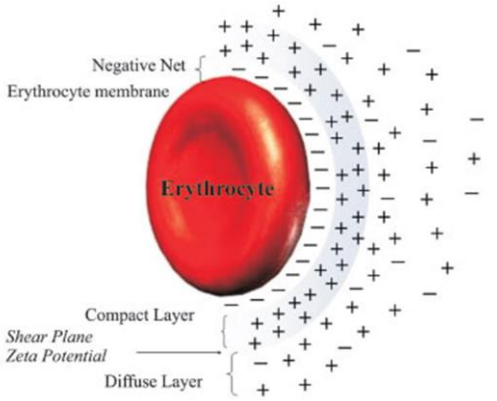
Chilean Society of Hematology
XX Congress of the Chilean Society of Hematology
X Congress of Transfusion Medicine



American Red Cross

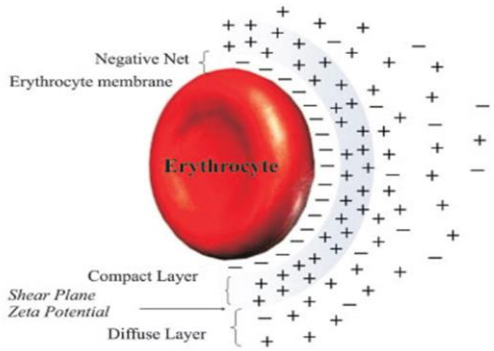
Special Techniques in the Immunohematology Reference Laboratory (IRL)

Sandra Nance, MS, MT(ASCP)SBB
Senior Director, IRL
Adjunct Assistant Professor, University of Pennsylvania
Senior Director, American Rare Donor Program

Conflict of Interest

Member of Standing Committee Grifols 2018

Application of Special Tests and Reagents



1. Enzymes
2. Enhancement Media
3. Adsorptions
4. Elutions
5. Titrations
6. Cell Separations
7. ELISA
8. Neutralization/Inhibition
9. Use of thiol reagents
10. Immunofluorescence
11. Solid phase
12. Column agglutination test
13. Monocyte Monolayer Assay
14. Flow Cytometry
15. Others

Red Cell Agglutination

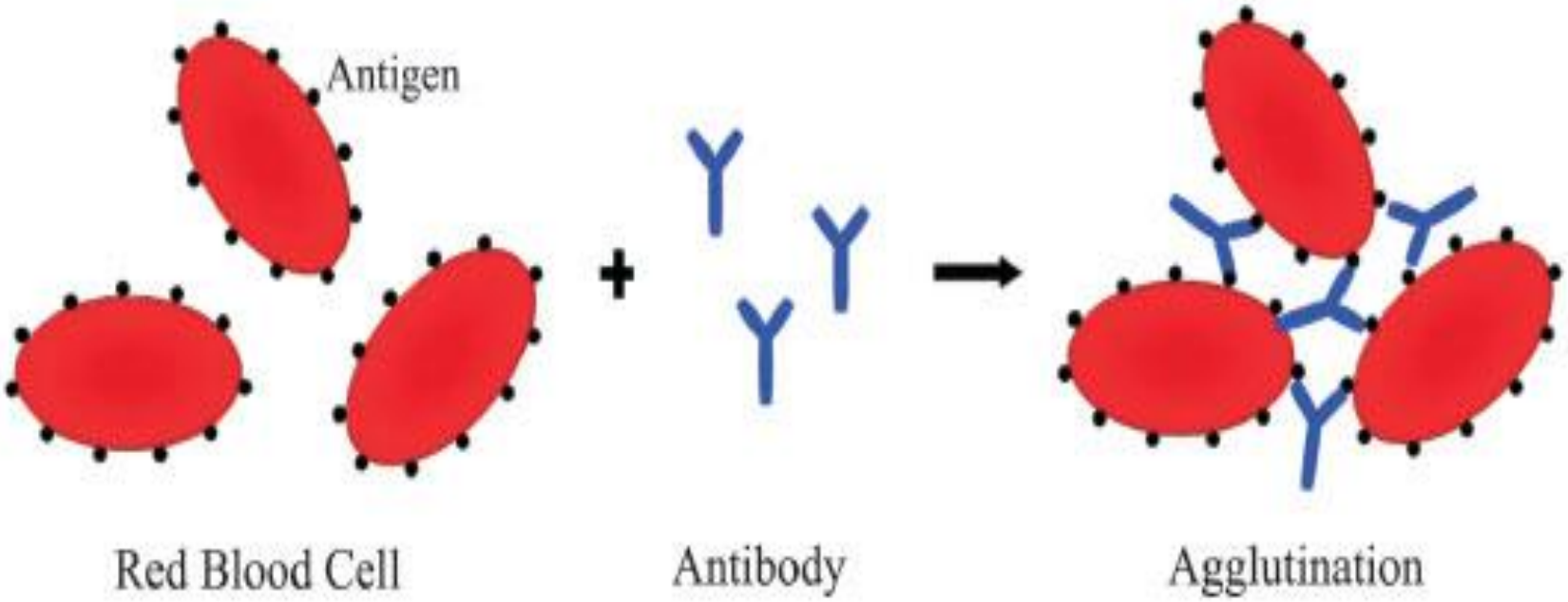


Figure 1 – Representation of the hemagglutination reaction. Blood group antigens and antibodies form a clumping of erythrocytes (modified from Parslow et al., 2004)[5]

American Red Cross

Fernandes HP; CesarI CL; Barjas-CastroII, ML
Revista Brasileira de Hematologia e Hemoterapia, Rev. Bras. Hematol. Hemoter. vol.33 no.4 São Paulo 2011
Electrical properties of the red blood cell membrane and immunohematological investigation

RBC Agglutination

The magnitude of the zeta potential depends on the net charge density of surrounding cations (ionic strength)

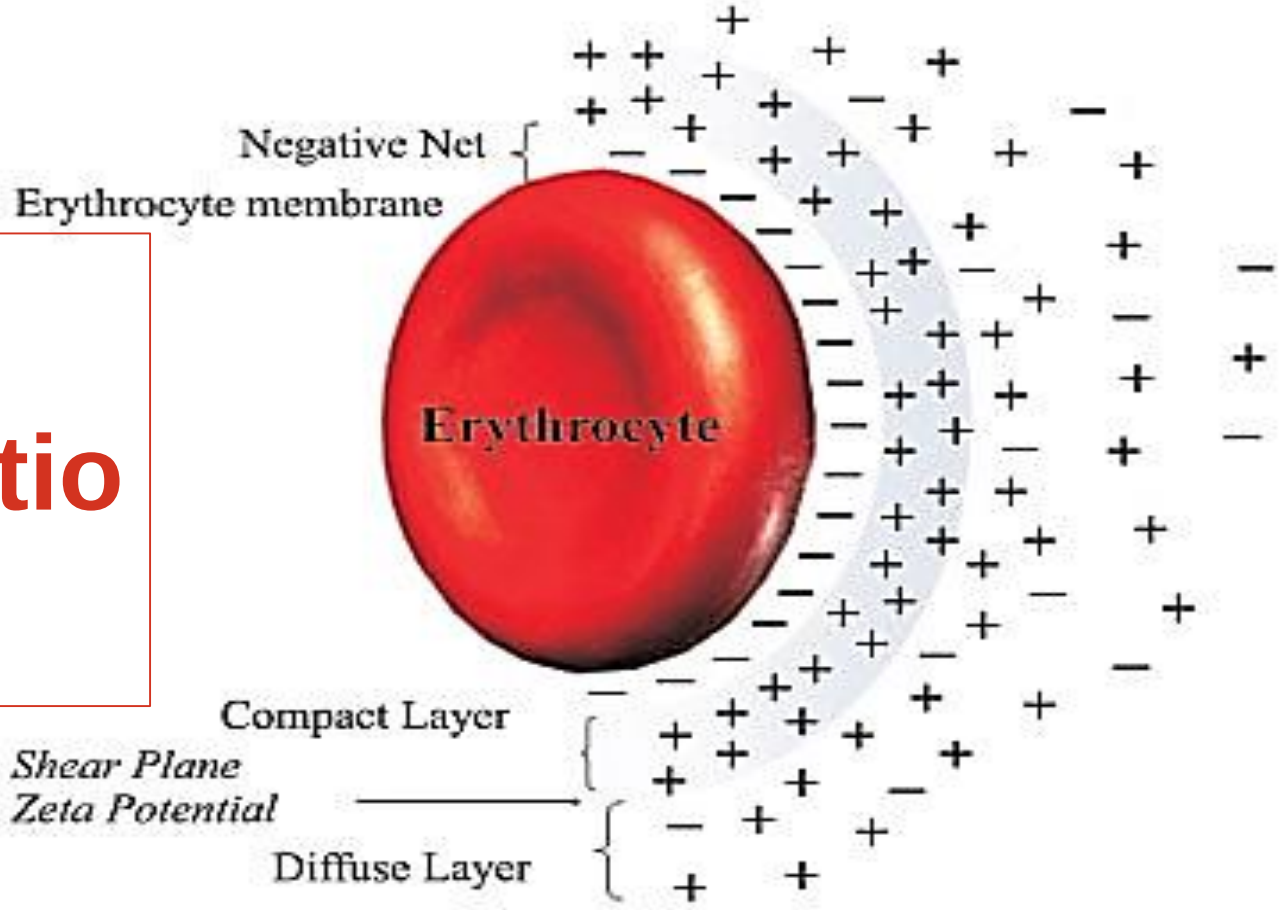


Figure 5 – Schematic representation of zeta potential. Erythrocytes (negative charges) in suspension causing a rearrangement of charges through the formation of two ionic layers that generate a electric potential difference between them, called the Zeta potential (Modified from Pollack & Reckel, 1977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

Fernandes HP; CesarI CL; Barjas-CastroII, ML

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Electrical properties of the red blood cell membrane and immunohematological investigation

