American Red Cross

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Case Studies 2022

Objectives

- **Show** an in depth look into patient workups performed at a reference laboratory
- **Analyze** various techniques used for antibody identification
- **Solve** complex immunohematologic problems

Case #1

- 48 year old Asian female
- Transfusion history reported as never transfused
- H/H is 11.8 & 36.7
- Hospital ABO O Positive
- Diagnosis: Dysmenorrhea

Antibody ID

- ABO/Rh
- DAT
- C, E, c,
- Serum studies

Case #1

- ABO:

	A	B	A,B	D	Cont	A1	A2	B
IS	0	0	0	2+	0	4+	4+	4+

- DAT:

Poly	IgG	C3	Control
0/0	NT	NT	0/0
0/0✓	NT	NT	0/0

Rh Phenotype

C	E	c	e
3+	0	4+	4+

Low Ionic Strength Saline(LISS)

- In 1964 Mollison and Polley discovered that by lowering ion strength of RBCs it would result in rapid uptake of antibodies.
- This helps greatly reduce the incubation time.
- Glycine included in LISS

LISS

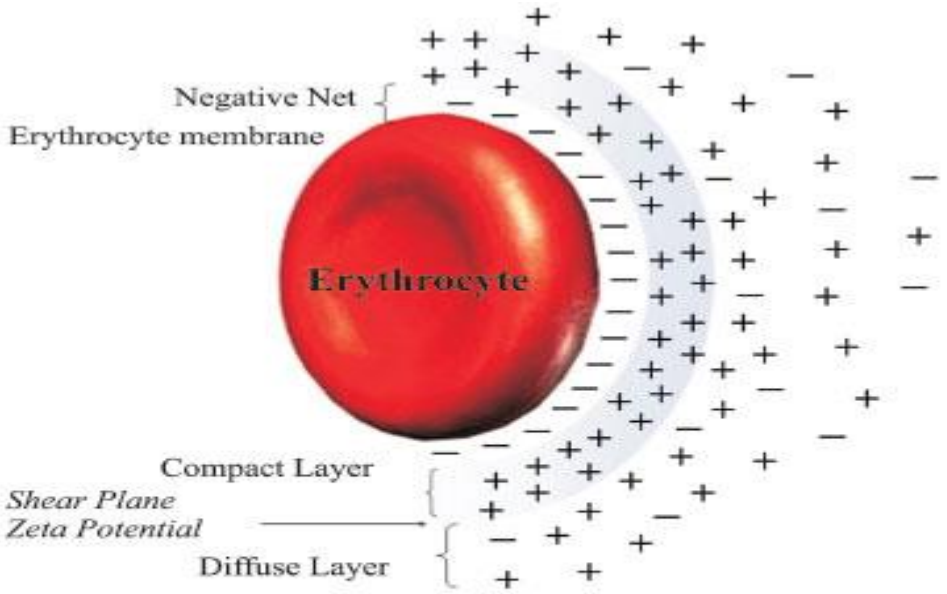


Figure 5 – Schematic representation of zeta potential. Erythrocytes (negative charges) in suspension causing a rearrangement of charges through the formation of two ionic layers that generate a electric potential difference between them, called the Zeta potential (Modified from Pollack & Reckel, 1977 and Rouger & Salmon, 1981).[1,13]

Polyethylene Glycol (PeG)

- Water soluble polymer
- Steric exclusion of water
- Increases antibody uptake
- Helps provide shorter incubation time(15 min)

