NHS
Blood and Transplant

Immune Haemolytic Anaemias

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NHS Blood and Transplant

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Definitions

- Haemolysis
  - A reduction of the average circulating red cell life-span (120 days +/- 10 days)
- DAT
  - Direct antiglobulin test
  - Shows the presence of antibodies on the surface of the red cells (nothing more!)

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Red Cells Coated with Antibody

- Maternal antibody coating foetal red blood cells
  - ?
- Recipient antibody coating transfused red blood cells
  - ?
- Patient's antibody coating autologous red blood cells
  - ?
- Donor antibody coating patient's red blood cells
  - ?

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Red Cells Coated with Antibody

- Maternal antibody coating foetal red blood cells
  - Haemolytic Disease of the Newborn - HDN
- Recipient antibody coating transfused red blood cells
  - Haemolytic transfusion reaction - HTR
- Patient's antibody coating autologous red blood cells
  - Autoimmune haemolytic anaemias - AIHA
- Donor antibody coating patient's red blood cells
  - Haemolytic transfusion reaction - HTR

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Haemolytic Anaemias

- **Non-serological**
  - Hereditary
    - spherocytosis
    - elliptocytosis
    - thalassaemias
    - haemoglobinopathies
    - Rhnull disease
  - Mechanical anaemias
  - Microangiopathic
  - Infectious agents
- **Serological**
  - AIHA
    - Warm-type
    - Cold-type
    - Paroxysmal cold haemoglobinuria - PCH
    - Paroxysmal nocturnal haemoglobinuria - PNH
    - Drug Induced
    - Combined warm/cold
  - HDN/HDF
  - HTR

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AIHA

- Diseases Associated with AIHA
  - Reticulo-endothelial neoplasms (CLL and lymphoma)
  - Myelodysplastic syndromes
  - Systemic lupus erythematosus
  - Infection (especially post-viral in childhood)
  - Immunological diseases

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Harmless Positive DATs

- Routine blood donors - positive DAT
  - 1 in 2175 [NBS-Colindale 2000]
  - 1 in 5400 [NBS-Birmingham 2002]
- Detected in crossmatch
  - Why no destruction?
- Is the DAT really positive?
  - Colloidal silica - autoclaved/storage in glass
  - Rouleaux formation - high levels of immunoglobulis
  - Wharton's jelly - collecting from cut cord

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Treatments

- Some treatments can cause positive DATs
  - Anti-Lymphocyte Globulin (ALG) and Anti-Thymocyte Globulin (ATG)
  - Anti-D and Immune Thrombocytopenia Purpura (ITP)
  - Any immunoglobulin given can give rise to DAT positive and free ‘auto / allo’ antibody

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Negative DATs in AIHA Patients

- Low affinity IgG auto-antibodies
  - More likely with gels due to high shear forces
- Low levels of bound auto-antibody
- IgM and IgA auto-antibodies
- IgA auto-antibodies
- IgM auto-antibodies

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Positive DATs in non-AIHA Patients

- 5% AIHA
- 16% malignancy
- 19% surgery/bleeding
- 10% MDS

Knight *et al* (2000)

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Why Investigate?

- To detect any antibodies of potential clinical significance
  - in the serum
  - eluted from the red cells
  - or both

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Clinical Significance

- Thermal range of antibody activity (37˚C)
- Antibody specificity (previous knowledge)
- Immunoglobulin class
- IgG sub-class
- Inhibitable by plasma
- Monocyte Monolayer Assay (MMA)
- Chemiluminescence

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Pathogenicity of Red Cell Antibodies

- Antibody characteristics
- Quantity of red blood cell-bound IgG and / or complement
- Target antigen characteristics
- Type of complement on circulating RBC
- Activity of reticuloendothelial system

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Warm AIHA

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WAIHA

- DAT: IgG +/- C3 coating cells
- ABO & Rh phenotyping
  - mAb
  - ZZAP
    - A mixture of Dithiothreitol (DTT) and Activated (cysteine) Papain that is used to remove Ig and complement from red blood cells
- Free antibody in serum?
  - allo, auto, or both?
  - strength?
- **Alloantibody detection priority**

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A Typical Panel

	ABO	Rh	M	N	S	s	P_1	Lu^a	Lu^b	K	k	Kp^a	Kp^b	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b		Sal RT	IAT 37	
1	O	$R_1^wR_1$	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+		0	5	
2	O	R_1R_1	+	-	+	+	+	+	+	-	+	-	+	-	+	-	+	-	+		0	5	
3	O	R_2R_2	+	-	-	+	+	-	+	-	+	-	+	-	+	+	-	+	-		0	5	
4	O	R_or	-	+	+	-	+	-	+	-	+	-	+	-	-	-	-	+	-		0	5	
5	O	r'r	-	+	+	-	-	-	+	-	+	-	+	+	-	+	-	+	+		0	5	
6	O	r''r	+	-	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+		0	5	
7	O	rr	-	+	+	-	-	-	+	-	+	+	+	-	+	+	-	+	-		0	5	
8	O	rr	+	-	+	+	+	-	+	-	+	+	+	+	-	-	+	+	-		0	5	
9	O	rr	-	+	-	+	+	+	+	-	+	-	+	+	-	+	-	-	+		0	5	
10	O	rr	-	+	+	-	-	-	+	+	-	-	+	-	+	+	+	-	+		0	5	
		Auto																			0	5	

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Methods of (possibly) sorting out the serology

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Methods

- Autoadsorptions
- Alloadsorptions
- Titrating the autoantibody
- Diluting the plasma

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Autoadsorptions

- By definition, autoadsorptions – using the patient's own cells to adsorb out antibody will only remove autoantibody
- If there are any alloantibodies "hiding" underneath the autoantibody then they will remain – as the patient will not possess the antigens to which alloantibodies are directed

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IMPORTANT

Autoadsorptions cannot be used if the patient has been recently transfused

Recently = _____

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Adsorptions

- To find if any alloantibodies are lurking underneath the autoantibody
- Volume of serum / cells available?
  - Autologous
    - ZZAP
    - Chloroquine
    - Citric acid
  - Allogeneic

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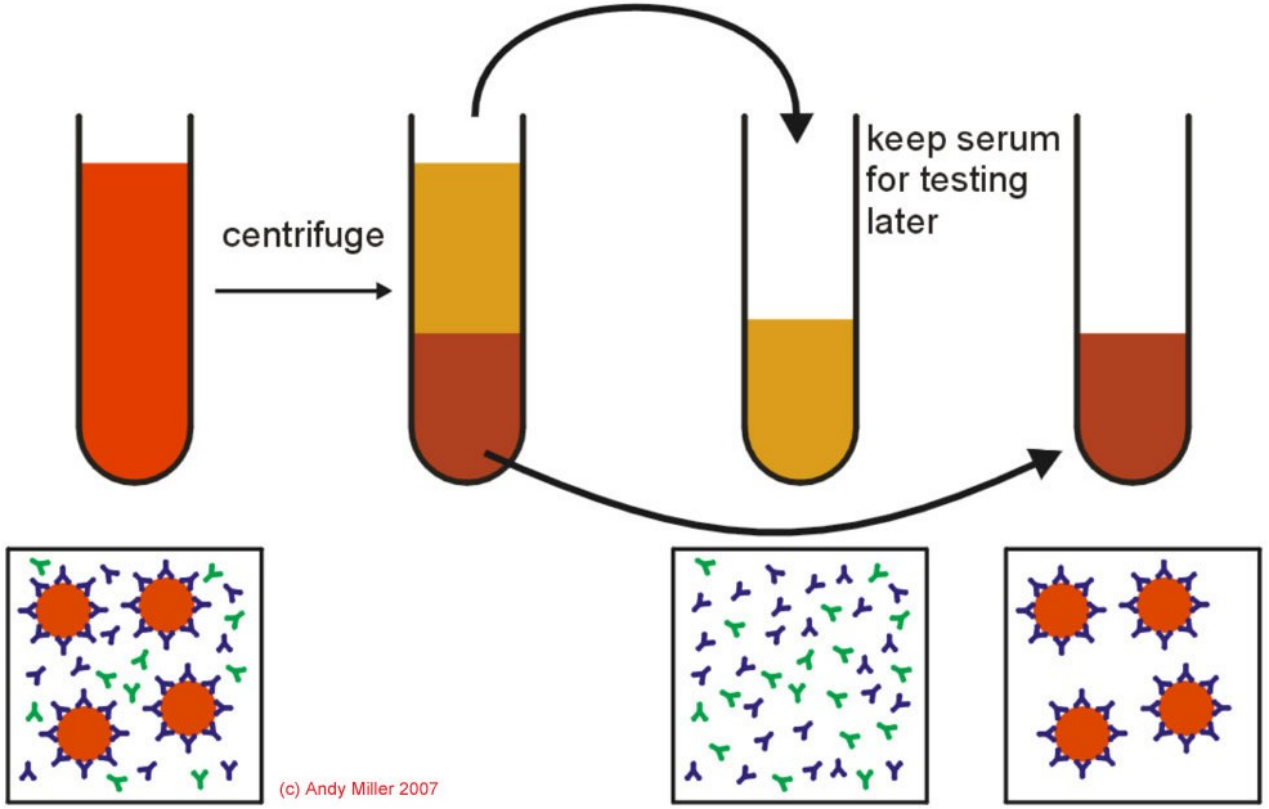
Autoadsorptions 1