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RBC Agglutination

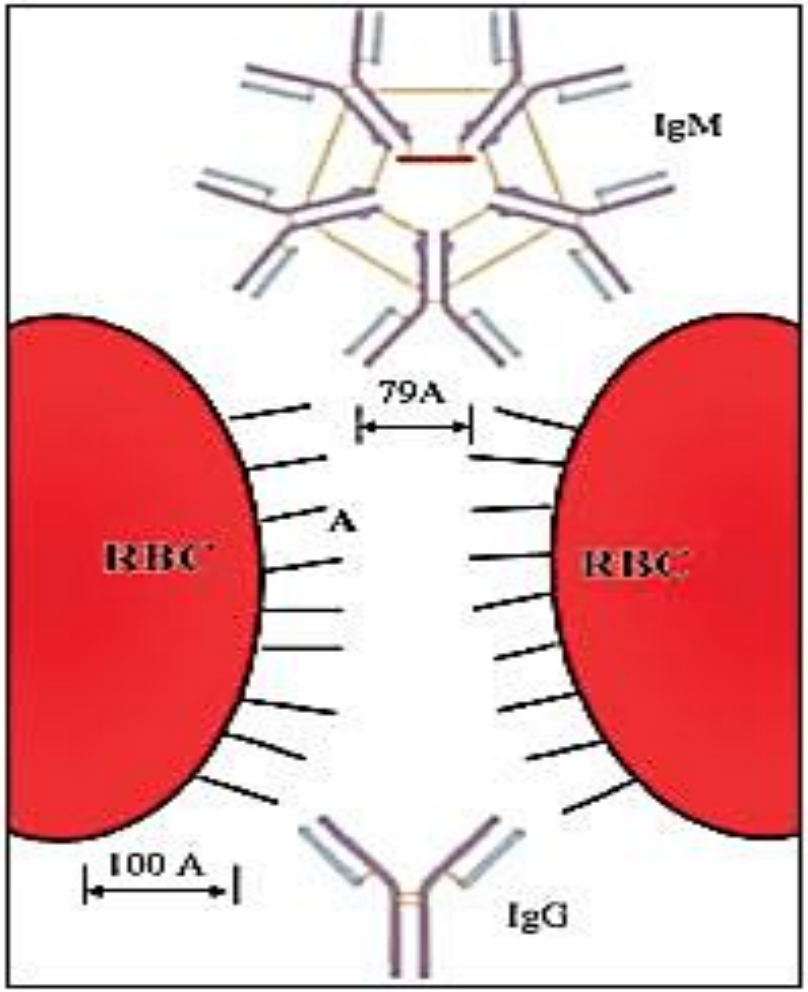

Figure 4 – The minimum distance for two red blood cells with IgG and IgM molecules to bind (scale 100Å) (Modified from Van Oss & Absolon, 1983).[4]

Fernandes HP; CesarI CL; Barjas-CastroII, ML

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Electrical properties of the red blood cell membrane and immunohematological investigation

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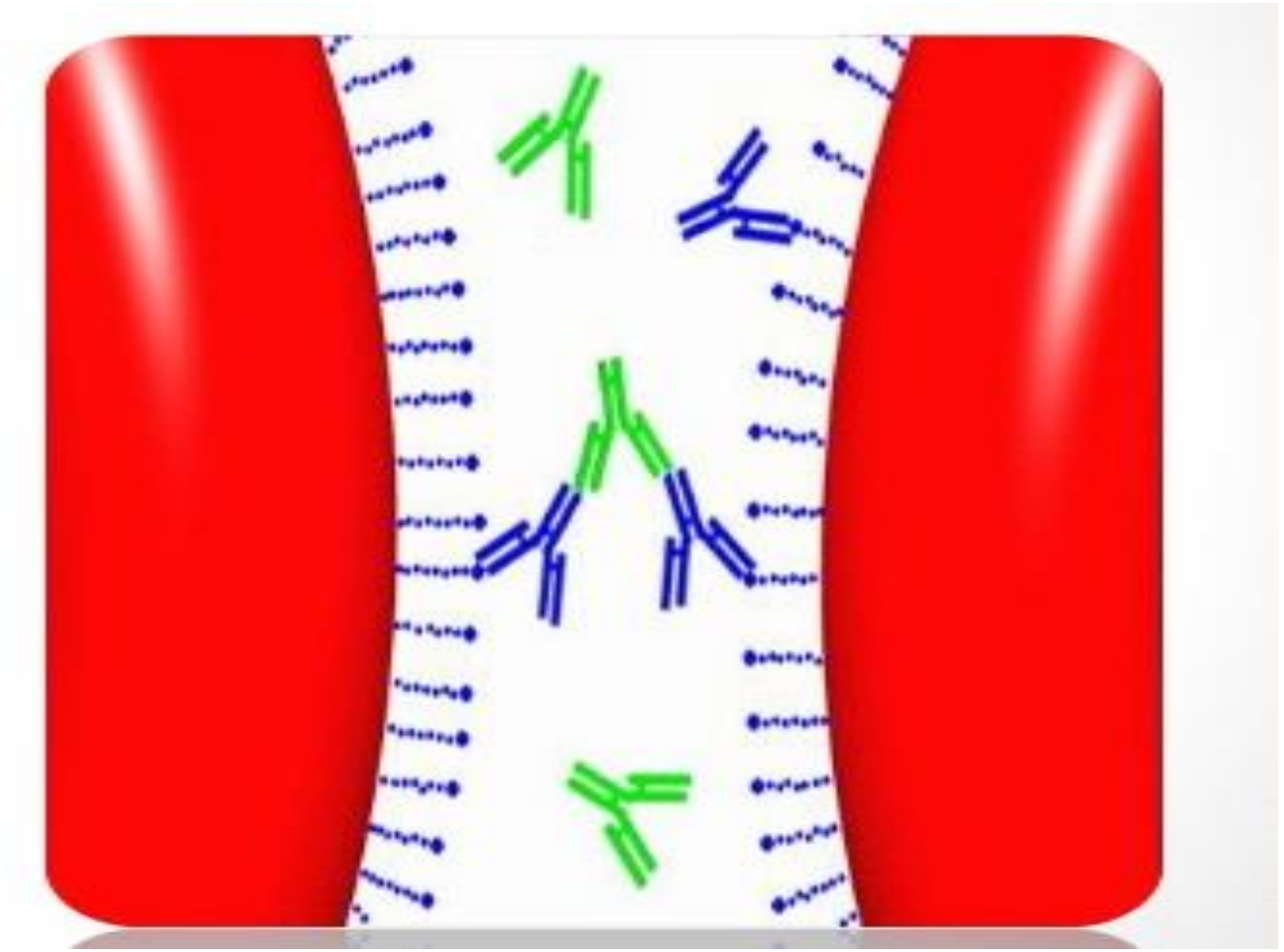
Addition of AHG to Sensitized RBCs

Blue - antibodies bound to RBCs

Green- AHG Fab portion of AHG Binds to Fc portion of IgG antibodies

Causing visible agglutination



Showing incomplete and complete Agglutination Reactions Dr.T.V.Rao MD 5; 6. Adding of Antiglobulin Serum

How Do Enzymes Work in RBC Agglutination Testing

- Remove glycoprotein fragments from the RBC membrane
- Enabling greater proximity between RBCs
- Better access of antibodies to antigens
- Sialic acid removal reduces the electrical charge of the erythrocyte surface which causes a reduction in zeta potential

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Fernandes HP; CesarI CL; Barjas-CastroII, ML

Revista Brasileira de Hematologia e Hemoterapia, Rev. Bras. Hematol. Hemoter. vol.33 no.4 São Paulo 2011

Electrical properties of the red blood cell membrane and immunohematological investigation

How Do Enzymes Work in RBC Agglutination Testing

Table 2 - Effects of enzyme treatment of red blood cells using bromelain, chymotrypsin, dispase, ficin, neuraminidase, pepsin and trypsin

Results were obtained from the zeta potential measurements (ζ) (Modified from Omi et al., 1994[21])

Enzyme	Zeta potential ζ (mV)	% Zeta potential Reduction
Bromelain	-6.05	55.8
Chymotrypsin	-7.91	42.3
Dispase	-9.04	34.0
Ficin	-4.38	68.0
Neuramidase	-1.31	90.4
Papain	-8.52	37.8
Trypsin	-7.28	46.8
Normal	-13.70	0

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Fernandes HP; Cesarl CL; Barjas-CastroII, ML
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Enzyme Treatment: Impact to RBC Antigens

- Common antigens denatured
  - Fy^a Fy^b M N S s (variable)
- Common antigens not denatured
  - D C c E e f K k Jk^a Jk^b Le^a Le^b
  - Fy3
- Antigens enhanced
  - RH System, LE System, JK System

Effect of Enzymes and DTT (Dithiothreitol) on Antigens in Antibody Identification

Possible antibody specificity is based on *general* patterns of reactions against enzyme and DTT treated (200mM) RBCs (assuming no anti-enzyme is present or an eluate is used).

Ficin/Papain	*Trypsin*	*α-chymotrypsin*	*DTT (200mM)*	*Possible specificity*
Neg	Neg	Neg	Pos	Bp^a; Ch/Rg; Xg
Neg	Neg	Neg	Neg	Indian; JMH
Neg	Neg	Pos	Pos	M, N, En^aTS; Ge2, Ge4
Neg	Pos	Neg	Pos	'N'; Fy^a, Fy^b
Variable	Pos	Neg	Pos	S, s
Variable	Pos	Neg	Weak or Neg	Yt
Neg	Pos	Pos	Pos	En^aFS
Pos	Neg	Neg	Weak or Neg	Lutheran; MER2
Pos - Papain Weak or neg - Ficin	Neg	Neg	Neg	Knops
Pos	Neg	Weak	Neg	Dombrock
Pos	Pos	Neg	Weak	Cromer
Pos	Pos	Neg	Pos	Some Diego (on 3rd loop)
Pos	Pos	Pos/Weak	Neg	LW
Pos	Pos/Weak	Pos/Weak	Pos	Scianna
Pos	Pos	Pos	Neg	Kell (but KALT & KYOR are trypsin sensitive)
Pos	Pos	Pos	Enhanced	Kx
Pos	Pos	Pos	Pos	ABO; En^aFR, U; $PP1P^k$; RH; Lewis; Fy3; Kidd; most Diego; Colton; H; Ge3; OK; I/i; P, FORS; JR; LAN; Cs^a; ER; LKE, PX2; VEL; At^a; Emm; AnWj; Sd^a; PEL; MAM; ABTI