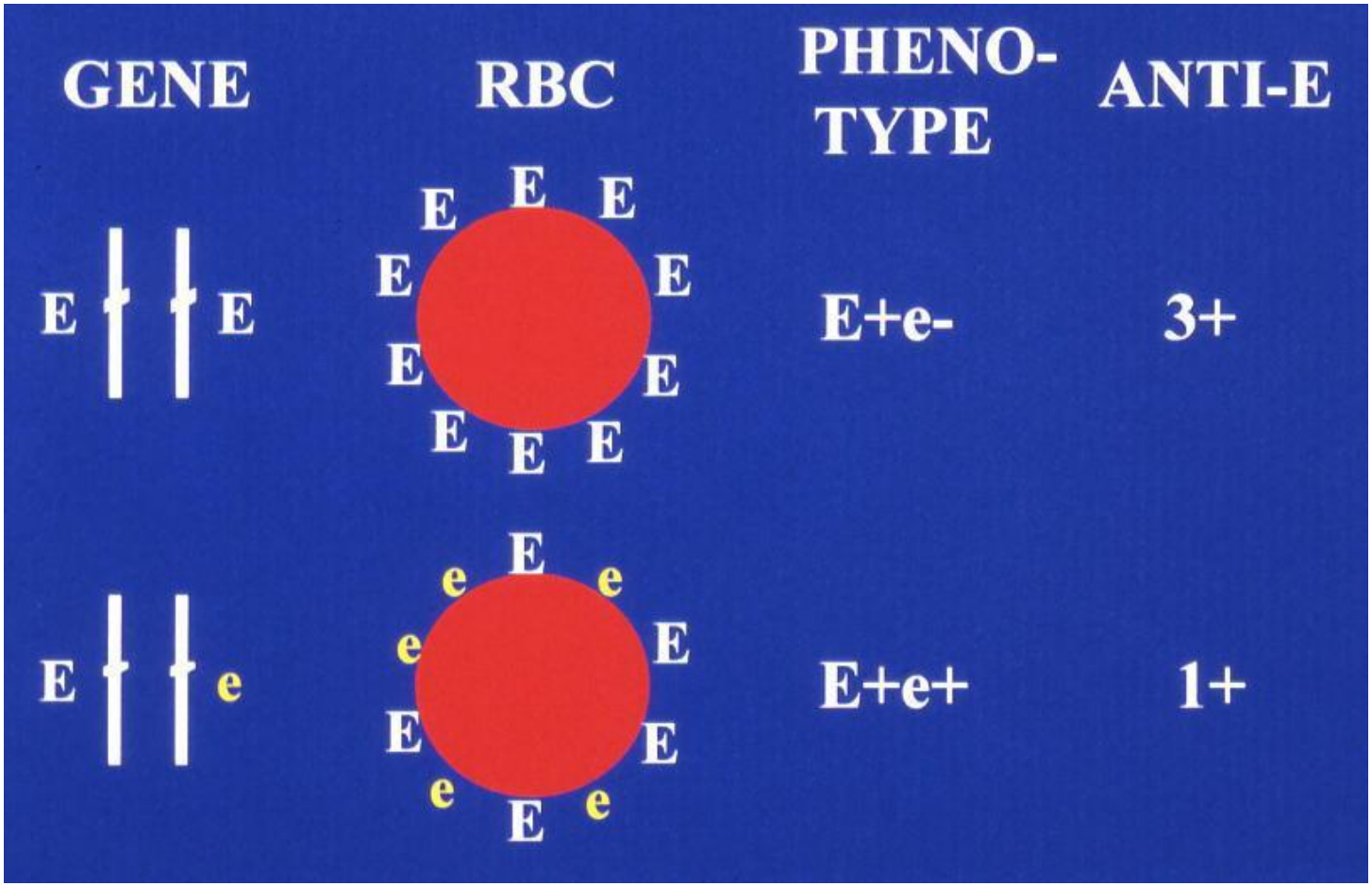
Polyethylene Glycol (PeG)

- Enhances detections of potential clinically significant antibodies
- Enhances detection of Warm-reactive autoantibodies
- Used to enhance reactivity of acid eluates
- Do not read at 37C.
- Use anti-IgG

Polyethylene Glycol (PeG)

- Elevated gamma globulins can produce nonreactive IgG-coated RBCs.
- Proteins may precipitate in samples from patients with exceptionally high globulins

Homozygous VS Heterozygous



Case #1

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	AHG
1	0	0	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	+	4+	4+
2	0	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	0	+	4+	4+
3	0	+	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	4+	4+
4	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	4+	4+
5	+	0	+	+	0	0	+	+	+	+	0	0	0	0	+	0	0	+	4+	4+
6	0	0	+	0	+	0	+	+	w	0	+	0	+	+	+	0	+	0	4+	4+
7	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	4+	4+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	4+	4+
9	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	4+	4+
Auto																			0	0✓

Case #1

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				PeG
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	IgG
1	0	0	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	+	4+
2	0	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	0	+	4+
3	0	+	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	4+
4	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	4+
5	+	0	+	+	0	0	+	+	+	+	0	0	0	0	+	0	0	+	4+
6	0	0	+	0	+	0	+	+	w	0	+	0	+	+	+	0	+	0	4+
7	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	4+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	4+
9	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	4+
Auto																			o✓