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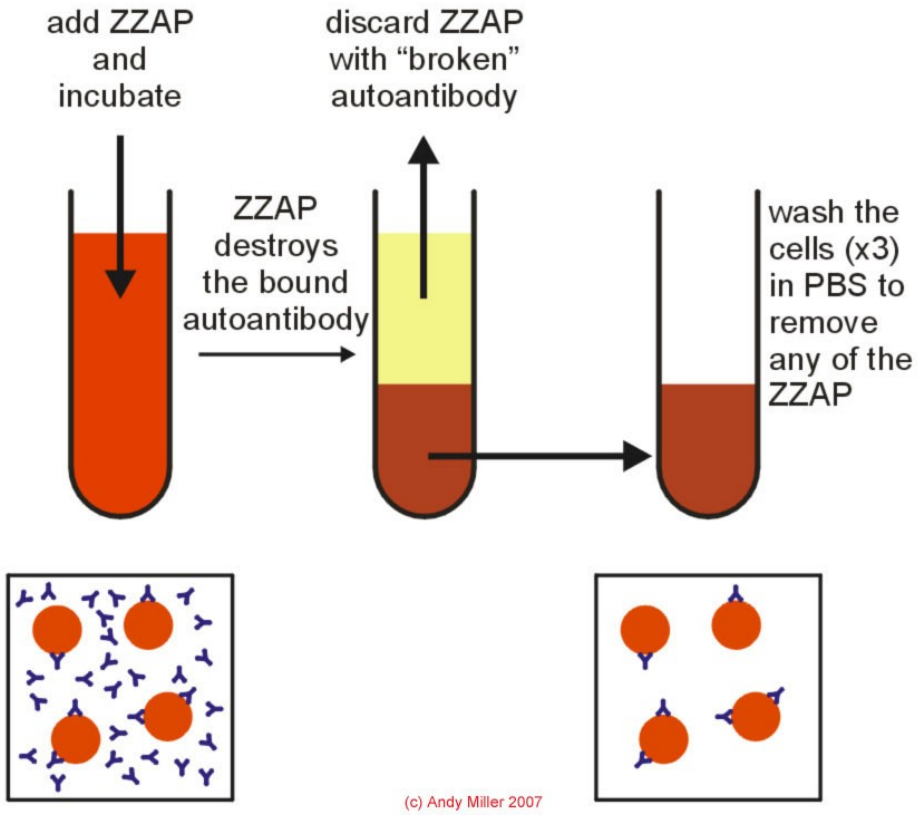
Autoadsorptions 2



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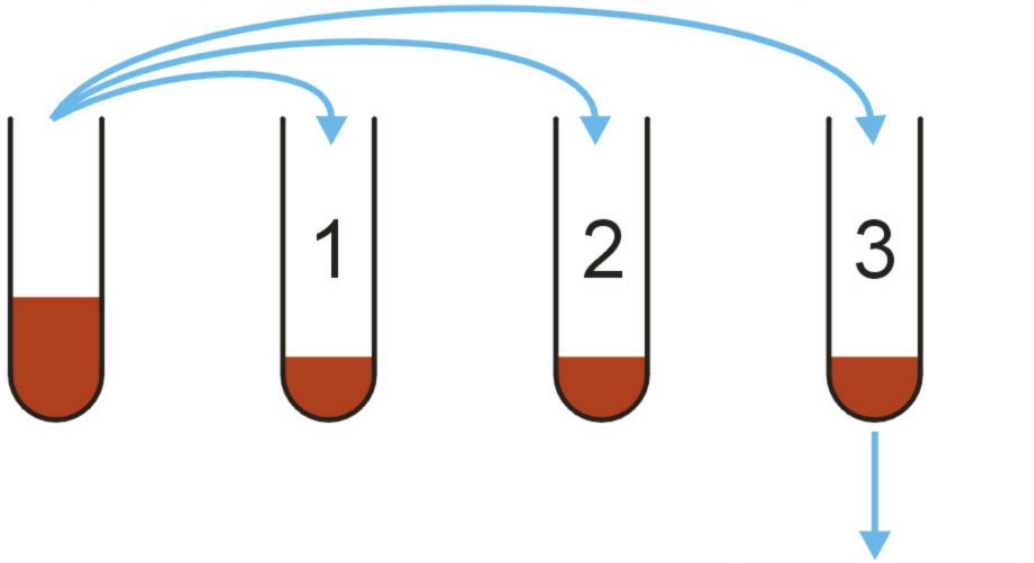
Autoadsorptions 3

split packed cells into as many decent-sized aliquots as possible - this depends upon the amount of cells you have in the first place



Repeat the number of absorptions for as many aliquots as you have - try and aim for a minimum of three

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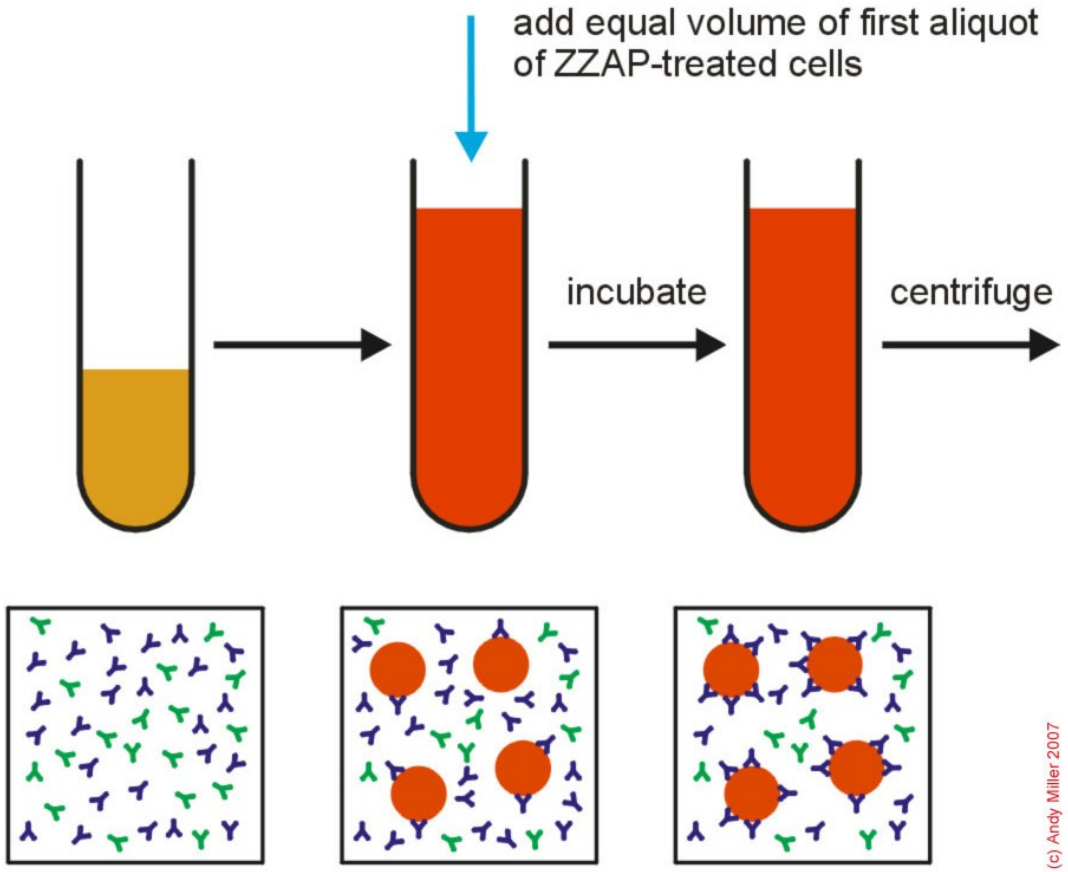
Autoadsorptions 4