Courtesy of Christine Lomas-Francis *Immunohematology* 2017, in press

Enhancement Media

- Albumin
- LISS
- PEG

Enhancement Media - Albumin

- 22% albumin commonly used, 30% described
- Reduces zeta potential
- Allows RBCs to come closer together
- Enhances agglutination
- Effective enhancement medium, especially with antibodies in the Rh system
- Does not destroy or inactivate antigens

Enhancement Media - LISS

- Low ionic strength saline solution (LISS) is a salt solution with 20% less sodium chloride concentration
- Formula described in literature
  - 0.17M saline (180 mL)
  - 0.15M phosphate buffer (20 mL)
  - 0.3M sodium glassine (800 mL)

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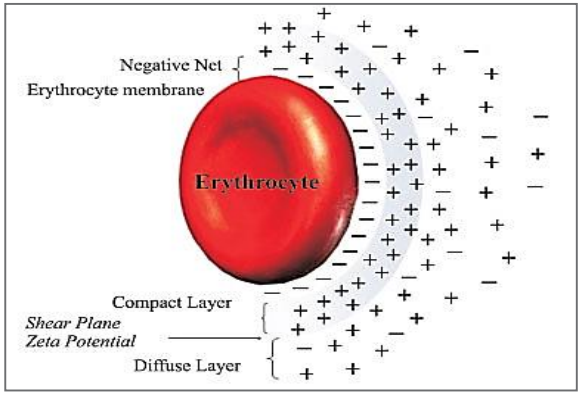
Fernandes HP; Cesarl CL; Barjas-CastroII, ML. Electrical properties of the red blood cell membrane and immunohematological investigation. Revista Brasileira de Hematologia e Hemoterapia, Rev. Bras. Hematol. Hemoter. vol.33 no.4 São Paulo 2011

Enhancement Media - LISS

- LISS decreases ionic strength reducing thickness of the double layer due to the increased cations density
- This reduces the zeta potential promotes non-covalent bonds that are dependent on the distance between antibody and antigens on the RBC membrane
- Less incubation time, but not stronger reactivity



Pollack W, Reckel RP. A reappraisal of the forces involved in Hemagglutination. Int Archs Allergy Appl Immun. 1977;54(1):29-42.

Fernandes HP; Cesarl CL; Barjas-CastroII, ML Revista Brasileira de Hematologia e Hemoterapia, Rev. Bras. Hematol. Hemoter. vol.33 no.4 São Paulo 2011 Electrical properties of the red blood cell membrane and immunohematological investigation

PEG – Polyethylene Glycol

- Water soluble linear polymer, prepared by the polymerization of ethylene oxide
- Formula: HO-(CH_2CH_2O)n- CH_2CH_2OH
- Hydroxyl group of PEG molecule provides a site for covalent bonds with other molecules
- PEG does not denature antigens

American Red Cross

Fernandes HP; CesarI CL; Barjas-CastroII, ML. Electrical properties of the red blood cell membrane and immunohematological investigation. Revista Brasileira de Hematologia e Hemoterapia, Rev. Bras. Hematol. Hemoter. vol.33 no.4 São Paulo 2011

Enhancement Media: PEG

- PEG in water acts as a very mobile molecule allowing exclusion of water and other polymers
- PEG removes water from the surface of RBCs, increasing antibody concentration and promoting the binding of antibodies with antigenic sites
- Although PEG is said to be non-antigenic and non-immunogenic, PEG-coated RBCs have been reported to stimulate antibodies when PEG treated RBCs have been transfused to patients

American Red Cross

Fernandes HP; CesarI CL; Barjas-CastroII, ML. Revista Brasileira de Hematologia e Hemoterapia, Rev. Bras. Hematol. Hemoter. vol.33 no.4 São Paulo 2011.Electrical properties of the red blood cell membrane and immunohematological investigation.

de Man AJ, Overbeeke MA. Evaluation of the polyethylene glycol antiglobulin test for detection of red blood cell antibodies. Vox Sang. 1990;58(3):207-10

Adsorptions - Autologous

- Uses:
  - Remove autoantibody to reveal alloantibodies in the serum (cold or warm incubation)
  - Best choice for untransfused patient with antibody reactive with autologous RBCs
- Autologous RBC treatment first to remove some autoantibody from RBCs to allow adsorption of more autoantibody
  - Gentle heat elution (45C)
  - ZZAP (DTT + Ficin)
  - WARM® (commercial)

Immucor Website

Adsorptions - Allogeneic

- Uses:
  - Remove serum autoantibody to reveal if there are alloantibodies
  - Remove serum allo-antibody to high prevalence antigen to reveal of there are alloantibodies
  - Isolate antibody to identify specificity – adsorption with subsequent elution – often antibody to high prevalence antigen to all use of ABO incompatible rare RBCs
- RBCs or RBC Stroma

Hypothetical Panel: To Demonstrate Allogeneic Adsorption

																							Saline		Albumin		Ficin	
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IS	RT	37	IgG	37	IgG
1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	0	0	0	0	2+	2+	3+
2	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+	0	0	0	2+	2+	3+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	0	2+	2+	3+
4	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	0	0	2+	2+	3+
5	+	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+	0	0	0	2+	2+	3+
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0	0	0	0	2+	2+	3+
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0	0	0	2+	2+	3+
AC																								0	0	0	0√	0	0√

AC=Autocontrol - Patient's RBCs and Patient's serum

American Red Cross

Hypothetical Panel

Saline		Albumin		Ficin	
IS	RT	37	IgG	37	IgG
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	0√	0	0√

What is the quickest way to the answer?

Need to rule out underlying alloantibodies to common antigens before testing rare RBCs

Depends on resources of lab

- Genotyping available?
- Does lab do adsorptions?
  - With allogeneic RBCs?
- RBC treatment options?

So that rare RBCs are not erroneously assumed to be positive due to another specificity when reactivity is really due to an antibody to a common antigen

Hypothetical Panel

Saline		Albumin		Ficin	
I S	RT	37	IgG	37	IgG
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	0√	0	0√

Allogeneic Adsorption

- Use three different donor cells
  - R1R1 D+ C+ E- c- e+
  - R2R2 D+ C- E+ c+ e-
  - rr D- C- E- c+ e+
  - And at least one is:
    - K-, Jk(a-) or Jk(b-), S- or s-
    - RBCs are <u>enzyme treated</u> for enhanced adsorption, so will be Fy(a-b-) M- N-

Hypothetical Panel

Saline		Albumin		Ficin	
IS	RT	37	IgG	37	IgG
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	0√	0	0√

Adsorbing RBC Set for this patient:

- R1R1 D+ C+ E- c- e+ K- Jk(a-) S-
- R2R2 D+ C- E+ c+ e- K- Jk(b-) s-
- rr D- C- E- c+ e+ K-

- R1 will adsorb:
  - anti- D, -C, -e, -Jk^b, -s (but possibly not)
- R1 will leave behind:
  - anti-E, -c, -K, -Jk^a, -S, -Fy^a, -Fy^b, -M, -N and possibly anti-s in adsorbed serum

Hypothetical Panel

Saline		Albumin		Ficin	
I S	RT	37	IgG	37	IgG
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	0√	0	0√

Adsorbing RBC Set for this patient:

- R1R1 D+ C+ E- c- e+ K- Jk(a-) S-
- R2R2 D+ C- E+ c+ e- K- Jk(b-) s-
- rr D- C- E- c+ e+ K-

- R2 will adsorb:
  - anti- D, -E, -c, -Jk^a, -S (but possibly not)
- R2 will leave behind:
  - Anti- C, -e, -K, -Jk^b, -s, -Fy^a, -Fy^b, -M, -N and possibly anti-S in adsorbed serum

Hypothetical Panel

Saline		Albumin		Ficin	
IS	RT	37	IgG	37	IgG
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	2+	2+	3+
0	0	0	0√	0	0√

Adsorbing RBC Set for this patient:

- R1R1 D+ C+ E- c- e+ K- Jk(a-) S-
- R2R2 D+ C- E+ c+ e- K- Jk(b-) s-
- rr D- C- E- c+ e+ K-

- rr will adsorb:
  - Anti-c, -e, -Jk^a, -Jk^b, -S, -s (possibly not anti-S or –s)
- rr will leave behind:
  - Anti- D, -C, -E, -K, -Fy^a, -Fy^b, -M, -N and possibly anti- S and –s) in adsorbed serum

R1 Adsorbed Serum

																								Albumin			
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			37	IgG		
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	1+		
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+			0	0√		
4	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+			0	1+		
5	+	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+			0	1+		
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0			0	2+		
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+			0	0√		
AC																										0	0√		

R1R1 D+ C+ E- c- e+ K- Jk(a-) S-

AC=Autocontrol - Patient's RBCs and Patient's serum

American Red Cross

R1 Adsorbed Serum

Cell #3 - Ruled out anti-E,-c, -Fy^{b}, -N
Cell #7 – Ruled out anti-M

Anti-Jka in R1 Adsorbed Serum

																								Albumin			
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			37	IgG		
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	1+		
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+			0	0√		
4	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+			0	1+		
5	+	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+			0	1+		
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0			0	2+		
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+			0	0√		
AC																										0	0√		

R1R1 D+ C+ E- c- e+ K- Jk(a-) S-

AC=Autocontrol - Patient's RBCs and Patient's serum

American Red Cross

R2 Adsorbed Serum

																								Albumin			
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			37	IgG		
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	2+		
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+			0	0√		
4	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+			0	0√		
5	+	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+			0	0√		
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0			0	0√		
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+			0	0√		
AC																										0	0√		

R2R2 D+ C- E+ c+ e- K- Jk(b-) s-

AC=Autocontrol - Patient's RBCs and Patient's serum

R2 Adsorbed Serum

Cell #3 Ruled out- anti Jk^b, -s
Cell #4 Ruled out – anti-Fy^a
Cell #5 Ruled out – anti-e, -K(het)
Anti-C in R2 Adsorbed serum

																								Albumin		
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			37	IgG		
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	2+		
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+			0	0√		
4	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+			0	0√		
5	+	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+			0	0√		
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0			0	0√		
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+			0	0√		
AC																										0	0√		