Saline Screen

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				
Cell	**D**	**C**	**c**	**E**	**e**	**K**	**k**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**Le^a**	**Le^b**	**P_1**	**M**	**N**	**S**	**s**	**IS**	**RT**
1	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	4+	4+
2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	+	+	0	4+	4+
3	+	0	+	0	+	+	+	0	0	0	+	0	0	+	+	0	0	+	4+	4+
AC																			0	0

Phenotype

K	k	M	N	S	s	P_1	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b
3+	2+	3+	3+	0	2+	3+	4+	0	3+	0	0	3+

Case #1

	Common Blood Groups																	Patient Test results		
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS	PeG
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	AHG	IgG
1	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	0	+	4+	4+	4+

Lectins

- Sugar-binding proteins of non-immune origin that may combine with cell surface antigens, causing agglutination.
- •Examples: *Dolichos biflorus* = anti-A1, *Ulex europaeus* = anti-H
- •Usually seeds from plants

Lectin Preparation

- Obtain raw seeds
- •Grind seeds in blender until they look like coarse sand
- •Place ground seeds in beaker of saline for 4-12 hours; mix occasionally
- •Collect and filter supernatant
- •Standardize to determine appropriate dilution

H Antigen

- H antigen expressed on group O RBCs
- H antigen is precursor to A and B antigens, so expression of H decreases by action of A and B transferases as H is converted to A or B antigen
- Order of reactivity of RBCs with anti-H(Ulex Europaeus lectin):

  group O > A_2 > B > A_2B > A_1 > A_1B
- H found on all RBCs except the rare null O_h (Bombay) phenotype

Case #1

- Typing with the H lectin was negative

H- Cells

	Common Blood Groups																	Patient Test results			
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs						
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	IS	LISS 37	LISS AHG	PeG IgG
1	+	+	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	0	0✓	0✓
2	+	0	+	+	0	0	+	+	0	0	+	0	0	+	+	0	0	+	0	0	0✓	0✓
3	0	0	+	0	+	+	+	0	0	+	0	0	+	+	+	0	+	0	0	0	0✓	0✓

Bombay (O_h) Phenotype

- RBCs not agglutinated by anti-H, -A, -B, or –A,B
- Genotype is hh, sese
- Anti-H has a wide thermal range (4-37C) and is clinically significant

Conclusion

- There were Oh cells present in the Dedham Frozen inventory.
- The surgery went well without any need for blood.

Case #2

- 62 year old Female
- Sample dated 3-17-23
- Trx with 4 units of red cells since 12-09-2022
- H/H is 10 & 30
- The hospital is requesting an antibody identification
- A pos
- Diagnosis: Wound infection / Hip

Case #2

- ABO:

	A	B	A,B	D	Cont	A1	A2	B
IS	4+	0	4+	3+	0	0	0	4+

- DAT:

Poly	IgG	C3	Control
0/0	NT	NT	0/0
0/0✓	NT	NT	0/0

Rh Phenotype

C	E	c	e
3+	4+	4+	4+

Case #2

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	IgG
1	+	+	0	0	+	0	+	0	0	0	+	+	0	+	+	0	0	+	0	o✓
2	+	0	+	+	0	0	+	+	w	0	0	0	+	+	+	+	0	+	0	o✓
3	+	0	+	0	+	0	+	0	0	0	+	0	0	+	+	0	0	+	0	1+
4	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	1+
5	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	0	1+
6	+	+	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	o✓
7	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	1+
8	0	0	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+	0	1+
9	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	0	+	0	1+
AC																			0	o✓

Case #2

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	IgG
1	+	+	0	0	+	0	+	0	0	0	+	+	0	+	+	0	0	+	0	o✓
2	+	0	+	+	0	0	+	+	w	0	+	0	+	+	+	+	0	+	0	o✓
3	+	0	+	0	+	0	+	0	0	0	+	0	0	+	+	0	0	+	0	1+
4	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	1+
5	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	0	1+
6	+	+	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	o✓
7	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	1+
8	0	0	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+	0	1+
9	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	0	+	0	1+
AC																			0	o✓