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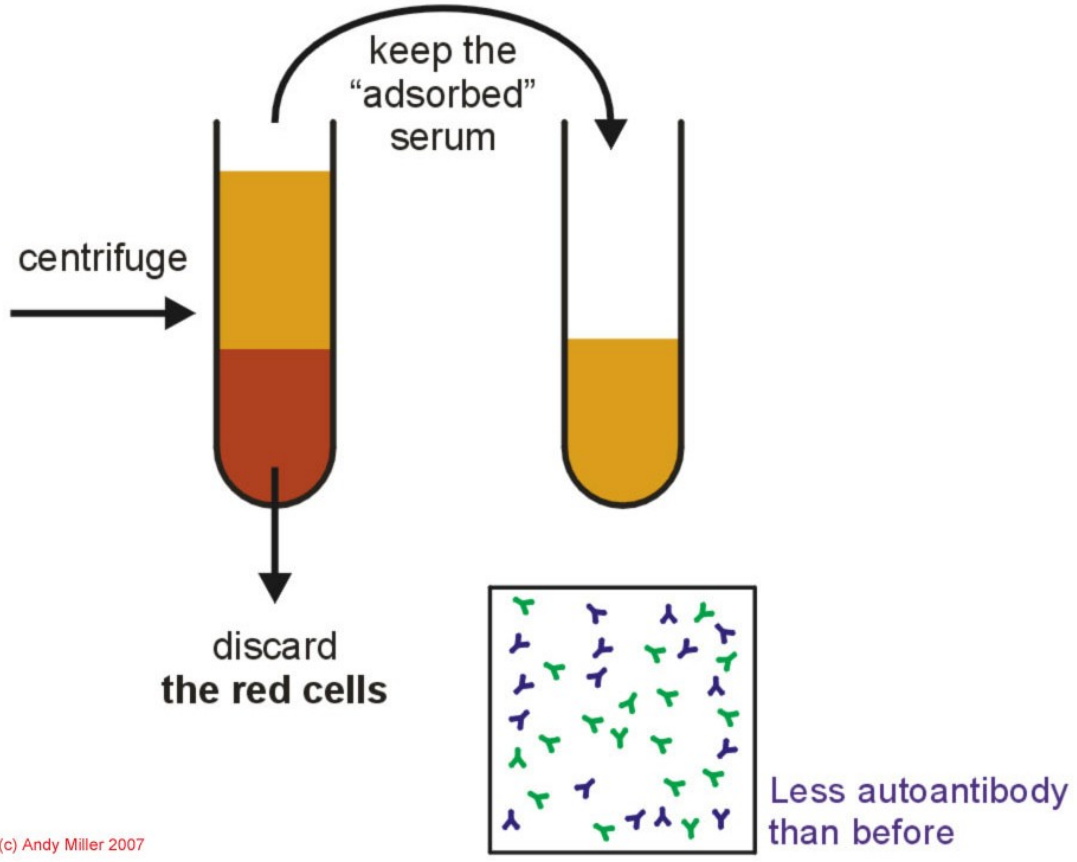
Autoadsorptions 5



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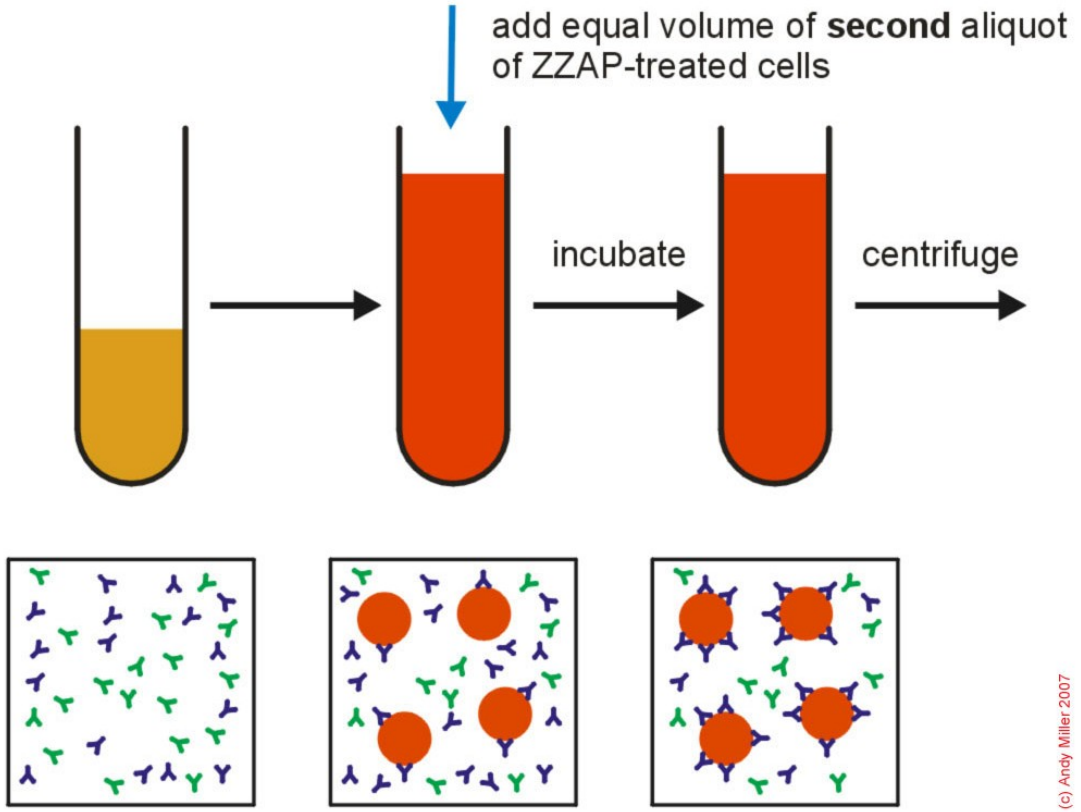
Autoadsorptions 6



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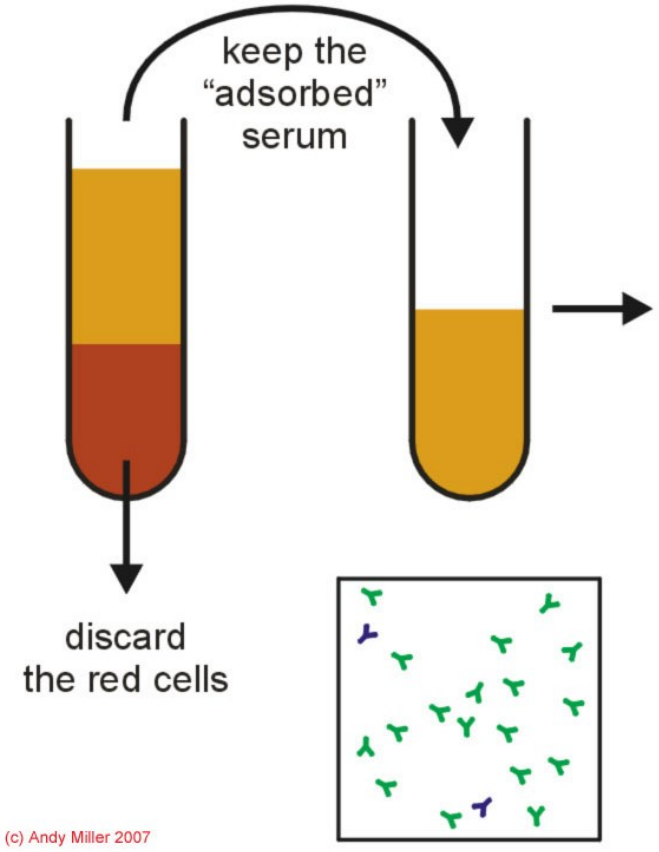
Autoadsorptions 7



Repeat autoadsorptions for as many aliquots of ZZAP-treated red cells as you have