R2R2 D+ C- E+ c+ e- K- Jk(b-) s-

AC=Autocontrol - Patient's RBCs and Patient's serum

rr Adsorbed Serum

Antibodies to common antigens not ruled out: anti- D, -C, -Jk^a, -S

R1 Ads serum showed anti-Jk^a

R2 Ads serum showed anti-C

																								Albumin		
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			37	IgG		
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	2+		
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+			0	0√		
4	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+			0	0√		
5	+	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+			0	0√		
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0			0	0√		
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+			0	0√		
AC																										0	0√		

rr D- C- E- c+ e+ K-

AC=Autocontrol - Patient's RBCs and Patient's serum

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Cell #3 Ruled out anti-D

rr Adsorbed Serum

rr Adsorbed serum shows anti-C

																								Albumin			
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s			37	IgG		
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	2+		
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+			0	0√		
4	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+			0	0√		
5	+	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+			0	0√		
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0			0	0√		
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+			0	0√		
AC																										0	0√		

rr D- C- E- c+ e+ K-

AC=Autocontrol - Patient's RBCs and Patient's serum

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Hypothetical Panel – Usual Recording

																							R1 Ads		R2 Ads		rr Ads	
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		IgG		IgG		IgG
1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	0		2+		2+		2+
2	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+		2+		2+		2+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+		0√		0√		0√
4	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+		2+		0√		0√
5	+	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+		2+		0√		0√
6	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0		2+		0√		0√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+		0√		0√		0√
AC																									0√		0√		0√

R1 D+ C+ E- c- e+ K- Jk(a-) S-
R2 D+ C- E+ c+ e- K- Jk(b-) s-
rr D- C- E- c+ e+ K-

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Hypothetical Panel – Usual Recording

																									R1 Ads			rr Ads	
#	D	C	E	c	e	f	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Le	Lec	P						IgG				IgG
1	+	+	0	0	+	0	0	+	0	+															2+		2+		2+
2	+	+	+	+	0																	+	0	0	+		2+		2+		2+
3																	+	0	0	+	0	+	0	+		0√		0√		0√
											0	+	+	0	+	+	0	0	+	0	+	0	+		2+		0√		0√
5					+	+	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+		2+		0√		0√
6		0	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	0		2+		0√		0√
7	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+		0√		0√		0√
AC																										0√		0√		0√

Anti C and anti-Jk^a present in addition to antibody to High Incidence Antigen

Caution: in testing rare RBCs, must be C- Jk(a-)

R1 D+ C+ E- c- e+ K- Jk(a-) S-
R2 D+ C- E+ c+ e- K- Jk(b-) s-
rr D- C- E- c+ e+ K-

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Adsorptions with Reagent Sera

- Detect weak antigens
- Useful for investigations of serologic discrepancies
  - With past historic types
  - With different sources of reagents
  - With molecular testing

Elutions - Uses

- Detect and identify antibody on autologous RBCs
  - Transfusion Reaction
  - Hemolytic Disease of the fetus and newborn
  - Autoantibody
  - Drug induced immune hemolytic anemia
- Detect presence of weak antigen – adsorption/elution with reagent antisera
- Identify anti-G with sequential adsorption/elution
- For reagent production to eliminate ABO antibodies from valuable group A, B or O sera

Elutions - Methods

- Heat: 45C or 56C, harvest supernatant
- Lui (freeze thaw) – rapid freeze to -30C, rapid thaw to 37C – harvest supernatant
- Acid elution ELUKit II (Commercial)
- EDTA-Glycine Acid - EGA
- Others less used:
  - Ultrasonic, Organic Solvents
    - Ether, Xylene, Chloroform
  - Chloroquine diphosphate

Titrations - Uses

- Cold agglutinin disease screen and thermal amplitude studies
- Maternal fetal monitoring serologic initial testing
- Antibody identification
  - HTLA-like antibody suspected
  - Neutralization studies with strong antibodies
- Plasma/Platelet products - anti-A, anti-B titers
- Drug antibody testing in suspected non-immunologic adsorption of protein

Titrations - Methods

- Master doubling dilutions (serial titration)
  - 1:1, 1:2, 1:4, 1:8 etc to 1:2048
  - Maternal serum for possible HDFN
  - HTLA-like reactivity
  - Inhibition studies for strongly reactive antibody
  - Thermal amplitude for cold agglutinin disease
- Point dilution
  - Cold Agglutinin screen
  - Products – Type O Platelets, Type A plasma

Cell Separations - Uses

- Obtain autologous RBCs from a transfused patient's sample
  - Antigen Typing
  - Testing with autologous serum or eluate
  - Adsorptions

Cell Separations - Methods

- Reticulocyte separation
  - Microhematocrit – differential centrifugation
  - Phthalate esters, Percoll-Renografin, Silicone oil*
- Hemoglobin S positive RBCs
  - Hypotonic wash - 0.3% NaCl

ThermoFisher website

Brown D. Transfusion 1988;28:21-23

Molecular typing recommended whenever possible

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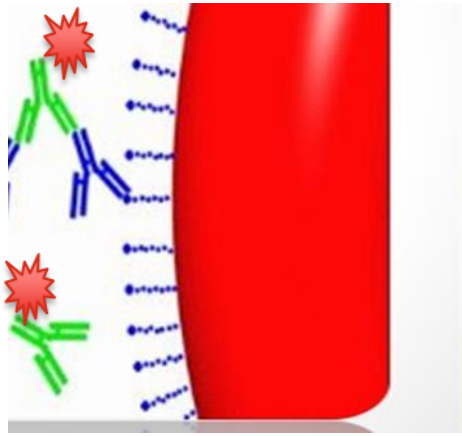
*Rushing D, Vengelen-Tyler V. Evaluation and comparison of four reticulocyte enrichment procedures. Transfusion 1987;27:86-89

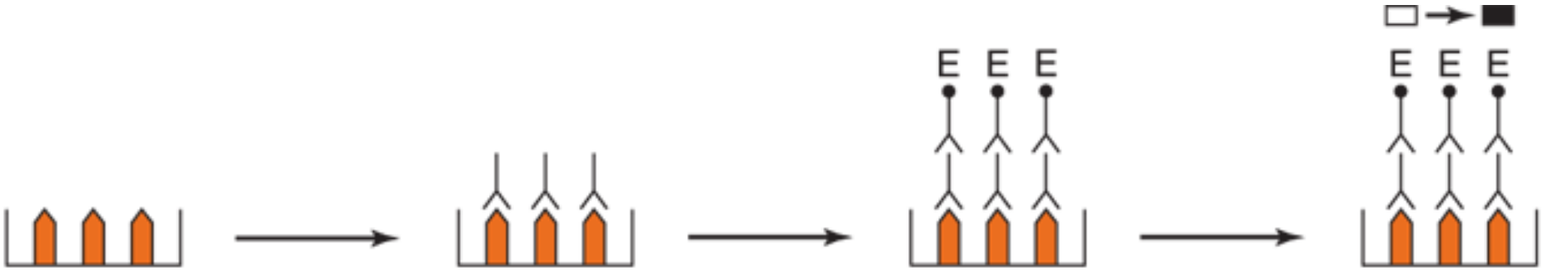
ELISA - Uses



- Detection of low level Ig in RBCs
  - IgA
  - IgM
  - IgG
- Quantitation of RBC antibody

Bodensteiner D, Brown P, Skikne B, Plapp F. The Enzyme-linked Immunosorbent Assay: Accurate Detection of Red Blood Cell Antibodies in Autoimmune Hemolytic Anemia. Am J Clin Pathol, 1983; 79: 182-18

ELISA - Method



Antigen is attached to the bottom of the well

Antibody in patient's serum attaches to antigen

Antibody to human IgG attaches to patient's IgG; the antibody to human IgG is enzyme-linked

Substrate for the enzyme is added, which changes color when acted upon by the enzyme

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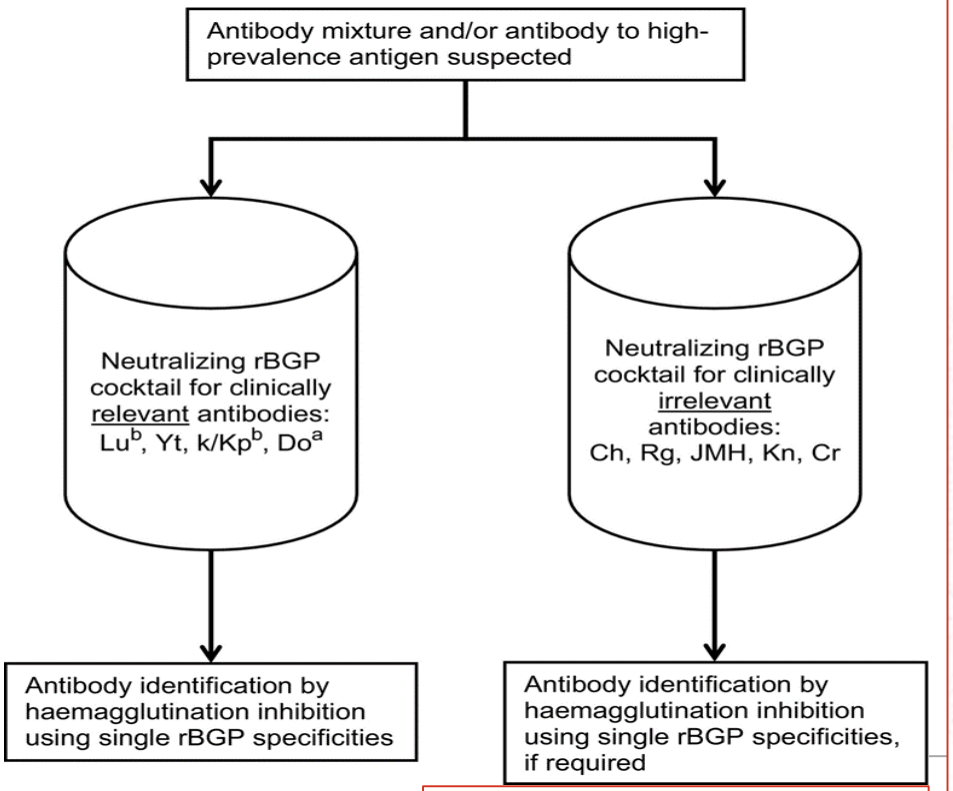
Source: Warren Levinson: Review of Medical Microbiology and Immunology, 14th Edition, www.accessmedicine.com