Initial Evaluation of Case #2

1. Is there a pattern?
2. What do we know?
3. Do we suspect alloantibodies?

Case #2

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				
																		LISS	
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	IgG
1	+	+	0	0	+	0	+	0	0	0	+	+	0	+	+	0	0	+	0	o✓
2	+	0	+	+	0	0	+	+	w	0	+	0	+	+	+	+	0	+	0	o✓
3	+	0	+	0	+	0	+	0	0	0	+	0	0	+	+	0	0	+	0	1+
4	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	1+
5	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	0	1+
6	+	+	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	o✓
7	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	1+
8	0	0	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+	0	1+
9	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	0	+	0	1+

Case #2

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs			LISS	
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	IgG
1	+	+	0	0	+	0	+	+	0	0	+	+	0	+	+	0	0	+	0	o✓
2	+	0	+	+	0	0	+	0	+	+	0	0	+	+	+	0	+	0	0	o✓
3	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	o✓

Case #2

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				Gel
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	IgG
1	+	+	0	0	+	0	+	0	0	0	+	+	0	+	+	0	0	+	0	o✓
2	+	0	+	+	0	0	+	+	w	0	0	0	+	+	+	+	0	+	0	o✓
3	+	0	+	0	+	0	+	0	0	0	+	0	0	+	+	0	0	+	0	1+
4	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	1+
5	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	0	1+
6	+	+	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	o✓
7	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	1+
8	0	0	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+	0	1+
9	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	0	+	0	1+

Case #2

- Transfusion recommendations
  - Blood negative for c or e
  - Frequency of c is 23%
  - Frequency of e is 3%
  - Since R1R2 there is no risk of sensitization to C or E

Case #3

- 78 year old Female
- Sample dated 06-23-2022
- Trx with 1 units of red cells since 06-17-2022
- H/H is 7.3 & 21.9
- The hospital is requesting an antibody identification
- O pos
- Antibody history of anti-Vel
- Diagnosis: Anemia

Case #3

- ABO:

	A	B	A,B	D	Cont	A1	A2	B
IS	0	0	0	3+	0	4+	4+	4+

- DAT:

Poly	IgG	C3	Control
0/0	NT	NT	0/0
0/0✓	NT	NT	0/0

Microcentrifugation

- Reticulocytes(young red cells) are harvested by microcentrifugation.
- Lower specific gravity
- Blood sample >72 hrs from last transfusion

Hypotonic wash

- 0.3% Sodium Chloride(NaCl)
- Red cells with HgbSS and HgbSC are resistant to lysis.
- Red cells with HgbAA or HgbAS are easily lysed.

Case #3

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	IgG
1	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	+	0	0	+	+	0	+	0	+	0	2+
4	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	2+
5	+	0	+	+	0	0	+	+	+	+	0	0	0	0	+	0	0	+	0	2+
6	0	0	+	0	+	0	+	+	w	0	+	0	+	+	+	0	+	0	0	2+
7	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	2+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	2+
9	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	2+
Auto																			0	o✓

