

Resolving immunohematology Case Studies

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Resolving immunohematology Case Studies

Program Objectives

- Describe the appropriate immunohematological techniques used to resolve a complex antibody
- Give examples of enhancement Medias used when trying to resolve antibody workup
- Recognize the importance of reactions at the different phases of serologic testing

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First Steps:

- Age
- Gender
- Race
- Diagnosis
- History
 - Medical
 - Transfusion
 - Medication



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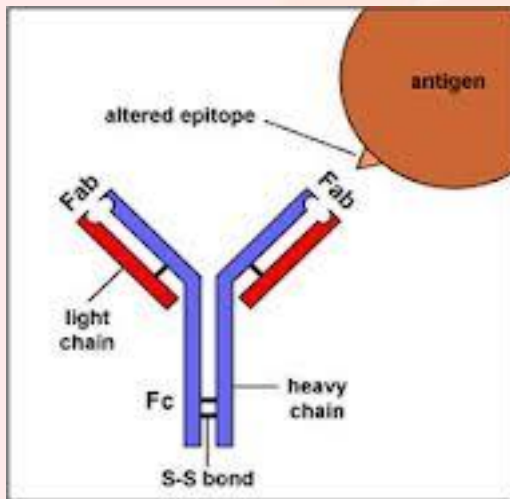
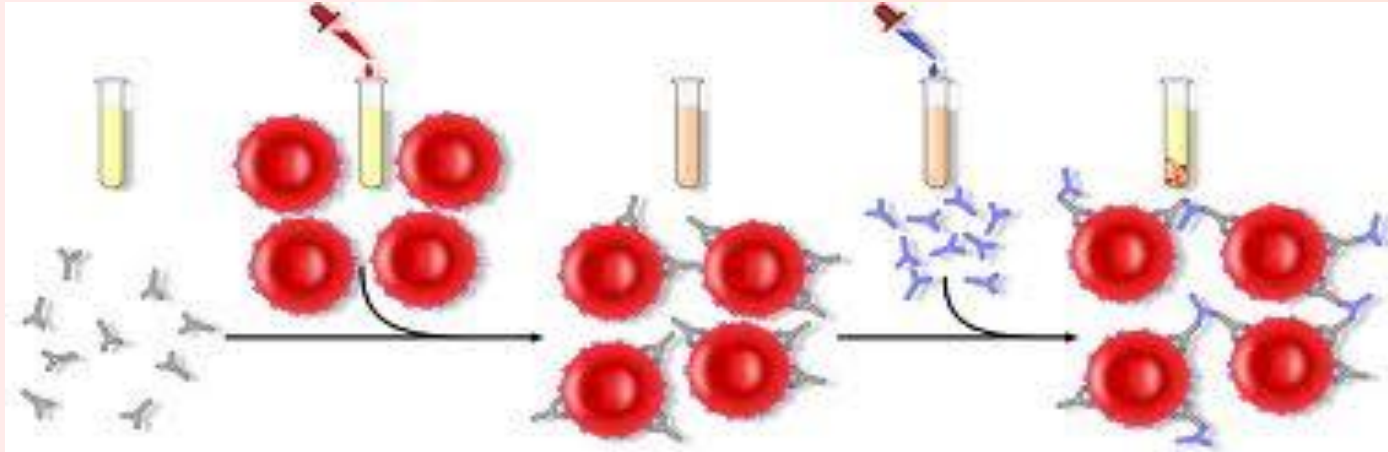
- What antibodies are identified on the panel?
- Is there a specificity?
 - (Homozygous/Heterozygous/is there a pattern)
- What phases did reactions occur?
- Did strength vary?
- Is there hemolysis?

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- What further testing would be helpful to identify antibody?
- How would you proceed in order to meet the patients need for transfusion?
- Always follow manufacture directions, your Standard Operating Procedures, Policies, and Technical Manual.

**Follow the manufacturer's
instructions**

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- The Epitope (portion of antigen) binds to the Paratope (Ig portion of the antibody that binds the antigen)
- **Mechanisms of Agglutination**
 - Sensitization
 - Lattice Formation
- **Agglutination** is affected by pH, Temperature, Time and ionic strength

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What techniques can we use:

- Gel, LISS, PEG, Albumin
- Elution Studies (Freeze-Thawing, Heat..)
- Cell Separation (Direct Separation, Phthalate Esters, Harvesting Autologous RBCs in Sickle cell patients...)
- Chemical Treatment – EGA/Chloroquine Diphosphate
- DTT – Serum and Cells, AET
- Enzyme Treatment (Ficin, Papain, Bromelain, Trypsin..)
- Adsorption Studies (Allogeneic, Autologous RBCs)
- Titration Studies (Pregnancy, “HTLA”)
- Inhibition/Neutralization Studies (Lewis, P1 substance, Sd^a...)
- Freezing rare RBCs – LN2 Preservation and Recovery