Neutralizations/Inhibitions – Substances and Actions

- Rabbit erythrocyte stroma – anti-I removal
- Human Platelet concentrate – HLA antibody removal
- Saliva – ABH, LE neutralization
- Breast Milk – I neutralization
- Pigeon egg white, Hydatid cyst fluid – P1 neutralization
- Urine (human and guinea pig) – Sd^a neutralization
- Pooled AB Plasma – Ch/Rg neutralization
- Blood Group substances - neutralization

  P1, LE, LU, KEL, FY, Yt^a, Xg^a, DO, LW, Rg, Ch, Cr, KN, In^b, JMH

Neutralizations - Inhibitions Blood Group Substances



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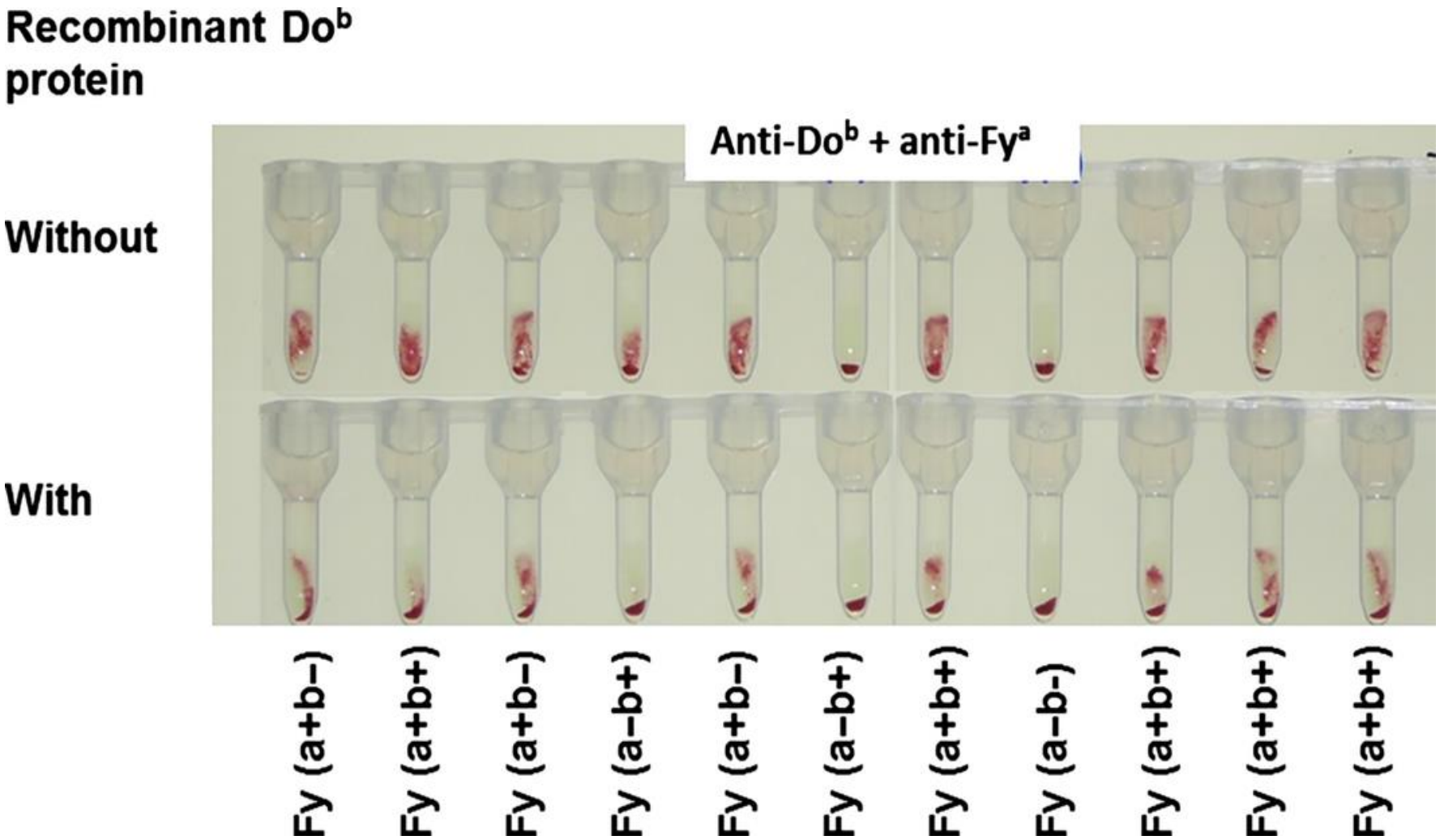
Seltsam A, Blasczyk R Recombinant blood group proteins in clinical practice – from puzzling to binary antibody testing. ISBT Science Series (2016) 11 (Suppl. 1), 243–249

Table 3 Currently available and approved recombinant blood group proteins

Name[a]	Blood group	Antigen(s) expressed
Lu^a	Lutheran	Lu^a, Lu3, Lu4, Lu5, Lu6, Lu8, Lu11, Lu12, Lu16, Lu17, Lu20, Lu21, LURC
Lu^b	Lutheran	Lu^b, Lu3, Lu4, Lu5, Lu6, Lu8, Lu11, Lu12, Lu16, Lu17, Lu20, Lu21, LURC
K/Kp^b	Kell	K, Kp^b, Ku, Js^b, K11, K12, K13, K14, K16, K18, K19, Km, K22, TOU, RAZ, KALI, KTIM, KUCI, KANT, KASH, KELP, KETI, KHUL
k/Kp^b	Kell	k, Kp^b, Ku, Js^b, K11, K12, K13, K14, K16, K18, K19, Km, K22, TOU, RAZ, KALI, KTIM, KUCI, KANT, KASH, KELP, KETI, KHUL
Fy^a	Duffy	Fy^a
Fy^b	Duffy	Fy^b
Yt	Yt	Yt^a
Xg^a	Xg	Xg^a
Sc	Scianna	Sc1, Sc3, STAR, SCER, SCAN
Do^a	Dombrock	Do^a, Gy^a, Hy, Jo^a, DOYA, DOMR, DOLG
Do^b	Dombrock	Do^b, Gy^a, Hy, Jo^a, DOYA, DOMR, DOLG
LW	Landsteiner-Wiener	LW^a, LW^ab
Rg	Chido/Rogers	Rg1, Rg2
Ch	Chido/Rogers	Ch1, Ch2, Ch3, Ch4, Ch5, Ch6
Cr	Cromer	Cr^a, Tc^a, Dr^a, Es^a, IFC, WES^b, UMC, GUTI, SERF, ZENA, CRON, CRAM, CROZ
Kn	Knops	Kn^a, McC^a, Sl^a, Yk^a, KCAM
In^b	Indian	In^b, INFI, INJA
JMH	JMH	JMH, JMHK, JMHL, JMHG, JMHM, JMHO

Recombinant Blood Group Proteins in Clinical Practice



ISBT Science Series
Volume 11, Issue S1, pages 243-249, 29 JAN 2016 DOI: 10.1111/voxs.12192
http://onlinelibrary.wiley.com/doi/10.1111/voxs.12192/full#voxs12192-fig-0003

Thiol Reagents - DTT, 2ME Use

- Antibody identification
  - Patients with multiple antibodies
  - Patients on anti-CD38 therapy
- Determination of IgG vs. IgM antibody presence
  - DTT or 2ME treated serum
  - Sephadex separation – affinity chromatography

Thiol Reagents – DTT, 2ME

Effect of Enzymes and DTT (Dithiothreitol) on Antigens in Antibody Identification

Possible antibody specificity is based on c[illegible]terns of reactions against enzyme and DTT treated (200mM) RBCs (assuming no ac[illegible]zyme[illegible]resent or an eluate is used).

Ficin/Papain	*Trypsin*	*α-chymotrypsi[illegible]*	*DTT (200mM)*	*Possible specificity*
Neg	Neg	Neg	Pos	[illegible]; Ch/Rg; Xg
Neg	Neg	Neg	Neg	In[illegible]n; JMH
Neg	Neg	Pos	Pos	M, [illegible] En^{a}TS; Ge2, Ge4
Neg	Pos	Neg	Pos	'N'; [illegible]^a, Fy^b
Variable	Pos	Neg	Pos	S, s
Variable	Pos	Neg	Weak or Neg	Yt
Neg	Pos	Pos	Pos	En^{a}FS
Pos	Neg	Neg	Weak or Neg	Luther[illegible]n; MER2
Pos - Papain Weak or neg - Ficin	Neg	Neg	Neg	Knops
Pos	Neg	Weak	Neg	Domb[illegible]ock
Pos	Pos	Neg	Weak	Crom[illegible]r
Pos	Pos	Neg	Pos	Som[illegible] Diego (on 3rd loop)
Pos	Pos	Pos/Weak	Neg	LW
Pos	Pos/Weak	Pos/Weak	Pos	S[illegible]nna
Pos	Pos	Pos	Neg	K[illegible] (but KALT & KYOR are trypsin sensitive)
Pos	Pos	Pos	Enhanced	[illegible]
Pos	Pos	Pos		ABO; En^{a}FR, U; PP1P^k; RH; Lewis; Fy3; Kidd; most Diego; Colton; H; Ge3; OK; I/i; P, FORS; JR; LAN; Cs^a; ER; LKE, PX2; VEL; At^a; Emm; AnWj; Sd^a; PEL; MAM; ABTI

DTT – Dithiolthreitol
2ME – 2 mercaptoethanol

15 Courtesy of Christine Lomas-Francis *Immunohematology* 2017, in press

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DTT Use for Testing Patients treated with Anti-CD38

- Recommendations for these patients
  - Pre-treatment antigen and antibody status
    - Extended phenotype determination by serologic or molecular methods
    - Antibody screen and identification (as required)
  - During treatment – test serum with:
    - DTT Treated RBCS
    - Trypsin treated RBCs
    - Phenotyped cord RBCs
    - Typed RBCs from other patients on anti-CD38

