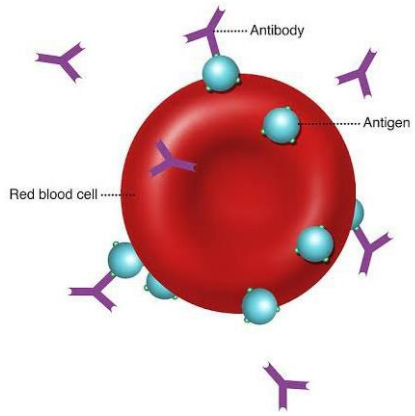
Additional Testing:

- cell separation to confirm if the plasma antibody is allo or auto
- cell separation to perform full phenotype
- alloadsorption to rule out underlying alloantibodies
- elution
  - Due to limited sample, the transfused cell population from the microhematocrit cell separation was retained for the elution.

Case Study #1
Elution

	D	C	E	c	e	f	C^w	M	N	S	s	P1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Eluate	Last Wash
I	+	+	o	o	+		o	+	o	o	+	W	o	+	o	+	o	+	o	+	o	+	1+	0✓
II	+	o	+	+	o		o	o	+	+	+	W	o	+	o	+	+	+	+	o	+	+	1+	0✓
III	o	o	o	+	+	+	o	+	+	+	o	+	+	o	o	+	o	+	+	+	+	o	1+	0✓

Case Study #1
Allogeneic Adsorptions



Allogeneic Adsorption Applications

- Resolution of reactivity due to autoantibody
- Confirmation of a weak subgroup or antigen via adsorption/elution
- Removal of a high prevalence antibody to allow for antibody exclusion
- Separation of multiple antibody specificities
- Removal of certain interfering therapeutic substances

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Case Study #1
Adsorbed Plasma Testing

	D	C	E	c	e	M	N	S	s	P1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	F1
R1R1	+	+	o	o	+	+	o	+	o	+	o	o	o		o	+	+	o	
R2R2	+	o	+	+	o	o	+	o	+	+	o	+	o		+	o	+	+	
rr	o	o	o	+	+	+	o	+	o	+	o	+	o		+	o	o	+	
R1R1																			
1	+	o	+	+	o	o	+	o	+	+	+	o	o		+	+	+	+	0✓
2	+	+	o	o	+	+	o	+	+	o	o	+	o		+	o	+	o	0✓
3	+	o	+	+	o	+	o	+	+	+	o	+	+	o	o	+	o	+	0✓
R2R2																			
1	+	+	o	o	+	+	o	+	o	+	o	+	+	+	o	+	+	o	0✓
2	+	o	+	+	o	+	+	+	+	+	+	o	o		+	+	+	+	0✓
rr																			
1	+	+	o	o	+	o	+	o	+	+	+	o	o		o	+	o	+	0✓
2	+	o	+	+	o	o	+	+	+	o	o	+	o		+	+	+	o	0✓
3	+	+	o	o	+	+	o	o	+	o	o	o	+	o	+	+	+	+	0✓

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F1: Allo adsorbed plasma x2 tested at Peg IgG

Identification of Warm Autoantibody Reactivity

- At least one of the following:
  - Auto Control Reactive at 37C and/or AHG
  - DAT is positive
- Serum studies are reactive at 37C and/or AHG.
- At least one of the following:
  - Neat serum reacts with DAT-negative autologous red blood cells.
  - Adsorption with autologous red blood cells removes autoantibody reactivity

Case Study #1
Autoantibody Confirmation

Neat plasma tested against DAT negative harvested reticulocytes

	Peg/IgG
Auto	0✓

- **Result**
  - Negative
- **Hypothesis**
  - Disproven
- **New Hypothesis**
  - High Frequency Antibody?

Case Study #1
Phenotype

Testing performed on reticulocytes harvested by microhematocrit centrifugation.

DAT	
	IgG
IS	0✓

C	E	c	e	K	Fya	Fyb	Jka	Jkb	M	N	S	s	P1	Lea	Leb
0	0	4+	4+	0	0	0	3+	0	0	2+	0	0	3+	0	0

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Case Study #1

The Clues

What we learned from additional testing:

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Case Study #1
The Clues

Patient's phenotype:

S	s
0	0

Could the patient have an anti-U???