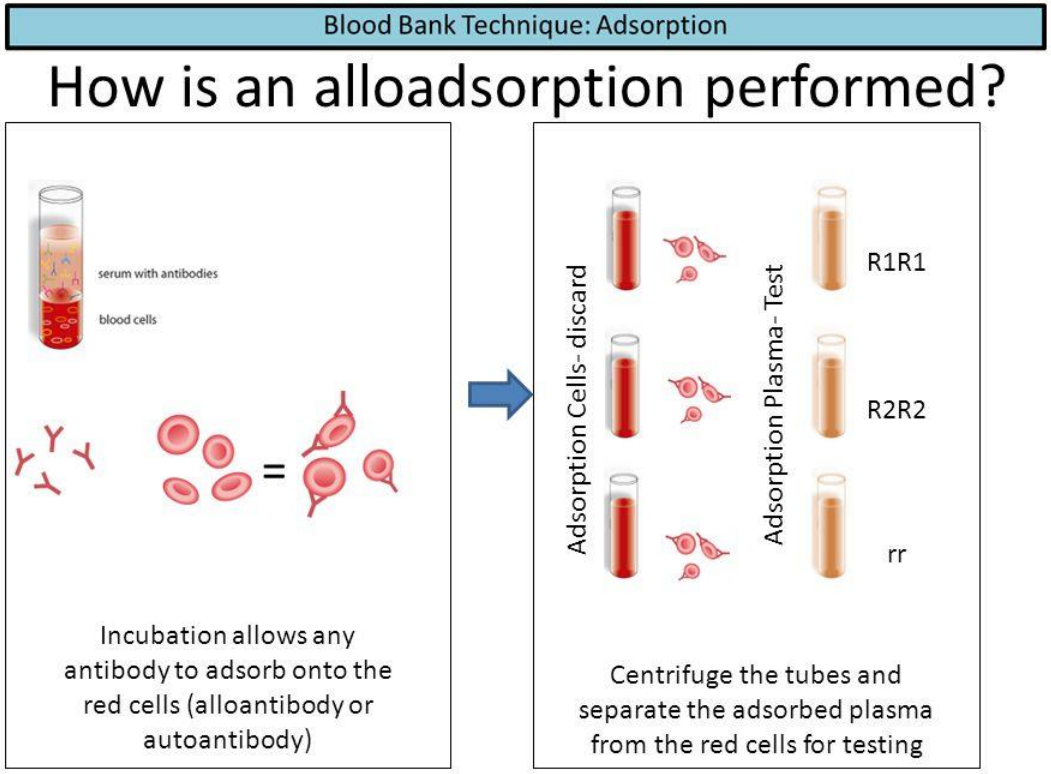
Case #3

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				PeG
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	IS	IgG
1	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	+	0	0	+	+	0	+	0	+	0	2+
4	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	2+
5	+	0	+	+	0	0	+	+	+	+	0	0	0	0	+	0	0	+	0	2+
6	0	0	+	0	+	0	+	+	w	0	+	0	+	+	+	0	+	0	0	2+
7	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	2+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	2+
9	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	2+
Auto																			0	o✓

Case #3

	Common Blood Groups																		Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs					
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s		IS	PeG IgG
1	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	0	VEL-	0	o✓
2	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	VEL-	0	o✓

AlloAdsorption



Adsorbing cells

	D	C	E	c	e	K	Fya	Fyb	Jka	Jkb	M	N	S	s
R1R1	+	+	O	O	+	O	+	O	+	O	O	+	O	+
R2R2	+	O	+	+	O	O	O	+	O	+	+	+	+	+
rr	O	O	O	+	+	O	+	+	+	+	+	O	+	O

Enzyme treat to increase adsorption efficiency and prevent adsorption of allo antibodies.

Adsorbing cells QC

	D	C	E	c	e	K	Fy a	Fy b	Jk a	Jk b	M	N	S	s	R1	R2	r
R1R1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0 ✓		
R2R2	+	0	+	+	0	0	0	+	0	+	+	+	+	+		0 ✓	
rr	0	0	0	+	+	0	+	+	+	+	+	0	+	0			0 ✓

Case #3

	Common Blood Groups																	Patient Test results		
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				Ficin Treated Ads at 37 X3	
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	LISS IgG R1R1	LISS IgG R2R2	LISS IgG rr
1	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	o✓	o✓	o✓
2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	+	0	+	o✓	o✓	o✓
3	0	0	+	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+	o✓	o✓	o✓
4	0	0	+	+	+	+	0	0	+	0	+	0	+	+	0	+	0	+	o✓	o✓	o✓

Vel Blood Group System

- Vel- phenotype occurs 1 in 4,000 people
- Locus on short arm of chromosome 1
- There is variable expression of the antigen on RBCs

Anti-Vel

- Anti-Vel is usually IgM + IgG
- It can bind complement
- Reactions at IAT and enzyme IAT
- Causes severe HTR
- Can cause a positive DAT but no clinical HDFN

Report Language

- Since the Vel antigen is found in over 99 % of the general population, there may be a delay in providing antigen negative units. If units are not available in our center's frozen inventory, then the American Rare Donor Program can be contacted, and additional units can be obtained from other ARC centers. If it becomes necessary to procure Vel negative units from outside our region, then a 24-48-hour delay may occur in order to obtain the products. Family members should be contacted and tested for the Vel antigen to determine whether they would be suitable donors. This laboratory would be interested in performing a family study to find antigen negative donors.