Solid Phase

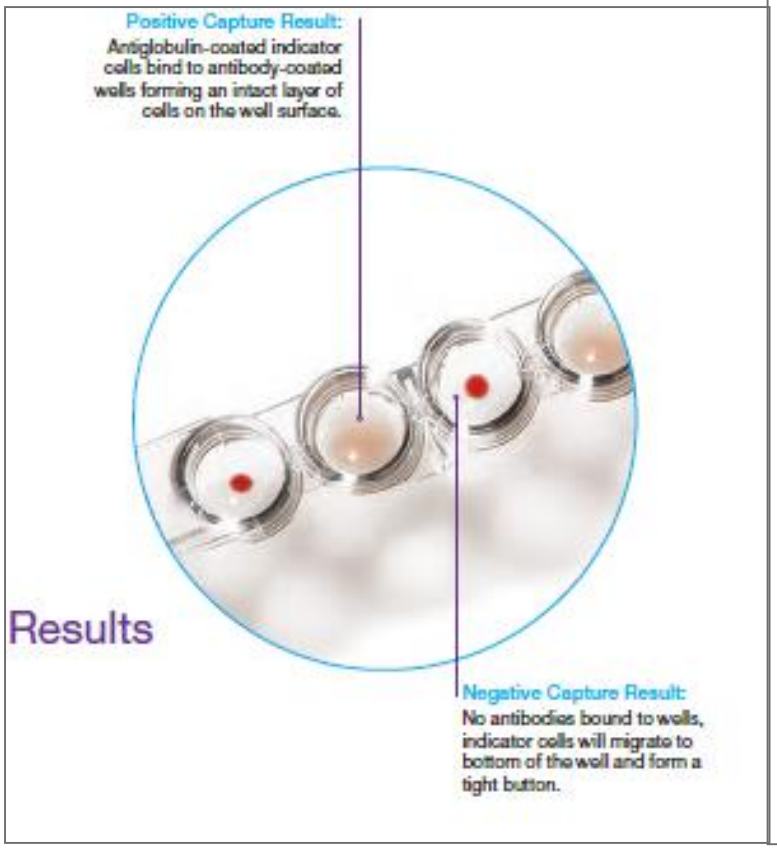
- Red cell antibody detection and identification
- Platelet/HLA antibody detection

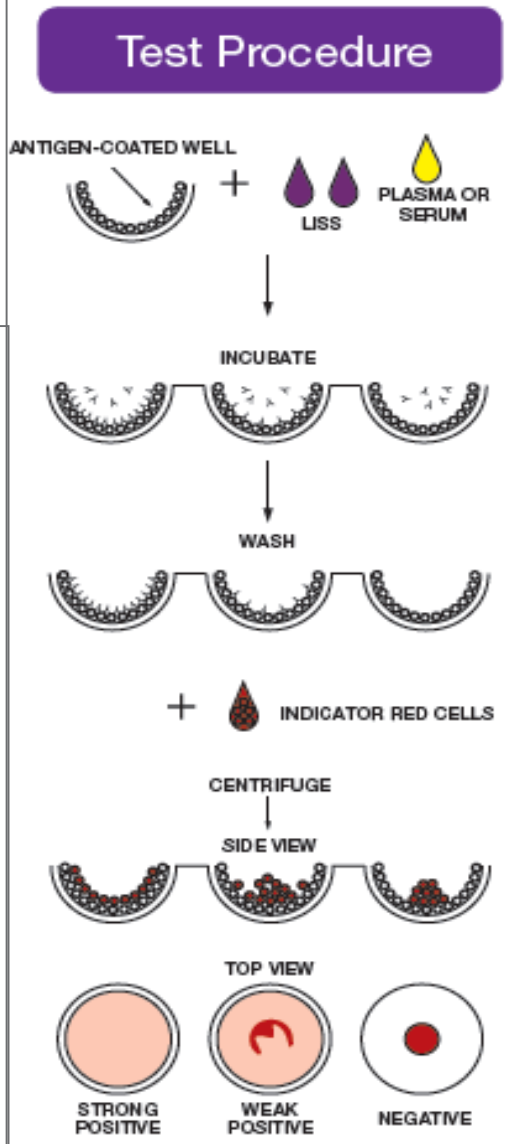
American Red Cross

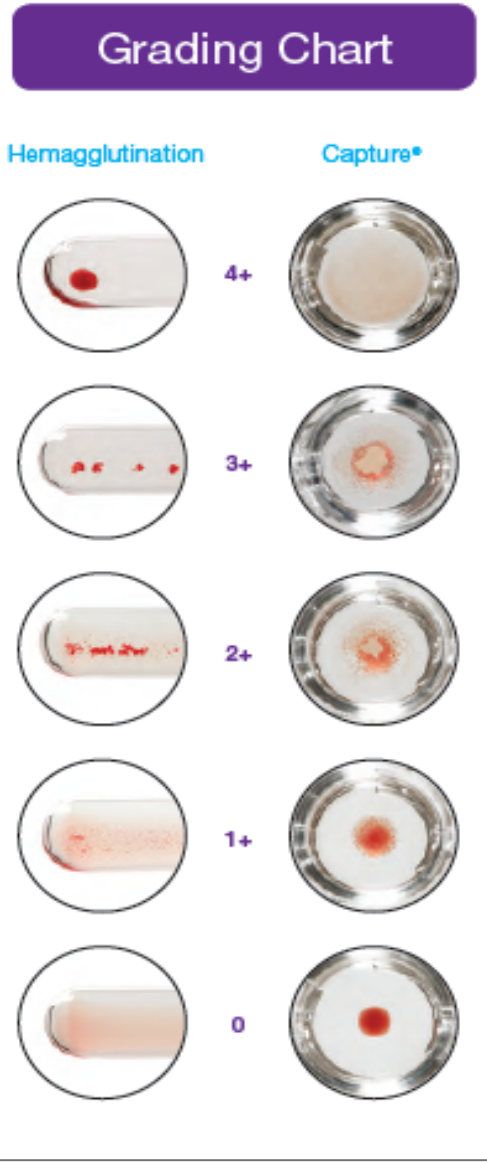
Immucor website

BIO-RAD website

Solid Phase







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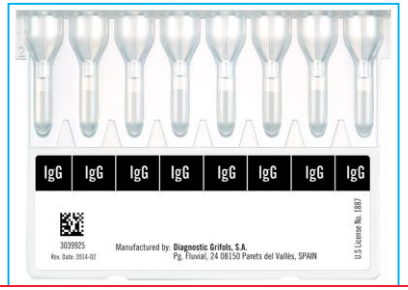
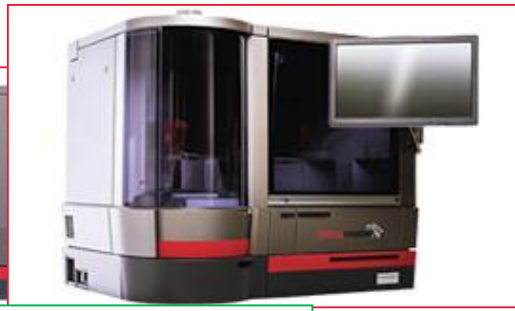
Immucor website – Capture Brochure

Column Agglutination Test

- Manual and automated systems
  - Grifols
  - Ortho
  - BioRad

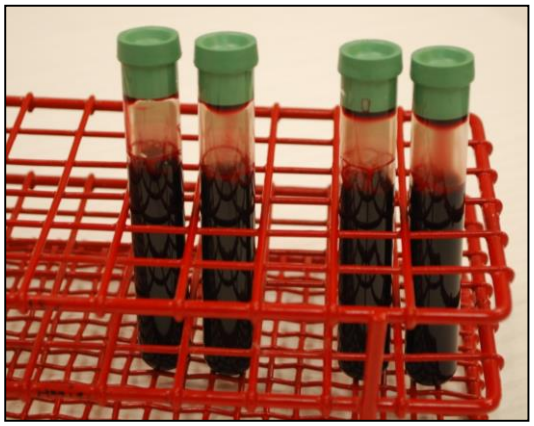


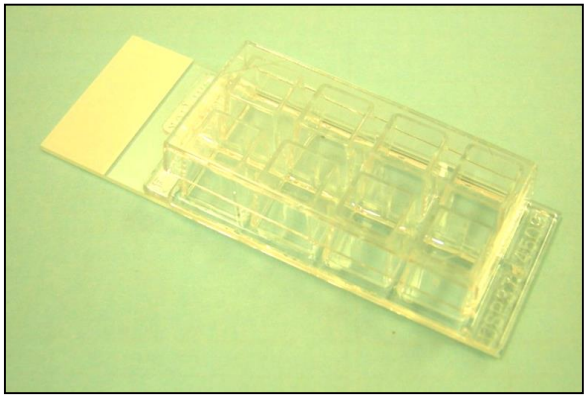


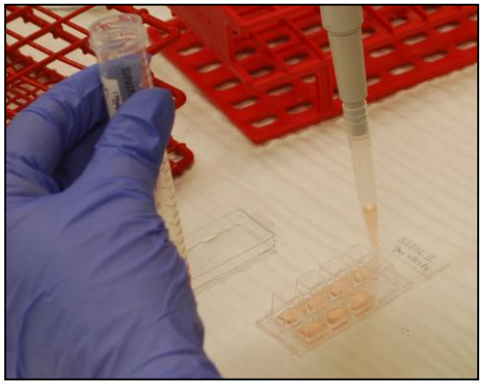
Monocyte Monolayer Assay (MMA)

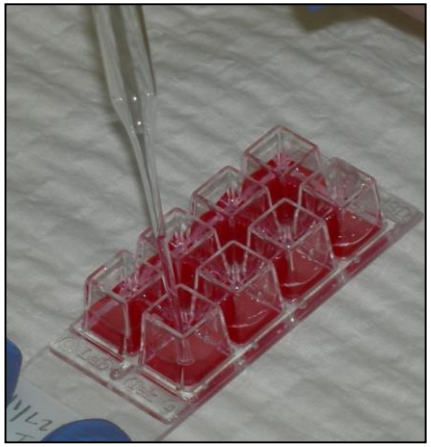
- **Overview**
  - Test to predict *in vivo* response to transfusion of incompatible RBCs
  - Tissue macrophages and peripheral blood monocytes mediate extravascular hemolysis of RBC in vivo
- **Indications**
  - Predict survival of antigen-positive RBCs
    - Antibody to high incidence antigen
    - RBCs unavailable and need to know clinical significance
- **Cannot be used to predict:**
  - HDFN – Hemolytic disease of the fetus and newborn
  - Autoantibody significance *in vivo*
  - Clinical significance of IgM antibodies