Test absorbed serum against an antibody identification panel - the autoantibody *should* be much weaker / absent and not interfere with the testing

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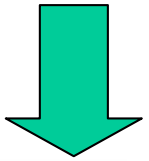
The Desired Result



	ABO	Rh	M	N	S	s	P_1	Lu^a	Lu^b	K	k	Kp^a	Kp^b	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b		Sal RT	IAT 37	IAT 37
1	O	$R_1^wR_1$	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+		0	5	3
2	O	R_1R_1	+	-	+	+	+	+	+	-	+	-	+	-	+	-	+	-	+		0	5	0
3	O	R_2R_2	+	-	-	+	+	-	+	-	+	-	+	-	+	+	-	+	-		0	5	3
4	O	R_or	-	+	+	-	+	-	+	-	+	-	+	-	-	-	-	+	-		0	5	3
5	O	r'r	-	+	+	-	-	-	+	-	+	-	+	+	-	+	-	+	+		0	5	3
6	O	r''r	+	-	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+		0	5	0
7	O	rr	-	+	+	-	-	-	+	-	+	+	+	-	+	+	-	+	-		0	5	3
8	O	rr	+	-	+	+	+	-	+	-	+	+	+	+	-	-	+	+	-		0	5	3
9	O	rr	-	+	-	+	+	+	+	-	+	-	+	+	-	+	-	-	+		0	5	0
10	O	rr	-	+	+	-	-	-	+	+	-	-	+	-	+	+	+	-	+		0	5	0
		Auto																			0	5	0
																							ADSORBED SERUM

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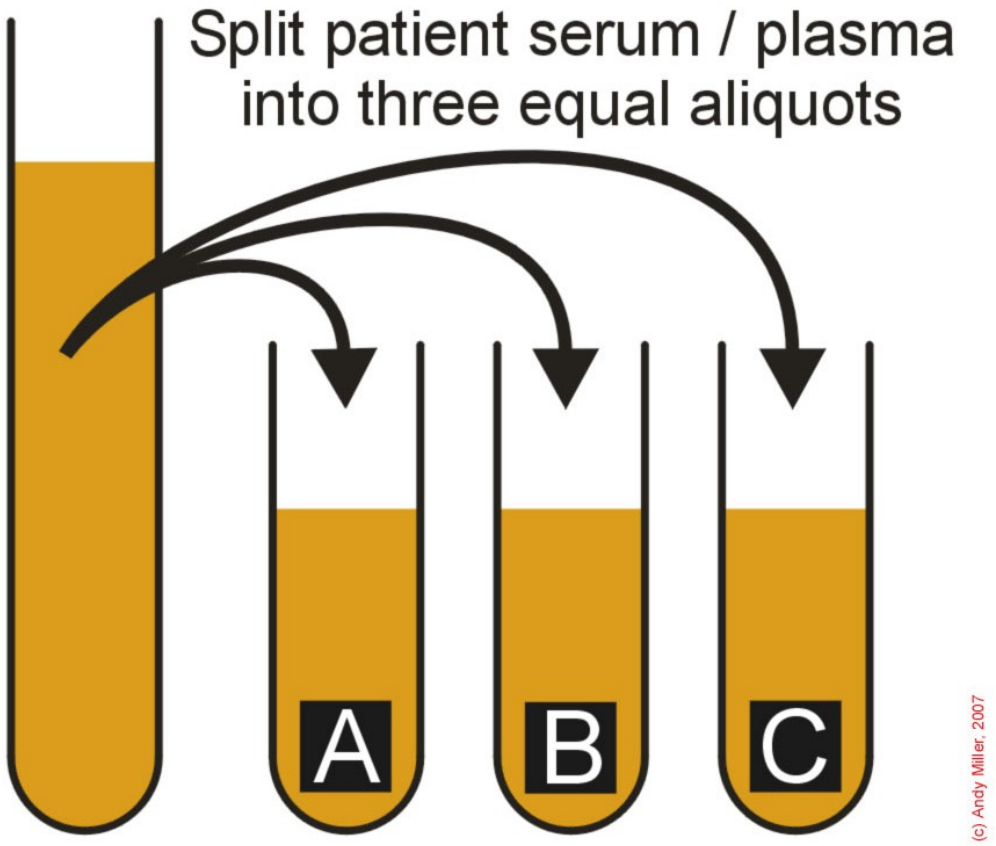
Alloadsorption 1



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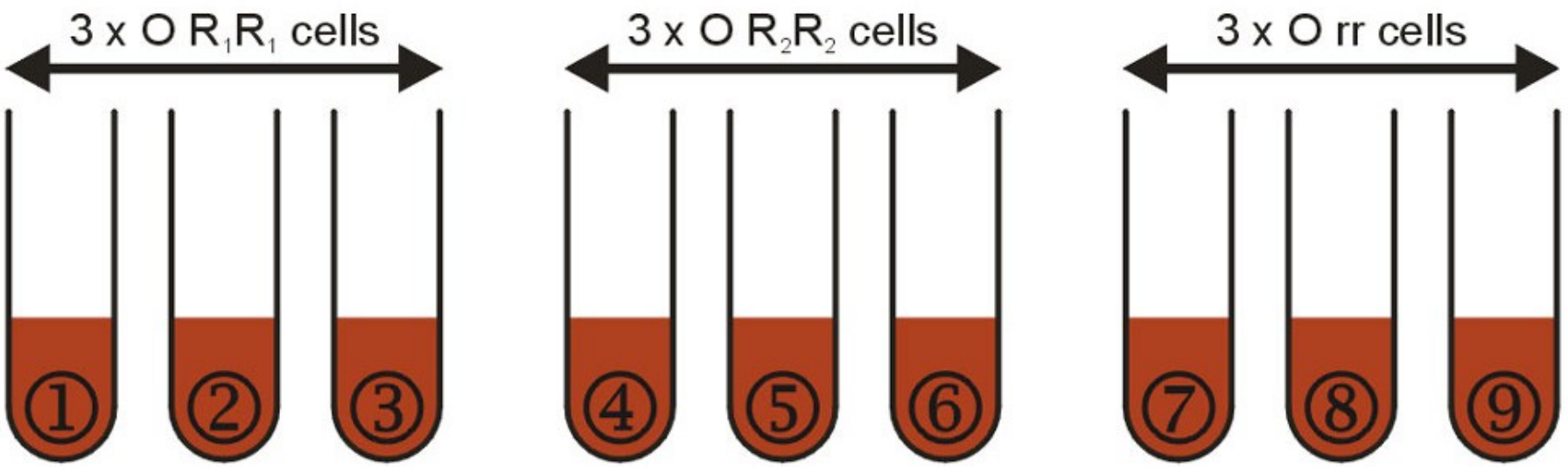
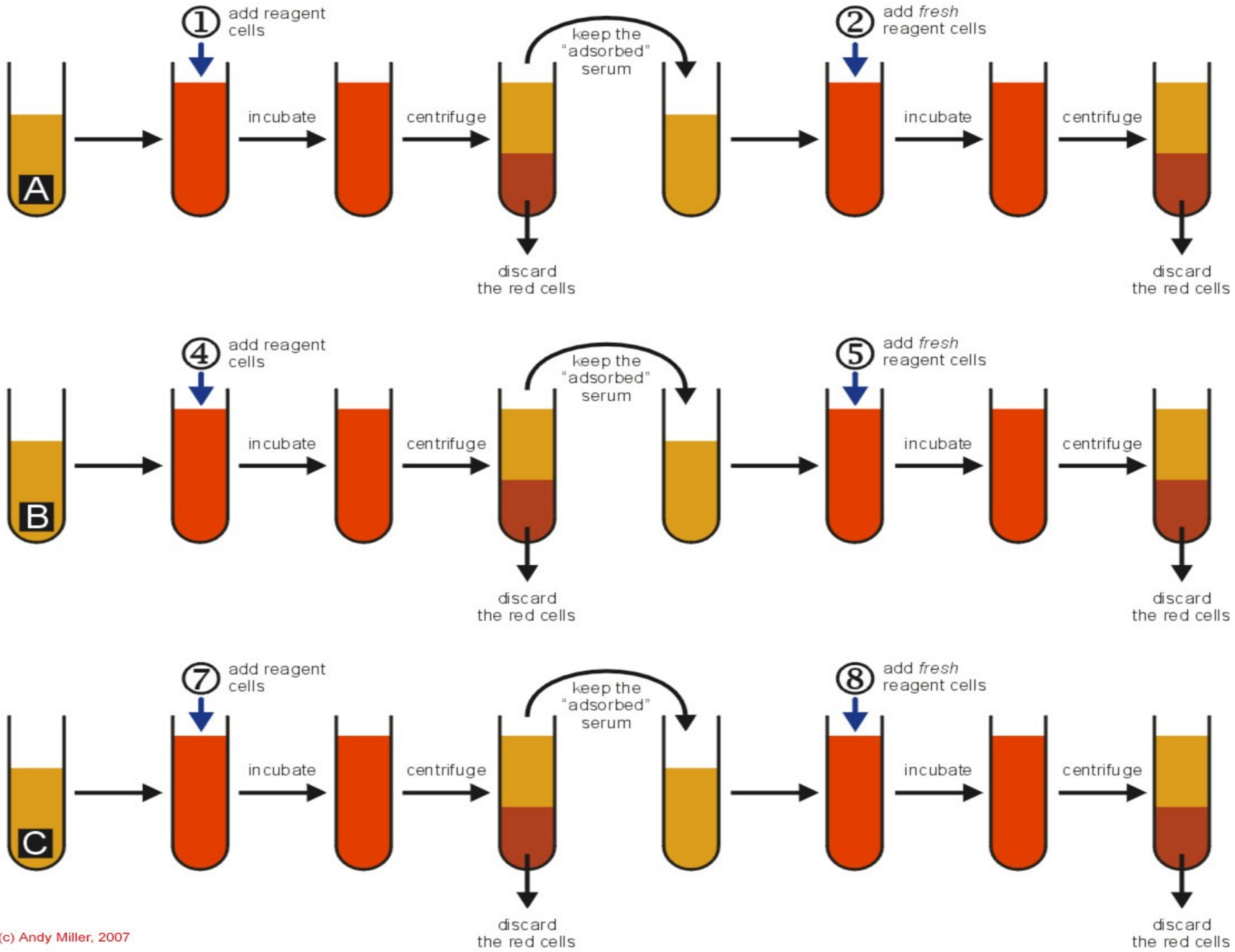
Alloadsorption 2