Case Study #1
Additional testing

	D	C	E	c	e	f	C^w	M	N	S	s	P1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	PEG IAT	Eluate	Last Wash
1	+	0	o	+	+		o	+	o	o	o	W	o	+	o	+	o	+	o	o	+	o	0✓	0✓	0✓
2	+	o	o	+	+		o	+	+	o	o	0	o	o	o	+	o	+	+	o	+	+	0✓	0✓	0✓
3	o	o	o	+	+	+	o	+	+	0	o	+	+	o	o	+	o	+	o	o	+	o	0✓	0✓	0✓

Testing with S-s-U- reagent red cells was nonreactive, confirming the presence of anti-U in both the serum and eluate

Case Study #1
Conclusion and Follow Up

Antibody confirmed as anti-U in serum and eluate

Additional follow up:

- Molecular testing to confirm if the patient is a true U- or Uvar
- Source U- compatible blood for transfusion
- The patient successfully received 2 units of U- RBCs and was discharged

This case demonstrated the complexity of identifying a high prevalence antibody when both the serum and eluate contain broad reactivity

Case Study #2
Patient Information

- Age: 60
- Medications: Not Provided
- Gender: Female
- Race: White
- Diagnosis: Sepsis/UTI
- Pregnancy History: None
- Transfusion History: Four units in the previous week
- Referring Facility's Results: History of anti-Fy^{a}

Case Study #2
Initial Testing

ABO/Rh								
Cell Typing						Serum Grouping		
	A	B	A,B	D	Control	A1	A2	B
IS	4+	0	4+	4+	0	0	0	3+

DAT				
	Poly	IgG	C3	Control
IS	1+	1+		0
5' RT	0		0✓	0

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Case Study #2
Initial Testing

	D	C	E	c	e	f	C^w	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	PEG IAT
1	0	0	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	+	W	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	+	W	+	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	W	+	0	+	+	0	+	0	+	0	+	0	2+
8	+	+	0	0	+		0	+	0	0	+	W	0	+	0	+	+	+	0	+	0	+	0	2+
9	+	+	0	0	+		+	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0✓
10	+	0	+	+	0		0	+	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	2+
11	+	+	+	0	+		0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	2+
Auto																							0	2+

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Case Study #2
Phenotype

Testing performed on reticulocytes harvested by microhematocrit centrifugation.

DAT	
	IgG
IS	W+

C	E	c	e
3+	3+	$1+^{mf}$	3+

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Case Study #2

The Clues

What we learned from patient information and initial testing:

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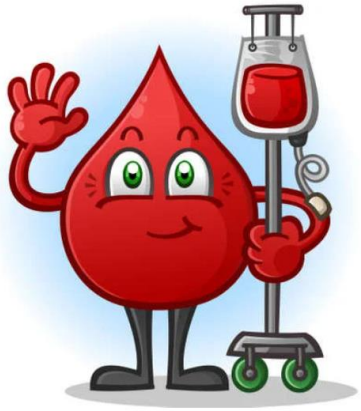
Case Study #2
The Clues



Clue
- DAT is positive for IgG

Prediction
- IgG antibodies are coating the red blood cells, patient has multiple recent transfusions

Addtional Testing
- Perform an elution

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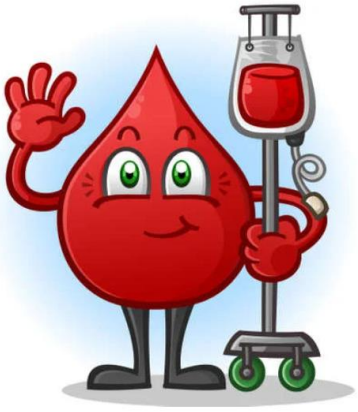
Case Study #2
The Clues



Clue
- Antibody panel is positive with all but one cell, including auto control

Prediction
- This could be a case of multiple alloantibodies

Additional Testing
- Additional selected cells are indicated as phenotype is unavailable at this time