Case #4

- 65 year old male
- Sample dated 07-01-2022
- No Transfusion History
- H/H is 9.0 & 26.0
- The hospital is requesting an antibody identification
- O Neg
- Diagnosis: Thrombocytopenia

Case #4

- ABO:

	A	B	A,B	D	Cont	A1	A2	B
IS	0	0	0	0	0	4+	4+	4+
AHG				0✓	0✓			

- DAT:

Poly	IgG	C3	Control
0/0	NT	NT	0/0
0/0✓	NT	NT	0/0

Rh Phenotype

C	E	c	e
0	0	4+	4+

Case #4

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37	IgG
1	0	0	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	+	0	W+
2	0	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	0	+	0	W+
3	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	W+
4	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	W+
5	+	0	+	+	0	0	+	+	+	+	0	0	0	0	+	0	0	+	0	W+
6	0	0	+	0	+	0	+	+	w	0	+	0	+	+	+	0	+	0	0	W+
7	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	W+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	W+
9	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	W+
Auto																			0	o✓

Case #4

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				PeG
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	IS	IgG
1	0	0	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	+	0	W+
2	0	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	0	+	0	W+
3	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	W+
4	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	W+
5	+	0	+	+	0	0	+	+	+	+	0	0	0	0	+	0	0	+	0	W+
6	0	0	+	0	+	0	+	+	w	0	+	0	+	+	+	0	+	0	0	W+
7	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	W+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	W+
9	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	W+
Auto																			0	o✓

Titer

1	2	4	8	16	32	64	128	256	512
W+	M+	M+	M+	M+	M+	M+	M+	o✓	o✓

HTLA Characteristics

- Weak reactions with IAT
- Reacts with all cells
- Strength can vary from microscopic to 2+
- HTLA stands for High Titer Low Avidity
- HTLA is not a blood group system
- Usually not clinically significant

HTLA Characteristics

- High titer is classified as greater than 64
- Low Avidity means
  - Antibody reacts weakly
  - Reacts the same strength for multiple consecutive dilutions

HTLA Characteristics

- HTLA antibodies can cause issues excluding alloantibodies
- Crossmatches are usually incompatible

Methods

- Neutralization tests
  - Pooled AB Plasma
- Enzymes
  - Ficin and papain
  - Pronase
  - Trypsin and chymotrypsin
- Chemical treatments
  - CDP (Chloroquine diphosphate)
  - DTT (Dithiothreitol)
  - AET (Aminoethylisothiouronium bromide)
- Use of Solid Phase Technology

Specificities

- Anti-Ch
- Anti-Rg
- Anti-Kn^{a}
- Anti-McC^{a}
- Anti-Sl^{a}
- Anti-Yk^{a}
- Anti-JMH
- Anti-Cs^{a}
- Anti-Yt^{a}
- Anti-Hy
- Anti-Gy^{a}
- Bg Antibodies

Pooled AB Plasma

- Pooled plasma can neutralize Ch, Rg, HLA, and Cr antibodies
- For the pool you need at least 5 individuals
  - Donors need to have negative antibody screens
- Needs two sets of tubes(Test and Control)
  - Test tube - 1 Volume patient plasma to 1 volume of pooled plasma
  - Control tube – 1 volume of patient plasma to 1 volume of 6% BSA
- Incubate both tubes at room temperature for 30 minutes
- Test a selected cell panel with test tube contents
- Test a selected cell panel with control tube contents

Selected Cells	Test	Control
#1	0✓	W+
#2	0✓	W+
#3	0✓	W+

AB Plasma Interpretation

Single Tube Inhibition		
If the test is...	**And the dilution control is...**	**Then interpret as...**
Positive	Positive	Antibody was not inhibited or underlying alloantibodies may be present.
Negative	Positive	Sample demonstrates inhibition.
Any result	Negative	Invalid testing. Antibody may have been diluted.