Commercial cells (OR_1R_1, OR_2R_2 and Orr) - designed for adsorptions



(c) Andy Miller, 2007


① add reagent cells
A
incubate
centrifuge
keep the "adsorbed" serum
discard the red cells
② add *fresh* reagent cells
incubate
centrifuge
discard the red cells
④ add reagent cells
B
incubate
centrifuge
keep the "adsorbed" serum
discard the red cells
⑤ add *fresh* reagent cells
incubate
centrifuge
discard the red cells
⑦ add reagent cells
C
incubate
centrifuge
keep the "adsorbed" serum
discard the red cells
⑧ add *fresh* reagent cells
incubate
centrifuge
discard the red cells
(c) Andy Miller, 2007

- Several different things can happen as you are not using the patient's own cells….

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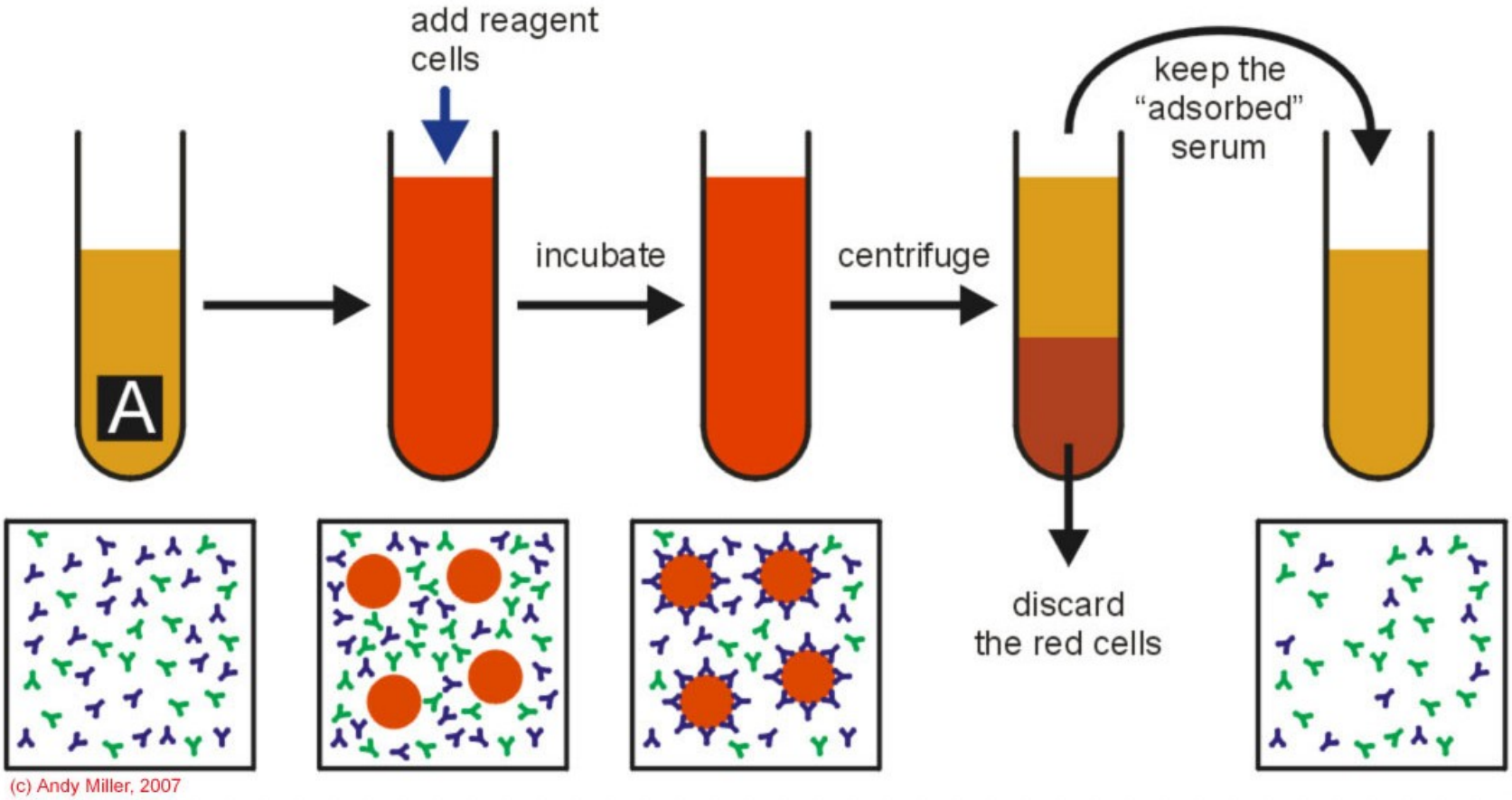
Auto adsorbed / Allo remains