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Case Study #2
Eluate Testing

	D	C	E	c	e	f	C^w	M	N	S	s	$P1$	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	PEG IAT
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	0	0✓
3	0	+	0	+	+	+	0	+	+	0	+	W	0	+	0	+	0	+	0	+	+	+	0	2+
4	0	0	+	+	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	2+
5	0	0	0	+	+	+	0	+	+	0	+	W	+	0	0	+	0	+	+	+	+	0	0	0✓
6	+	0	0	+	+	+	0	0	+	+	+	+	+	0	0	+	0	+	0	0	+	+	0	2+
7	+	+	0	0	+		0	+	0	+	0	W	+	0	+	+	0	+	+	+	0	+	0	2+
8	+	+	0	0	+		0	+	0	0	+	W	0	+	0	+	+	+	+	+	0	+	0	2+
9	+	+	0	0	+		+	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0✓
10	+	0	+	+	0		0	0	+	0	+	+	0	0	0	+	0	+	+	0	+	0	0	0✓
11	+	+	+	0	+		0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	2+
Auto																							0	2+

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Case Study #2
Initial Testing

	D	C	E	c	e	f	C^w	M	N	S	s	$P1$	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	PEG IAT
1	0	0	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	+	W	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	+	W	+	0	0	+	0	+	0	+	+	0	0	2+
6	+	0	0	+	+	0	0	0	+	+	+	+	+	0	0	+	0	+	0	0	+	+	0	2+
7	+	+	0	0	+		0	+	0	+	0	W	+	0	+	+	0	+	0	+	0	+	0	2+
8	+	+	0	0	+		0	+	0	0	+	W	0	+	0	+	+	+	0	+	0	+	0	2+
9	+	+	0	0	+		+	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0✓
10	+	0	+	+	0		0	+	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	2+
11	+	+	+	0	+		0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	2+
Auto																							0	2+

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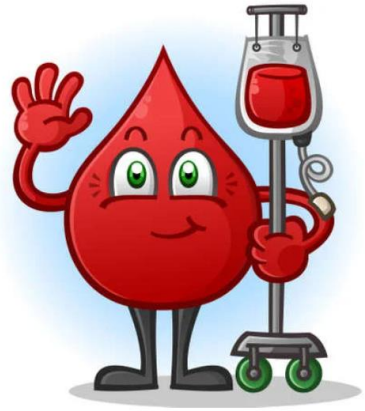
Case Study #2
The Clues



Clue
- Reactivity is at PEG/IAT

Prediction
- Anti-M, -N, -S, Lea are cold reactive
- Anti-Fy^{b} is unlikely in a Caucasian patient with anti-Fy^{a}

Hypothesis
- Test for anti-c

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Case Study #2
Testing Fy(a-), Jk(b-) RBCs

	D	C	E	c	e	f	Cʷ	M	N	S	s	P1	Le^{a}	Le^{b}	Lu^{a}	Lu^{b}	K	k	Fy^{a}	Fy^{b}	Jk^{a}	Jk^{b}	IS	PEG IAT
1	0	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	+	W	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	+	W	+	0	0	+	0	+	0	+	+	0	0	0✓
6	+	+	0	0	+	0	0	0	+	+	0	+	+	0	0	+	0	+	0	0	+	0	0	0✓
7	+	+	0	0	+		+	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	2+
8	+	+	0	0	+	0	0	+	+	+	+	+	0	+	0	+	0	+	+	+	+	0	0	2+

American Red Cross

Case Study #1
Adsorbed Plasma Testing

	D	C	E	c	e	M	N	S	s	P1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	F1
R1R1	+	+	0	0	+	+	0	+	0	+	0	0	0		0	+	+	0	
R2R2	+	0	+	+	0	0	+	0	+	+	0	+	0		+	0	+	+	
rr	0	0	0	+	+	+	0	+	0	+	0	+	0		+	0	0	+	
rr																			
1	+	0	+	+	0	0	+	+	+	0	0	+	0		+	+	+	0	0✓
2	+	+	0	0	+	+	0	0	+	0	0	0	+	0	+	+	+	+	0✓

In this case, we decided to adsorb with just the rr cells, which are c+, Fy(a+) and Jk(b+) and E-, K-

Cells were tested with the adsorbed serum to rule out the presence of anti-E and anti-K

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Case Study #2
The Conclusion?