Enzymes

ficin/papain	trypsin	chymotrypsin
M, N, S, s* Fy^a, Fy^b, Fy6 Ch, Rg, JMH Yt^{a*}	M, N Ch, Rg, JMH Lu, Yk^a, Knops	S, s Fy^a, Fy^b, Fy6 Ch, Rg, JMH Yt^a, Lu, Knops Cr
Xg^a, In^b, Pr Ge2, Ge4	Xg^a, In^b , Pr Ge2, Ge3*, Ge4	Xg^a, In^b, Pr
En^aTS, En^aFS	En^aTS	

DTT

Ficin/ Papain	DTT	Possible Specificity
neg	pos	M, N, S, s*; Ge2, Ge4; Xg^a; Fy^a, Fy^b; Ch/Rg
neg	neg	Indian; JMH
pos	weak	Cromer; Knops; Lutheran; AnWj; MER2
variable	neg	Yt^a
pos	neg	Kell; LW; Dombrock
pos	pos	A, B; H; P_1; Rh; Lewis; Kidd; Fy3; Diego; Scianna; Co; Ge3; Ok^a; I, i; P, LKE; At^a; Cs^a; Er^a; Jr^a; Lan; Vel*; Sd^a

*variable

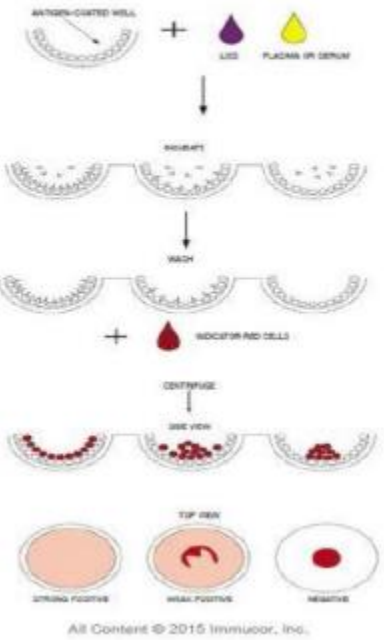
CDP

- Chloroquine Diphosphate
- Removes IgG
- Destroys Bg antigens

Solid Phase

Capture-R® Ready Technology
Basic Test Principle

- Red cells are bound to the microwell surface in the manufacturing process
- Plasma and potentiator (LISS) are added
- Antibodies to red cell antigens are "*captured*" on the microwell during incubation
- Unbound immunoglobulins are rinsed from the wells
- Indicator red cells are added, and "sandwich" the "captured" antibodies, making them visible
- *Centrifugation* brings the indicator red cells in contact with antibodies bound to the reagent red cell membranes.



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Solid Phase

Some IgG antibodies have been shown to react poorly in solid phase red blood cell adherence assays. These include examples of antibodies to Bg^a, Bg^b, Kn^a, Cs^a, Yk^a, JMH, McC^a, Ch and Rg. [7,8] Weak examples of clinically relevant antibodies may fail to react by Capture-R Ready Screen, even though the antibodies are detected by an alternative technique. Passively administered anti-D may fail to react by Capture-R Ready-Screen, even though the antibodies are detected by an alternative technique. NO ONE TEST METHOD IS CAPABLE OF DETECTING ALL ANTIBODIES.

Case #4

	Common Blood Groups																	Patient Test results	
	Rh					Kell		Duffy		Kidd		Lewis		P1	MNSs				
Cell	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	LISS IgG	Solid Phase
1	0	0	+	0	+	+	0	0	+	+	+	0	+	+	+	+	0	+	W+	0
2	0	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	0	+	W+	0
3	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	W+	0
4	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	W+	0
5	+	0	+	+	0	0	+	+	+	+	0	0	0	0	+	0	0	+	W+	0
6	0	0	+	0	+	0	+	+	w	0	+	0	+	+	+	0	+	0	W+	0
7	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	W+	0
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	W+	0
9	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	W+	0
Auto																			o✓	o

Conclusion

- Since Solid Phase was able to provide a rule out, it was not attempted to identify the HTLA specificity
- The antibody was reported out as HLTA-like

Report Language

- An antibody of the HTLA group was detected (titre 128). This antibody did not react in Solid Phase. The in vitro test for compatibility, unless performed in Solid Phase, cannot be used to predict the survival of transfused red cells since all units will appear incompatible.
- Anti-( ) is one of the HTLA group of antibodies and has not been known to cause decreased red cell survival. However, care must be taken to ensure that this antibody is not masking the reactivity of an alloantibody which can cause decreased red cell survival.