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Case Studies

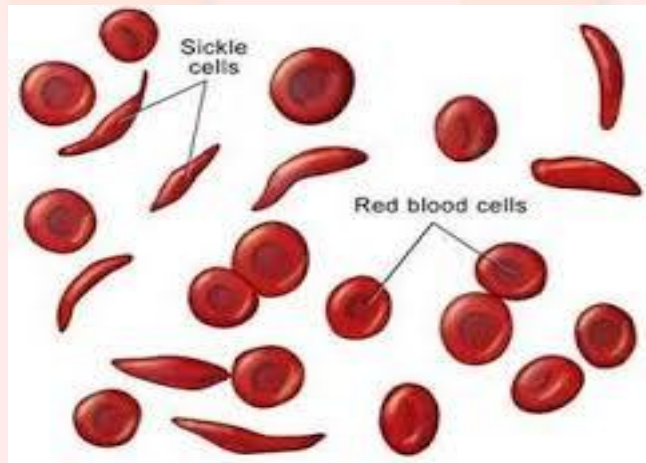
Case 1 - Panel Results

Panel Cell	D	C	E	c	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	Lu a	Lu b	P1	Gel	IS	37	AHG	CC
1	+	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	2+				
2	+	+	0	+	0	0	+	+	0	+	0	0	+	+	+	0	+	+	2+				
3	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	0	+	+	2+				
4	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+	2+				
5	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	0	+	+	2+				
6	0	0	0	+	+	+	+	+	0	+	+	0	+	+	0	0	+	+	2+				
7	0	0	+	+	+	0	+	0	0	0	+	0	+	+	0	+	+	0	2+				
8	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	2+				
9	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	0	+	+	2+				
Auto																			0				

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Recently transfusion-less than 3months

- Microhematocrit cell separation
 - Reticulocytes have lower specific gravity than transfused cells
- Hypotonic Saline Wash
 - Hemoglobin SS or SC are resistance to lysis

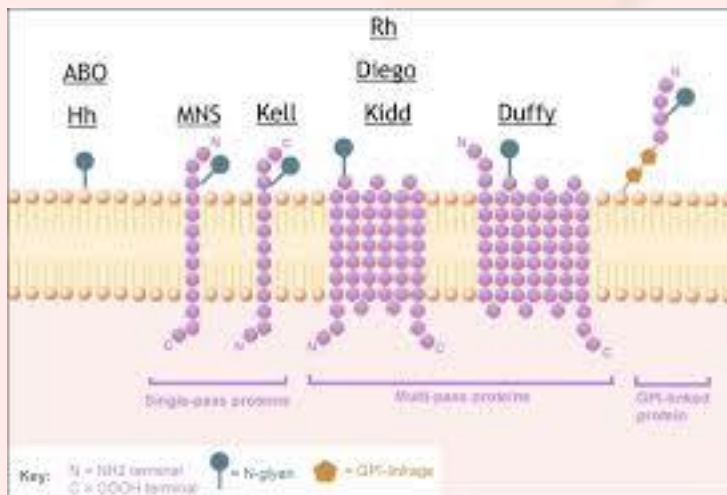


Resolving immunohematology

Case Studies

Case 1 – Patient's complete phenotype

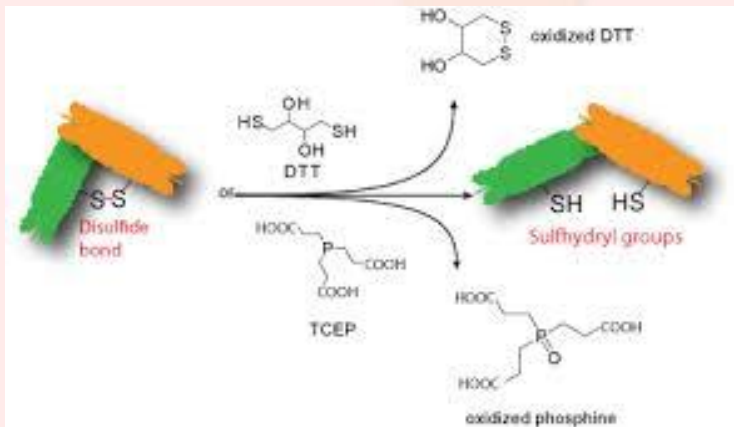
D	C	E	c	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	Lua	Lub	P1				
+	+	0	+	+	+	0	0	0	+	+	+	+	+	0	NT	NT	NT				