We have now identified anti-Jkb in the patient's eluate and anti-c, Fy^a, and Jk^b in the patient's serum.

Prior to reporting our test results, we perform a robust self-review of testing to ensure it adheres to standards – and we notice…

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Case Study #2
The Conclusion?

We have not ruled out anti-f!

The f antigen is a compound antigen expressed on RBCs having c and e antigens in the same haplotype.

It is usually made by individuals with the R1R2 phenotype (C+E+c+e+)

American Red Cross

Case Study #2
Testing Fy(a-), Jk(b-) RBCs

	D	C	E	c	e	f	C^w	M	N	S	s	P1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	PEG IAT
1	o	o	o	+	+	+	o	+	+	+	o	o	o	+	o	+	o	+	o	o	+	o	o	2+
2	o	o	o	+	+	+	o	+	+	+	+	o	+	o	o	+	+	+	o	+	+	o	o	2+
3	+	+	o	o	+	o	o	+	o	o	+	W	o	+	o	+	o	+	o	+	+	o	o	o✓
4	+	+	o	o	+	o	o	o	+	o	+	+	o	+	o	+	o	+	o	+	+	o	o	o✓
5	+	+	o	o	+	o	o	+	+	o	+	W	+	o	o	+	o	+	o	+	+	o	o	o✓
6	+	+	o	o	+	o	o	o	+	+	o	+	+	o	o	+	o	+	o	o	+	o	o	o✓
7	+	+	o	o	+		+	+	o	+	+	o	o	+	o	+	o	+	+	o	+	o	o	2+
8	+	+	o	o	+	o	o	+	+	+	+	+	o	+	o	+	o	+	+	+	+	o	o	2+

Looking back at our selected cells, we notice that both of the c+ cells used to confirm anti-c are rr cells, which are f antigen positive. Let's run a few more cells…

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Case Study #2
Testing f-, Fy(a-), Jk(b-) RBCs

	D	C	E	c	e	f	C^w	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	PEG IAT
1	+	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	2+

Some reagent manufacturers do not test for the f antigen and will leave that result blank on the manufacturer's antigen profile. However, based on the phenotype of the cell, you know whether the f antigen is present. Any cell with c and e on the same haplotype will carry the f antigen.

The adsorption with rr cells would have removed anti-f as well as the other alloantibodies present. We ran one additional f positive reagent cell with neat serum, just to confirm our identification!

Case Study #2
Conclusion and Follow Up

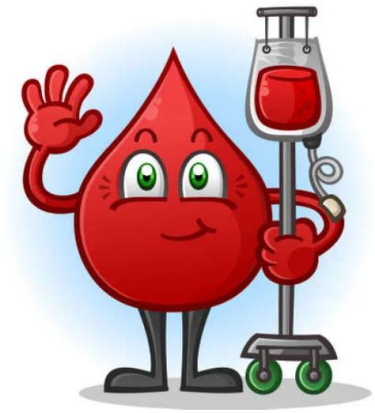
Antibodies confirmed as anti-Jk^b in the eluate and anti-f, -Fy^a, -Jk^b in the serum

Additional follow up:

- A subsequent untransfused sample was sent about six months later and the patient phenotype was confirmed as R1R2
- The patient successfully received 2 units of c-, Fy(a-), Jk(b-) RBCs and was discharged

This case demonstrated the complexity of identifying multiple alloantibodies including anti-f

Eureka!



Objective
- Antibody ID is unpredictable, and the laboratory uses many clues to come to a valid conclusion

Challenge
- Patient demographics and history and initial testing may point in the wrong direction

Conclusion
- Antibody identification can be challenging, rewarding, frustrating, and exhausting – and we love it!

Questions