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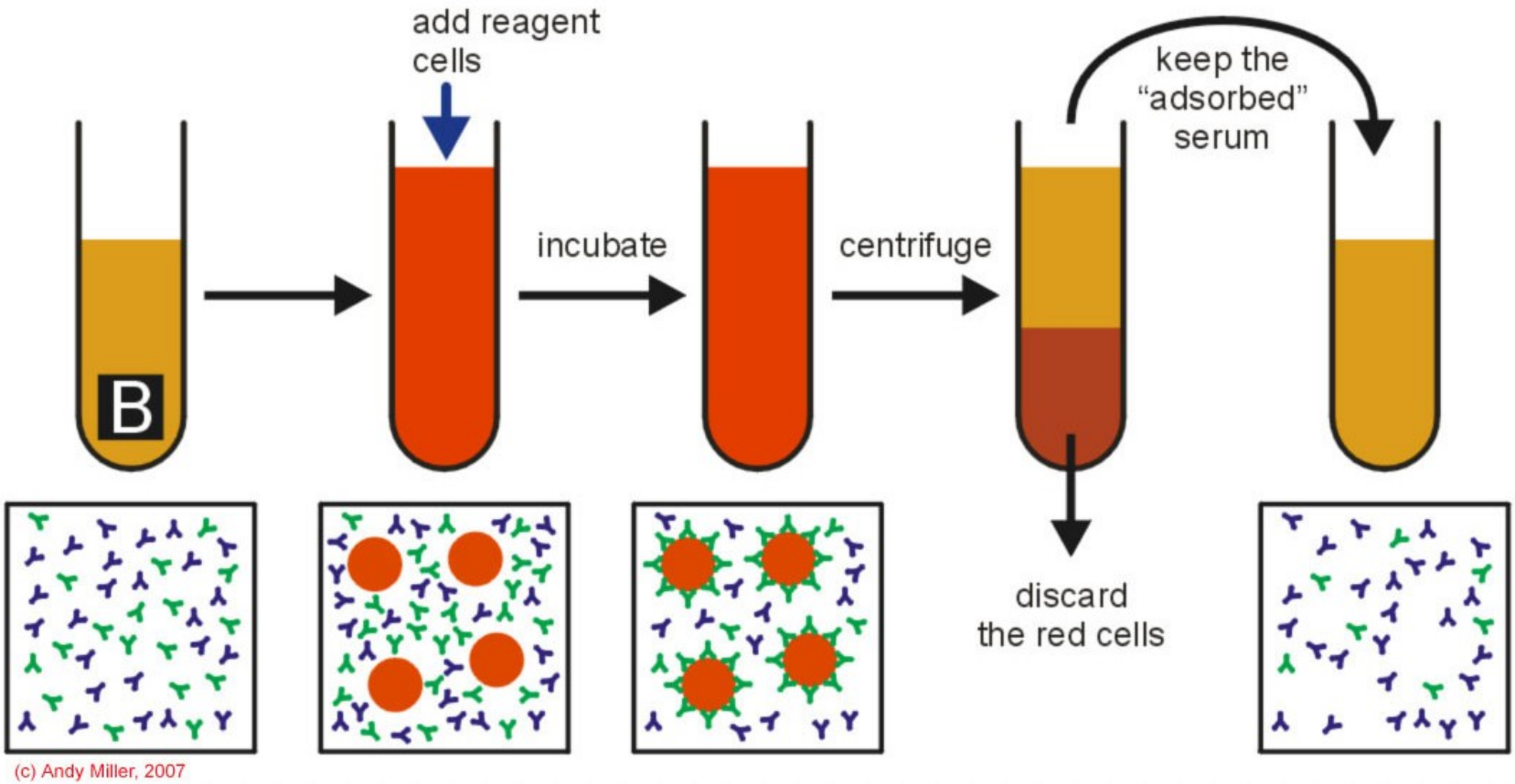
Allo adsorbed / Auto remains



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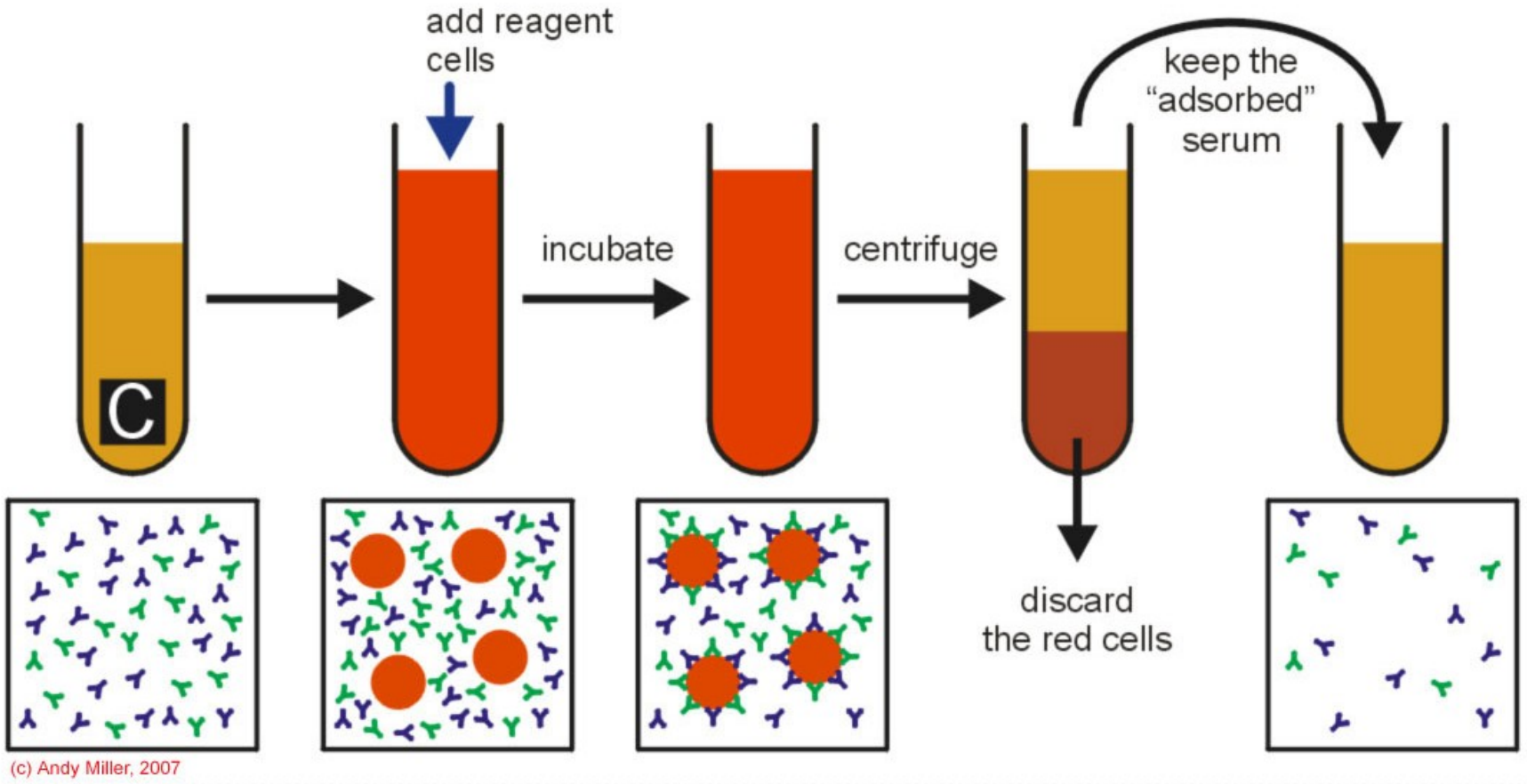
Allo and auto adsorbed



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What you Hope For

- The alloadsorption reagent cells remove the autoantibody completely
- At least one of the alloadsorption reagent cells leaves any underlying alloantibodies behind

Alloadsorption #0

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT				
									a	b			a	b	a	b	a	b	a	b							
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	4				
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	4				
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	4				
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	4				
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	4				
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	+	0	+		0	4				
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	4				
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	4				
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	0	+	+	+		0	4				
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	4				
Auto																						0	4				

A reminder of the problem

Alloadsorption #1

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT		R1R1	R2R2	rr
									a	b			a	b	a	b	a	b	a	b							
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	4		0	0	0
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	4		0	0	0
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	4		2	0	0
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	4		2	0	0
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	4		2	0	0
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	+	0	+		0	4		2	0	0
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	4		2	0	0
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	4		2	0	0
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	0	+	+	+		0	4		2	0	0
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	4		2	0	0
Auto																						0	4		/	/	/

Patient is 56y male, group B R_1R_1 K-

Alloadsorption #2

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT		R1R1	R2R2	rr
									a	b			a	b	a	b	a	b	a	b							
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	4		1	3	3
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	4		1	3	3
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	4		4	1	1
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	4		1	1	1
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	4		1	3	3
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	+	0	+		0	4		1	1	1
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	4		1	1	1
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	4		1	1	1
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	0	+	+	+		0	4		1	1	1
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	4		1	1	1
Auto																						0	4		/	/	/

Patient is 44y female, group A R_2r K-

Alloadsorption #3

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT		R1R1	R2R2	rr
									a	b			a	b	a	b	a	b	a	b							
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	4		4	4	4
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	4		4	4	4
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	4		4	4	4
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	4		4	4	4
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	4		4	4	4
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	+	0	+		0	4		4	4	4
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	4		4	4	4
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	4		4	4	4
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	+	0	+	+		0	4		4	4	4
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	4		4	4	4
Auto																						0	4		/	/	/

Patient is 50y female, group O rr K-

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AIHA Autoantibody Specificity