Resolving immunohematology Case Studies

Case 1 – DTT – Selected cells

Panel Cell	D	C	E	c	e	K	k	Fy a	Fyb	Jka	Jkb	M	N	S	s	Lu a	Lub	P1	Gel	IS	37	AH G	CC
1	+	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+				0	2+
2	+	+	0	+	0	0	+	+	0	+	0	0	+	+	+	0	+	+				0	2+
3	+	0	+	+	+	0	+	0	0	0	+	+	0	+	0	0	+	+				1+	
4	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+				0	2+
5	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	0	+	+				0	2+



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Case 1 – DTT selected cells

Panel Cell	D	C	E	c	e	K	k	Fy a	Fyb	Jka	Jkb	M	N	S	s	Lu a	Lu b	P 1	Ge 1	I S	3 7	AH G	C C
6	+	0	+	+	+	0	+	0	0	0	+	+	0	+	0	0	+	+				1+	
7	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+				0	2+
8	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	0	+	+				0	2+
9	0	0	+	+	+	+	+	+	0	+	+	0	+	+	0	0	+	+				1+	



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Case 1 - Panel Results – Anti-E, Anti- k

- DTT - Dithiothreitol is a very efficient reducing agent that can disrupt the tertiary structure of proteins by irreversibly reducing disulfide bonds to free sulfhydryl groups.
- RBCs treated with DTT will not react with antibodies in the Kell blood group systems
- Other Antigens antibodies will not react to are Knops systems, and examples of Anti-LW^a, -Yt^a, -Yt^b, -Do^a, -Do^b, -Gy^a, -Hy and -Jo^a

Resolving immunohematology Case Studies

Case 2 – Panel Results

Panel Cell	D	C	E	c	e	K	k	Fy _a	Fyb	Jka	Jkb	M	N	S	s	Lua	Lub	P1	Gel	IS	37	AHG	CC
1	+	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	3+				
2	+	+	0	+	+	0	+	+	0	+	0	0	+	+	+	0	+	+	3+				
3	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	0	+	+	3+				
4	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+	3+				
5	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	0	+	+	2+				
6	0	0	0	+	+	+	+	+	0	+	+	0	+	+	0	0	+	+	2+				
7	0	0	+	+	W+	0	+	0	0	0	+	0	+	+	0	+	+	0	0				
8	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	2+				
9	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	0	+	+	3+				
Auto																			0				

Resolving immunohematology Case Studies

Case 2 - Selected Cells

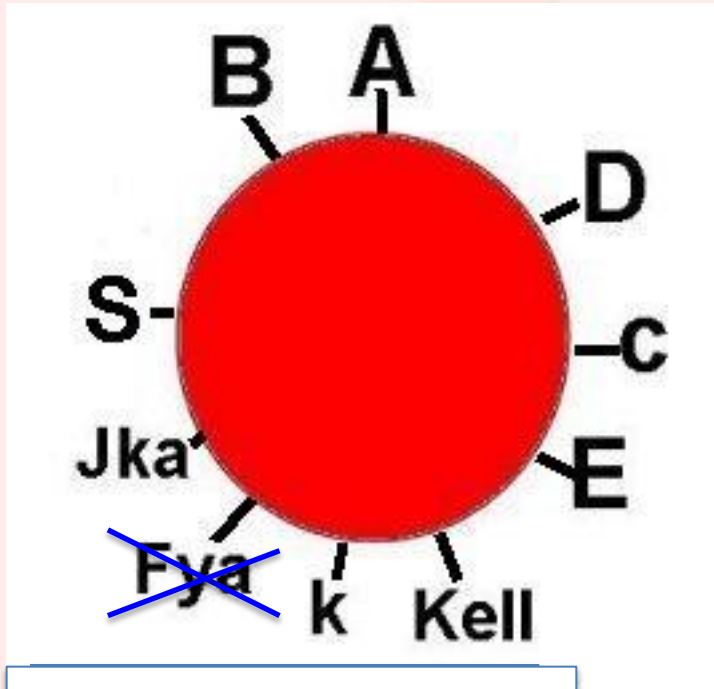
Panel Cell	D	C	E	c	e	K	k	Fy _a	Fy _b	Jka	Jkb	M	N	S	s	Lua	Lub	P1	Gel	IS	37	AHG	CC
1	0	0	0	+	+	0	+	0	0	+	0	0	+	+	0	0	+	+	0				
2	0	0	0	+	+	+	+	+	0	+	+	0	+	+	0	0	+	+	2+				
3	0	0	+	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	0				
4	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	2+				
5	0	0	+	+	+	0	+	+	+	0	+	+	+	+	0	0	+	+	2+				
6	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	2+				
7	0	+	0	+	+	0	+	+	0	+	0	0	+	+	+	0	+	+	2+				