Case #5

- 60 year old Male
- Sample date 6-16-22
- Trx with 1 unit of red cells 5-13-22
- H/H is 10 & 30
- History of HTLA, Cold Auto, anti-E,K,C^W, Le^a
- A pos
- Diagnosis: Stomach Cancer

Case #5

- Full phenotype with Reticulocytes
  - C+E-c+ C^w- K- Fy(a+b-) Jk(a+b-) M-N+S+s+ Le(a-b-)

Case #5

	Common Blood Groups																			Patient Test results	
	Rh						Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS	
Cell	D	C	c	E	e	CW	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37°	AHG
1	+	0	+	0	+	0	0	+	0	0	+	0	0	0	+	+	0	0	+	0	1+
2	0	0	+	0	+	0	0	+	0	+	+	0	0	+	+	0	+	0	+	0	0✓
3	0	0	+	0	+	0	0	+	+	0	0	+	0	+	+	+	+	0	+	0	0✓
4	0	0	+	0	+	0	0	+	+	0	+	0	+	0	0	0	+	0	+	0	0✓

Case #5

	Common Blood Groups																			Patient Test results
	Rh						Kell		Duffy		Kidd		Lewis		P1	MNSs				PeG
Cell	D	C	c	E	e	CW	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	IgG
1	+	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	0	+	0	+	+	+	+	0	+	2+
4	0	0	+	0	+	0	0	+	+	0	+	0	+	0	0	0	+	0	+	2+
Auto																				2+

Warm Autoantibody

- Warm autoantibodies are normally reactive with reds cells at 37C.
- The autoantibody is usually IgG
- Typically reactive with all cells by IAT.
- DAT negative autocontrol is reactive

Case #5

	Common Blood Groups																			Patient Test results	
	Rh						Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS	
Cell	D	C	c	E	e	CW	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37°	AHG
1	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	+	+	0	+	0	0✓

Case #5

	Common Blood Groups																			Patient Test results	
	Rh						Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS	
Cell	D	C	c	E	e	CW	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37°	AHG
1	+	0	+	0	+	0	0	+	0	0	+	0	0	0	+	+	+	0	+	0	1+
2	0	0	+	0	+	0	0	+	0	+	+	0	0	+	+	+	0	0	+	0	0✓
3	0	0	+	0	+	0	0	+	+	0	0	+	0	+	+	+	0	0	+	0	0✓

Case #5

	Common Blood Groups																			Patient Test results	
	Rh						Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS	
Cell	D	C	c	E	e	CW	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37°	AHG
1	+	0	+	0	+	0	0	+	0	0	+	0	0	0	+	+	+	0	+	0	1+
2	+	0	+	0	+	0	0	+	0	0	+	0	0	0	+	+	0	0	+	0	1+
Auto	+	+	+	0	+	0	0	+	+	0	+	0	0	0	+	0	+	+	+	0	0✓

Case #5

- 1940 Landsteiner & Weiner had what they called anti-Rh acquired from rabbits and guinea pigs. This reacted with 85% of adult human bloods.

Anti-LW

- Anti-LW is an anti-D like antibody
- There is more LW antigen sites on adult D+ than D- RBCs
- DTT and AET are used to help differentiate between anti-D and anti-LW

Case #5

	Common Blood Groups																			Patient Test results	
DTT Treated	Rh						Kell		Duffy		Kidd		Lewis		P1	MNSs				LISS	
Cell	D	C	c	E	e	CW	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	37°	AHG
1	+	0	+	0	+	0	0	+	0	0	+	0	0	0	+	+	+	0	+	0	0✓
2	+	0	+	0	+	0	0	+	0	+	+	0	0	+	+	+	0	0	+	0	0✓

DTT

- DTT(Dithiothreitol) is used to modify red cells by disrupting the disulfide bonds
- This is effective for Kell, LW, Lutheran, Dombrock, and Cromer blood group antigens

LW Phenotypes

Phenotype	Frequency
LW (a+b-)	97%
LW (a+b+)	3%
LW (a-b+)	Rare

anti-LW^a is actually what is called anti-LW

Anti-LW

- Reactive by IAT, mostly IgG
- No anti-LW has been shown to cause HDFN or HTR
- There have been successful transfusions with crossmatch-incompatible D- RBCs
- Auto-anti-LW is common with WAIHA

References

- AABB Technical Manual; 18th edition
- The Blood Group Antigen Facts Book, 3rd edition
- Blood Group Antigens & Antibodies
- AABB Association Bulletin #16-02

Special Thanks

- The MA and CT IRL Lab Staff