- Usually, the quoted specificities are merely preferences
  - The antibody may be adsorbed to exhaustion by antigen negative cells
  - The “specificity” merely indicates that the antibody titres further with a particular antigen
    - Anti-e is the most common

Alloadsorption #0

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT				
									a	b			a	b	a	b	a	b	a	b							
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	4				
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	4				
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	4				
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	4				
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	4				
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	+	0	+		0	4				
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	4				
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	4				
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	0	+	+	+		0	4				
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	4				
Auto																						0	4				

Same old problem

NHS
Blood and Transplant

Titration

Autoantibody / Eluate titration tested by IAT

Cell	Dilution										
	N	2	4	8	16	32	64	128	256	512	1024
O R_1R_1	4	4	4	4	4	4	3	2	1	0	0
O R_1R_1	4	4	4	4	4	4	3	2	1	0	0
O R_2R_2	4	4	3	2	1	0	0	0	0	0	0
O R_2R_2	4	4	3	2	1	0	0	0	0	0	0
O rr	4	4	4	4	4	4	3	2	1	0	0
O rr	4	4	4	4	4	4	3	2	1	0	0

This autoantibody shows a “preference” for -

NHS
Blood and Transplant

Diluting the Patient Plasma 1:3

- This is an advanced technique and should only be performed by advanced serologists
- Experience has shown that many weak autoantibodies are “diluted out” when the plasma is diluted 1 in 3 with saline
- Experience has shown that most weak alloantibodies are not “diluted out” when the plasma is diluted 1 in 3 with saline
- Use IAT technique only for this

Diluting out the autoantibody #1 – **EXPERIENCED SEROLOGISTS ONLY**

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT		
									a	b			a	b	a	b	a	b	a	b					
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	2		
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	2		
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	2		
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	2		
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	2		
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	+	0	0	+		0	2		
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	2		
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	2		
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	+	0	+	+		0	2		
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	2		
Auto																						0	2		

Problem – weaker autoantibdy

Diluting out the autoantibody #1 – **EXPERIENCED SEROLOGISTS ONLY**

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT		LISS IAT 1:3
									a	b			a	b	a	b	a	b	a	b					
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	2		0
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	2		0
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	2		0
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	2		0
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	2		0
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	+	0	0	+		0	2		0
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	2		0
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	2		0
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	+	0	+	+		0	2		0
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	2		0
Auto																						0	2		0

Patient is 39y male, group A R_2r

Diluting out the autoantibody #2 – **EXPERIENCED SEROLOGISTS ONLY**

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT		LISS IAT 1:3
									a	b			a	b	a	b	a	b	a	b					
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	2		1
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	2		2
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	2		0
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	2		1
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	2		2
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	+	0	+		0	2		0
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	2		0
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	2		1
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	0	+	+	+		0	2		1
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	2		2
Auto																						0	2		0

Patient is 57y female, group B R_1r

Diluting out the autoantibody #3 – **EXPERIENCED SEROLOGISTS ONLY**

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk			SAL 20	LISS IAT		LISS IAT 1:3
									a	b			a	b	a	b	a	b	a	b					
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	+	+	+		0	2		1
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	+	+	+	0		0	2		1
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	+	0	0	+		0	2		2
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	0	+	+		0	2		1
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	+	+	0		0	2		1
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	+	0	+		0	2		1
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	+	0	+		0	2		1
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	+	+	+		0	2		1
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	0	+	+	+		0	2		1
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0		0	2		1
Auto																						0	2		1

Patient is 89y male, group O rr K-

NHS Blood and Transplant

Transfusing AIHA Patients

- Transfusion is of limited value and carries increased risk of :-
  - Inducing the formation of allo-antibodies
  - Increasing the potency of the auto-antibodies
  - Inducing haemoglobinuria due to antibody-mediated red cell destruction

NHS
Blood and Transplant

Crossmatching

- Always select cells of the same ABO, Rh and K type
- Units must be antigen negative for any significant alloantibodies detected
- Match against both native and adsorbed serum
- Label blood units ‘suitable for…’

NHS
Blood and Transplant

Cold AIHA (CHAD)

NHS
Blood and Transplant

Diseases Associated with CHAD

- Mycoplasma pneumoniae infection
- Lymphoma
- Infectious mononucleosis
- Waldenströms macroglobulinaemia
- Paroxysmal cold haemoglobinuria
- Viral infection
- Syphilis

NHS Blood and Transplant

WARNING

- The cells and serum must be separated at 37˚C
  - Agglutination can be total and virtually instantaneous once the antibody is within thermal range
  - Agglutination can sometimes be reversed by re-warming the sample
  - A valid negative control must be achieved to validate any test!

Typical CHAD Panel

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk				SAL 20	LISS IAT	PAP 37
									a	b			a	b	a	b	a								
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	2		+			5	5	5
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	0	2		0			5	5	5
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	0	2		+			5	5	5
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	2		+			5	5	5
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	2		0			5	5	5
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	2		+			5	5	5
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	2		+			5	5	5
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	2		+			5	5	5
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	0	2		+			5	5	5
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	0	2		0			5	5	5
Auto																							5	5	5

Typical CHAD Panel ?

	ABO	Rh		M	N	S	s	P1	Lu		K	k	Kp		Le		Fy		Jk				SAL 20	LISS IAT	PAP 37
									a	b			a	b	a	b	a								
1	O	$R1^w$	R1	+	0	+	0	4	0	+	+	+	0	+	+	0	0	2		+			5	5	5
2	O	R1	R1	+	+	0	+	3	0	+	+	+	0	+	+	0	0	2		0			5	5	5
3	O	R2	R2	+	0	0	+	1	0	+	0	+	+	+	0	+	0	2		+			5	5	5
4	O	Ro	r	0	+	0	+	1	0	+	0	+	0	+	0	+	0	2		+			5	5	5
5	O	r′	r	0	+	0	+	4	0	+	0	+	0	+	0	+	0	2		0			5	5	5
6	O	r″	r	0	+	0	+	4	0	+	+	+	0	+	+	0	0	2		+			5	5	5
7	O	r	r	+	0	+	0	2	+	+	0	+	0	+	0	+	0	2		+			5	5	5
8	O	r	r	+	+	+	0	3	0	+	0	+	0	+	0	+	0	2		+			5	5	5
9	O	r	r	0	+	0	+	2	0	+	0	+	0	+	+	0	0	2		+			5	5	5
10	O	r	r	0	+	0	+	0	0	+	0	+	0	+	0	+	0	2		0			5	5	5
Auto																							0	0	0

NHS
Blood and Transplant

CHAD Autoantibody Specificities

- Cold AIHA specificities are true specificities
  - Anti-I, i, IH, ABO, P
- Factors affecting degree of haemolysis
  - Degree & duration of exposure to cold
  - Thermal range of autoantibody activity
  - Efficiency of complement activation at different temperatures
  - Complement utilisation & synthesis
  - Level of factor I (C3b inactivator)

CHAD – the big problem

NHS Blood and Transplant

A

Forward Group			Reverse Group			RhD Group	
Anti-A	Anti-B	AB Serum	A cells	B cells	O cells	Anti-D	Anti-D
5	5	5	5	5	5	5	5

B

Forward Group			Reverse Group			RhD Group	
Anti-A	Anti-B	AB Serum	A cells	B cells	O cells	Anti-D	Anti-D
5	0	5	0	5	0	0	0

C

Forward Group			Reverse Group			RhD Group	
Anti-A	Anti-B	AB Serum	A cells	B cells	O cells	Anti-D	Anti-D
5	1	5	1	5	1	1	1

NHS
Blood and Transplant

Transfusing CHAD Patients

- Often clinically ineffective, as the transfused donor cells are rapidly haemolysed by active C3b in the serum, which binds to virgin CR1 sites on transfused cells
- The autologous cells are relatively resistant to C3b haemolysis as all CR1 sites are blockaded by C3d/g moieties
- The transfusion of donor blood causes a significant release of complement complexes which haemolyse autologous, as well as transfused cells (reactive haemolysis)
- ? Blood warmer and keep patient warm

NHS
Blood and Transplant

Paroxysmal Cold Haemoglobinurea (PCH)

NHS
Blood and Transplant

- Exposure to cold leads to a sudden attack of intravascular haemolysis
- Haemoglobin is found in the urine
- Known since 1865
- Diagnostic test is the Donath-Landsteiner Test (1905)
- In the past – common in adults during the late stages of untreated syphilis
- Now a very rare condition (70 cases in 30 years) – almost always in young children post-viral illness