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Proteolytic enzymes – Ficin and Papain

- **Destroy or weaken red blood cell antigens**
 - M, N, Fya, Fyb, Xga, JMH, Ch, Rg, Yta
- **Enhance the reactivity of some antibodies**
 - Rh, P, I Kidd, and Lewis



Resolving immunohematology Case Studies

Case 2 - Selected Cells – **Enzyme** Treated cells

Panel Cell	D	C	E	c	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	Lua	Lub	P1	Gel	IS	37	AH G	CC
1	0	0	0	+	+	0	+	0	0	+	0	0	+	+	0	0	+	+	0				
2	0	0	0	+	+	+	+	+	0	+	+	0	+	+	0	0	+	+	0				
3	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0				
4	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	3+				
5	0	0	+	+	+	0	+	+	+	0	+	+	+	+	0	0	+	+	0				
6	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	3+				
7	+	0	0	+	+	0	+	+	0	+	0	0	+	+	+	0	+	+	4+				



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Case Studies

Case 2 - Panel Results:

Anti-D, Anti- C, Anti-Fya

- Proteolytic enzymes (Ficin) modify antigens of the red blood cell membrane: cleaves proteins at specific amino acids
- Enhances the reactivity of some antigens with antibodies
 - Rh (Anti-D and Anti-C)
- Abolish antigen reactivity
 - Fya antigen



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Case 3 – Panel Results

Panel Cell	D	C	E	c	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	Lua	Lub	P1	Gel	IS	37	AHG	CC
1	+	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	W+				
2	+	+	0	+	0	0	+	+	0	+	0	0	+	+	+	0	+	+	0				
3	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	0	+	+	W+				
4	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+	0				
5	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	0	+	+	W+				
6	0	0	0	+	+	+	+	+	0	+	+	0	+	+	0	0	+	+	0				
7	0	0	+	+	+	0	+	0	0	0	+	0	+	+	0	+	+	0	1+				
8	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0				
9	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	0	+	+	W+				
Auto																			1+				

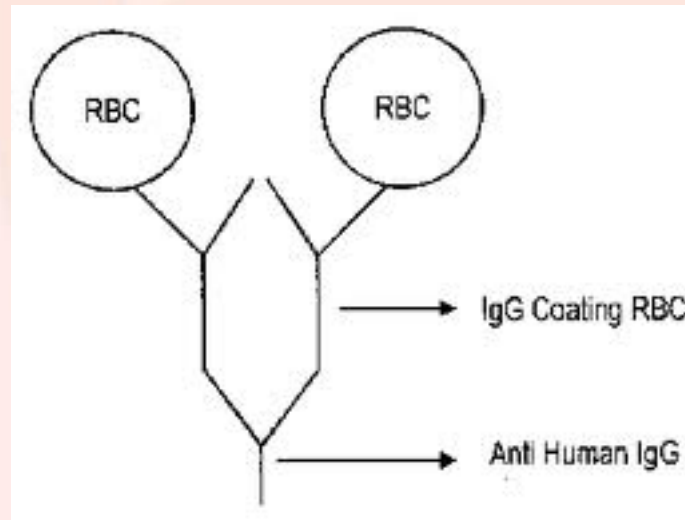


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Case Studies

Case 3 - DAT

Poly	IgG	C3	Control
1+	1+	0	0



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Case 3 – Eluate

Panel Cell	D	C	E	c	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	Lua	Lub	P1	Gel
1	+	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	2+
2	+	+	0	+	0	0	+	+	0	+	0	0	+	+	+	0	+	+	2+
3	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	0	+	+	2+
4	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+	2+
5	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	0	+	+	2+
6	0	0	0	+	+	+	+	+	0	+	+	0	+	+	0	0	+	+	2+
7	0	0	+	+	+	0	+	0	0	0	+	0	+	+	0	+	+	0	2+
8	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	2+
9	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	0	+	+	2+

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Case Studies

Case 3 - Selected Cells - LISS



Panel Cell	D	C	E	c	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	Lua	Lub	P1	Ge 1	IS	37-LISS	AHG	CC
1	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+		1+	0	0	2+
2	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	0	+	+		0	W+	0	2+
3	+	0	0	+	+	0	+	0	0	0	+	+	0	+	0	0	+	+		1+	W+	0	2+
4	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+		1+	W+	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	1+	
8	+	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+		1+	0	0	2+
9	+	+	0	+	0	0	+	+	0	+	0	0	+	+	+	0	+	+		0	0	0	2+