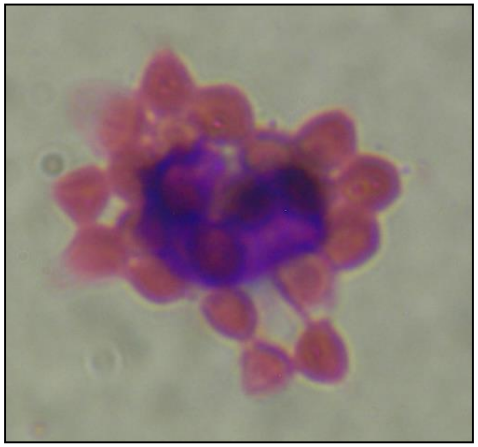
Monocyte Monolayer Assay (MMA)

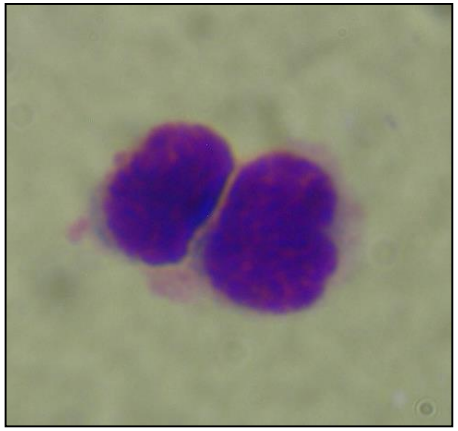












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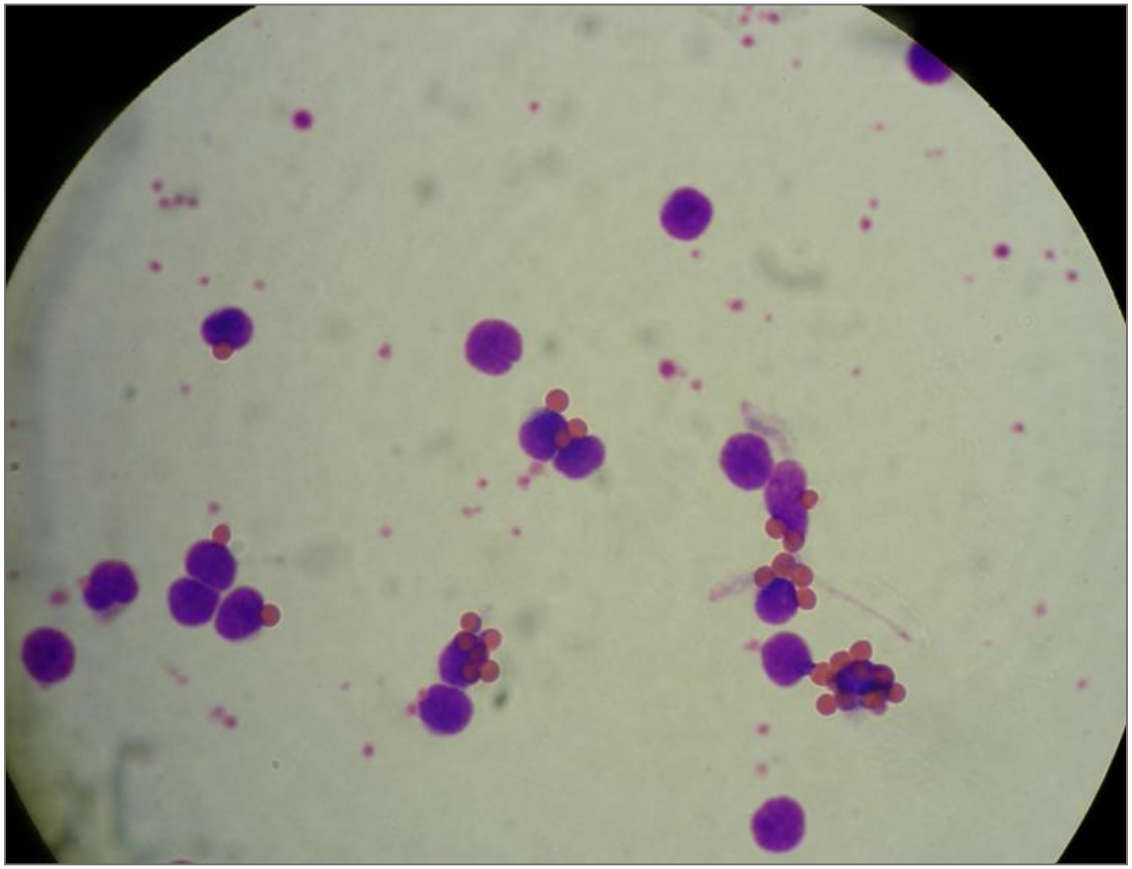
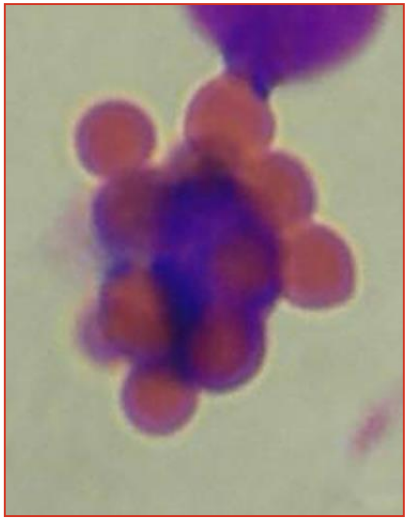
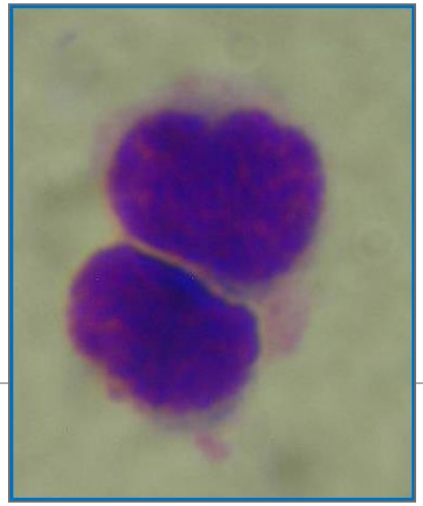
Monocyte Monolayer Assay (MMA)







Photography by Sandra Nance

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Monocyte Monolayer Assay Data

National Reference Laboratory for Blood Group Serology (NRLBGS) 1995-2017

Anti-	TT	POS	NEG
AnWj	2	1	1
At^a	4	3	1
Au^a	1	0	1
Co^a	2	2	0
Cr^a	4	4	0
Di^b	11	8	3
Do^b	5	1	4
E	1	1	0
e	3	2	1
GE Sys	31	16	15
hr^B	3	2	1
hr^S	7	4	3
Hy	9	7	2
I	5	1	4
Jk3	1	0	1
Jo^a	10	4	6
Jr^a	15	9	6

Anti-	TT	POS	NEG
Js^b	1	1	0
Kp^b	6	2	4
Ku	1	1	0
Lan	11	7	4
LU Sys	21	19	2
Lu^b	14	12	2
Lw	3	2	1
M	11	5	6
N	2	1	1
$PP1P^k$	1	1	0
RH Sys	1	1	0
s	1	0	1
Sc1	1	1	0
Tc^a	2	1	1
U	4	2	2
Vel	13	10	3
Yt^a	195	119	76

Maurer J, Nance S, Nickle P. Monocyte Monolayer Assay (MMA) to Predict Clinical Significance of Alloantibodies: a 22 year Review. AABB Abstract 2018

Monocyte Monolayer Assay Data

NRLBGS 1995-2017

Anti-	TT	POS	NEG
AnWj	2	1	1
At^{a}	4	3	1
Au^{a}	1	0	1
Co^{a}	2	2	0
Cr^{a}	4	4	0
Di^{b}	11	8	3
Do^{b}	5	1	4
E	1	1	0
e	3	2	1
GE Sys	31	16	15
hr^{B}	3	2	1
hr^{S}	7	4	3
Hy	9	7	2
I	5	1	4
Jk3	1	0	1
Jo^{a}	10	4	6
Jr^{a}	15	9	6

Anti-	TT	POS	NEG
Js^{b}	1	1	0
Kp^{b}	6	2	4
Ku	1	1	0
Lan	11	7	4
LU Sys	21	19	2
Lu^{b}	14	12	2
Lw	3	2	1
M	11	5	6
N	2	1	1
P1Pk	1	1	0
RH Sys	1	1	0
s	1	0	1
Sc1	1	1	0
Tc^{a}	2	1	1
U	4	2	2
Vel	13	10	3
Yt^{a}	195	119	76

Maurer J, Nance S, Nickle P. Monocyte Monolayer Assay (MMA) to Predict Clinical Significance of Alloantibodies: a 22 year Review. AABB Abstract 2018