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Case Studies

Case 3 - Selected Cells - LISS

Panel Cell	D	C	E	c	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	Lua	Lub	P1	Ge 1	IS	37-LISS	AHG	CC
1	+	+	0	0	+	0	+	0	+	+	0	0	+	+	+	+	+	+		0	0	1+	
2	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	+	+	+		0	0	1+	
3	0	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	+	+		W+	0	0	2+
6	+	0	+	+	+	0	+	+	0	+	0	0	+	0	+	0	+	+		0	0	0	2+

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Case 3 - Crossmatch

Unit #	IS	37	AHG	CC
¹ W140914123456 (M, Lua Neg)	W +	0	0	2+
² W140914123505 (M, Lua Neg)	0	0	0	2+

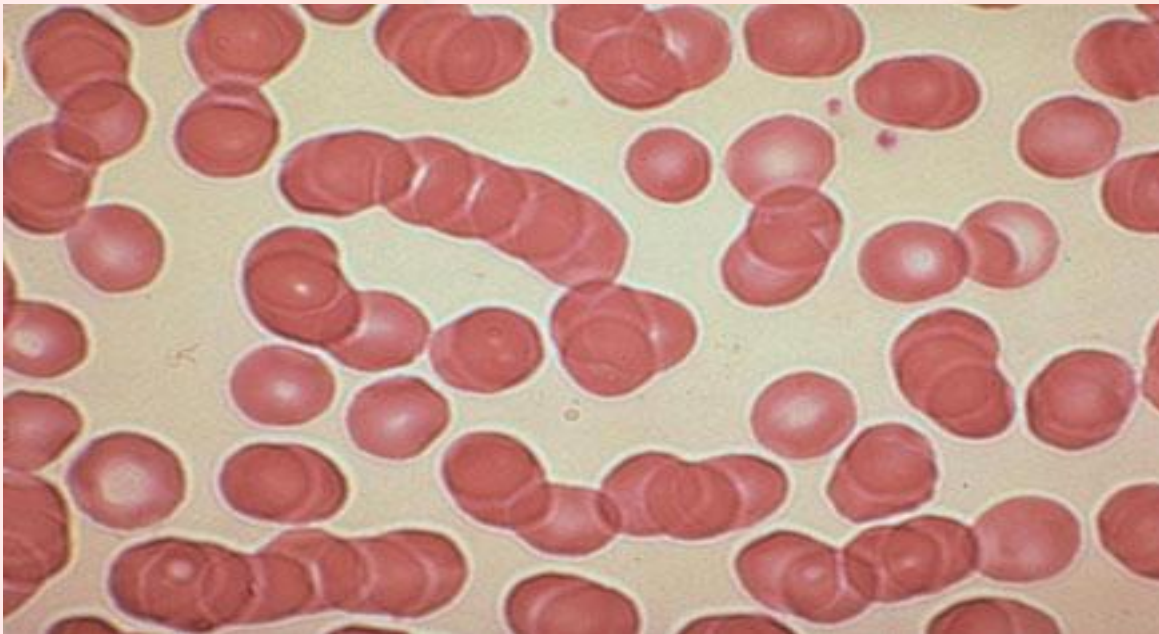


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Case Studies

Case 3

Rouleaux – abnormal concentration of serum proteins giving a stack coin appearance under the microscope



QUALTEX
LABORATORIES

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Case 3 – Crossmatch-*Saline Replacement

Unit #	IS	37	AHG	CC
¹ W140914123456 (M, Lu ^a neg)	W+/*0	0	0	2+

1. Re-centrifuge the tube
2. Remove the serum – leaving the RBC button
3. Add two (2) drops of saline
4. Re-suspend the button gently
 1. If rouleaux- disperse- Negative reaction
 2. If true agglutination – Positive reaction



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Case 3 - Panel Results:

- Anti-M, Anti-Lua, slight Rouleaux, Positive Eluate
- Some examples of Anti-M react stronger with double dose expression than heterozygous cells
- Other methods - Tube testing – saline agglutination
- Examples reacting at 37°C or Antiglobulin phase should be considered potentially clinically significant
- Anti-Lua is an antibody to a low frequency antigen (Lu (a+ b-) .15%, Lu (a+ b+) 7.5%)
 - Has not been reported to cause HDFN or HTRs

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Case Studies

References

- Issitt, P.D., & Anstee D.J. (1988). Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications,
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- Judd, J.W., Johnson S.T., Storry J.R. (2008). Judd's Methods in Immunohematology. 3rd ed. Bethesda, MD: AABB Press

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