Monocyte Monolayer Assay Data

NRLBGS 1995-2017

Anti-	TT	POS	NEG
AnWj	2	1	1
At^{a}	4	3	1
Au^{a}	1	0	1
Co^{a}	2	2	0
Cr^{a}	4	4	0
Di^{b}	11	8	3
Do^{b}	5	1	4
E	1	1	0
e	3	2	1
GE Sys	31	16	15
hr^{B}	3	2	1
hr^{S}	7	4	3
Hy	9	7	2
I	5	1	4
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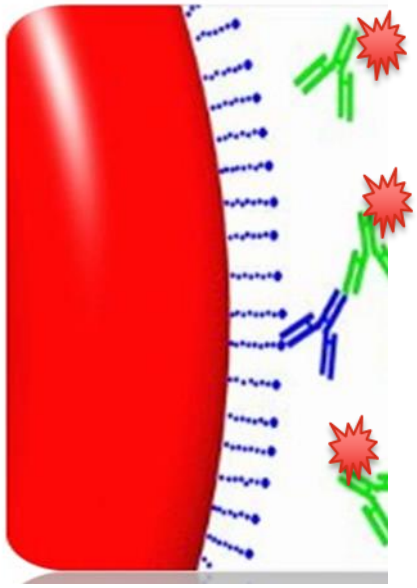
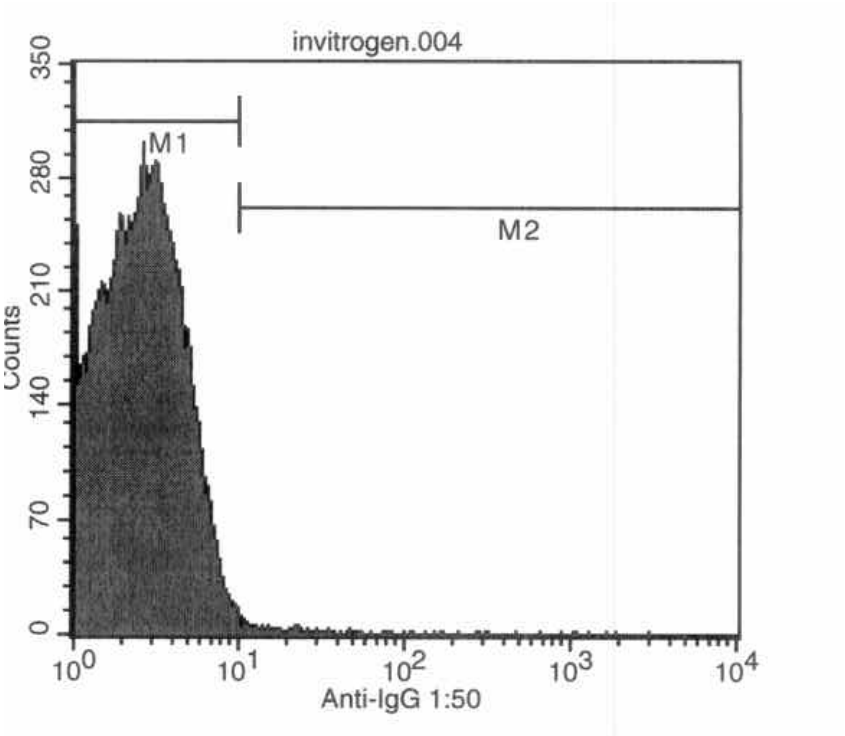
Maurer J, Nance S, Nickle P. Monocyte Monolayer Assay (MMA) to Predict Clinical Significance of Alloantibodies: a 22 year Review. AABB Abstract 2018

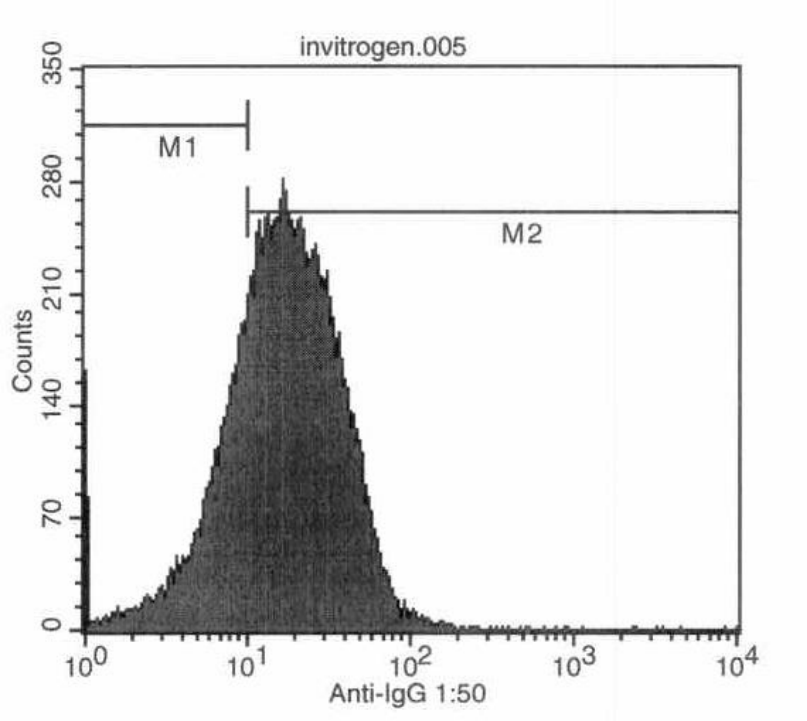
Flow Cytometry

- Detection of RBC, Platelet, Neutrophil antigens & antibodies
- Measurement of red cell bound IgG, IgA, IgM
- Determination of RBC zygosity
- Determination of RBC survival



Anti-IgG DAT by Flow Cytometry





Negative - %M2 = 0.5%

Positive - %M2 = 75.86%

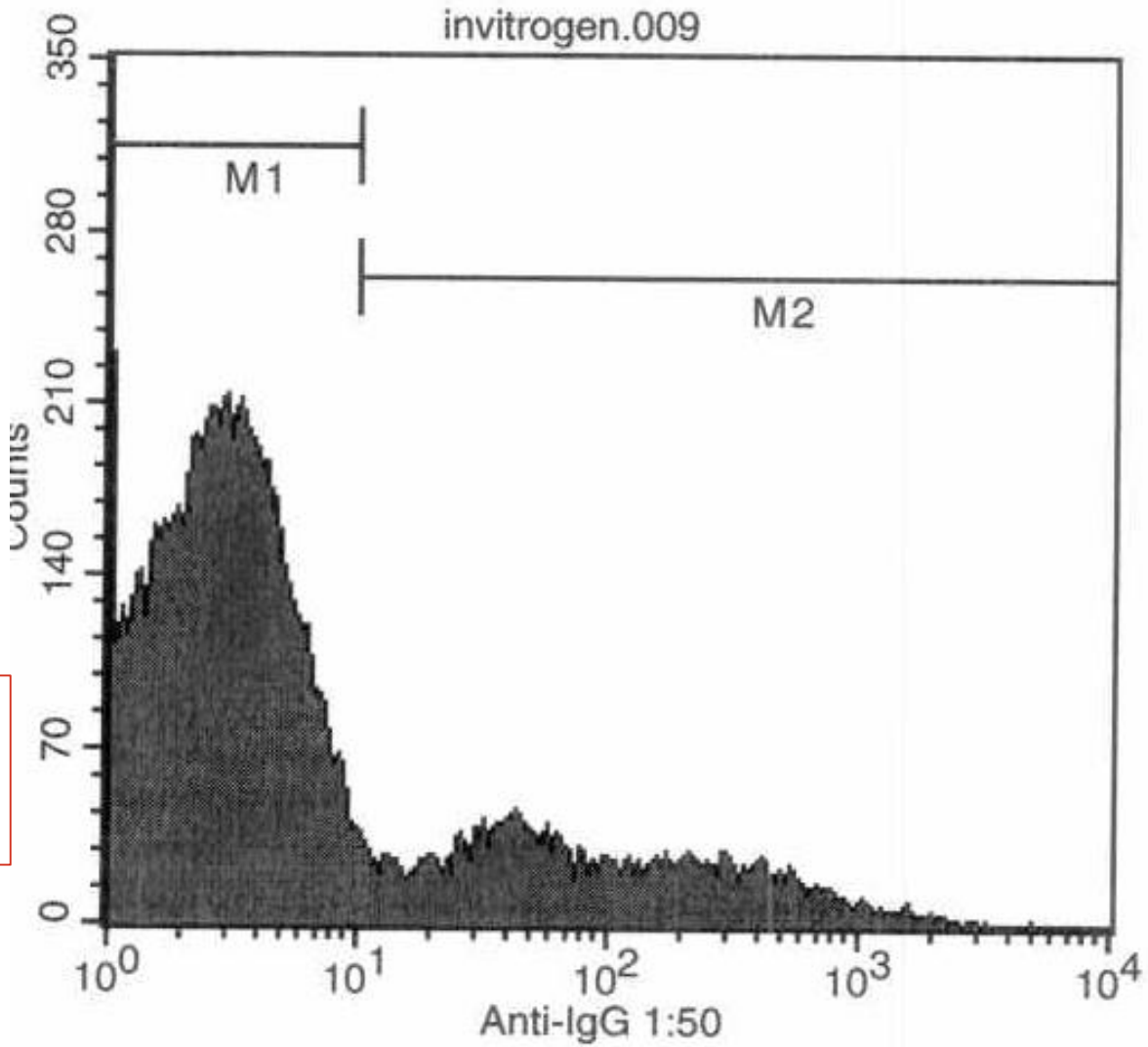
CONTROLS

American Red Cross

Anti-IgG by Flow Cytometry

IgG detected
% M2 = 20.70%

Patient sample
Looks like 2 populations
Uncoated and Coated RBCs



Other Special Techniques - CDP

- Chloroquine diphosphate (CDP)
  - Removes 80% of HLA antigens from RBCs or Platelets for testing
  - Removes IgG from RBCs in 83% of cases
    - Caution should be used with monoclonal sera typing of RBCs
    - Long incubation could affect RBC antigen reactivity with non-AHG sera
  - Removes IgG from Platelets

American Red Cross

Edwards JM, Moulds JJ, Judd WJ. Chloroquine dissociation of antigen-antibody complexes :A new technique for typing red blood cells with a positive direct antiglobulin test. TRANSFUSION 1982;22:59-61.

Other Special Techniques - Polybrene

- Sensitization phase with 1mL LIM (Dextrose and EDTA) – 1 min
- Polybrene agglutination phase – 15 seconds
- Resuspension phase – 10 seconds
- Supplementary AHG phase will detect KEL system antibodies (initial phase noted to not detect all KEL system antibodies)

Lalezari P, JIANG AF. The Manual Polybrene Test: A Simple and Rapid Procedure for Detection of Red Cell Antibodies. Transfusion 1980;20:206-211
First publication 1968 Transfusion 1968, 8:372-380

Other Special Techniques – Saline Replacement and DIDS for Rouleaux

- Alteration in the normal albumin:globulin ratio in a patient's plasma can be seen in multiple myeloma, macroglobulinemia, cryoglobulinemia, hyperfibrinogenemia, also with plasma volume expander infusion (dextran and hydroxyethyl starch)
- Dispersement of Rouleaux formation:
  - DIDS treated RBCs. DIDS (diisothiocyanatostilbene-2,2'-disulfonic acid) inhibits the formation of serum- or plasma-induced rouleaux through its ability to bind to band 3 on red cell membranes
  - Saline replacement – centrifuge, remove serum and add saline, resuspend, centrifuge, re-read

Norris S, Allen DD, Neff TP, Wilkinson SL. Evaluation of 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid in the inhibition of rouleaux formation. Transfusion 1996;36:109-112.

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