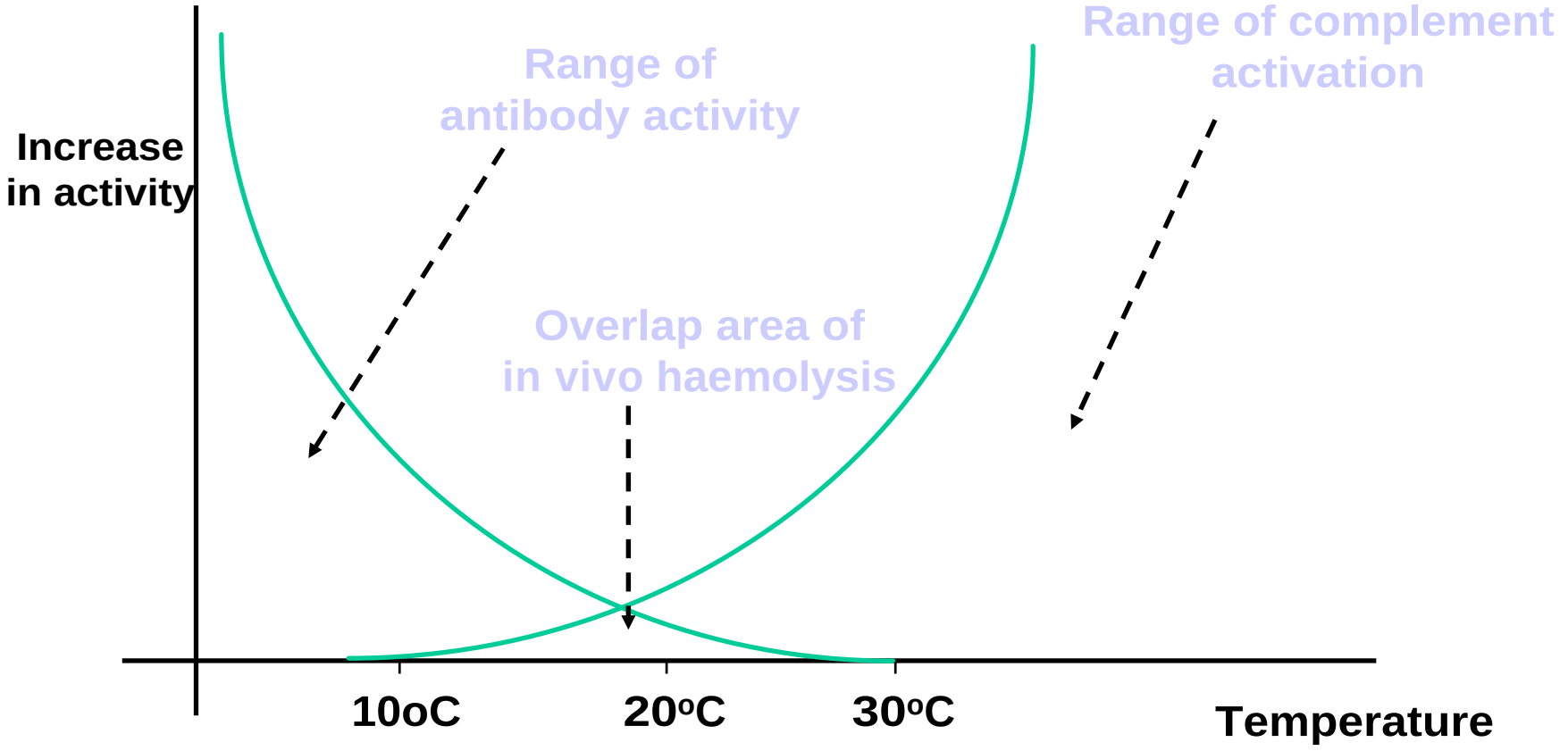
NHS Blood and Transplant

Donath-Landsteiner Reaction

- IgG biphasic antibody, with anti-P specificity
- Adults usually have a chronic condition
- DAT Positive - C3d +/- C3c, may detect weak IgG if cooled sample used, especially using gel column test.
- Weak or no cold autoantibody at room temperature.
- 37˚C tests are usually negative.
- Confirmation of diagnosis is made using the Donath-Landsteiner test with WARM SEPARATED SERUM.

NHS Blood and Transplant

Degree of Haemolysis

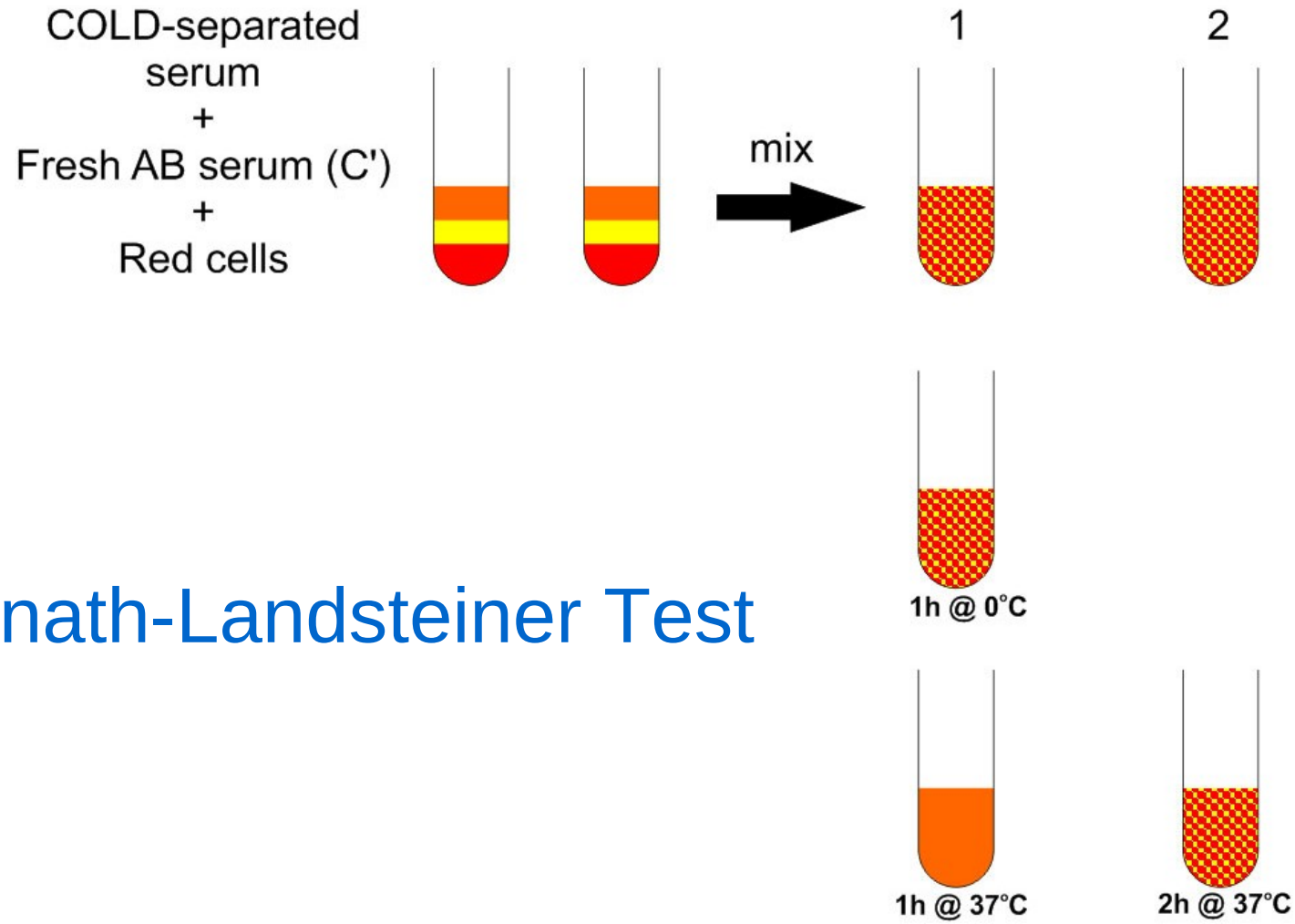


NHS
Blood and Transplant

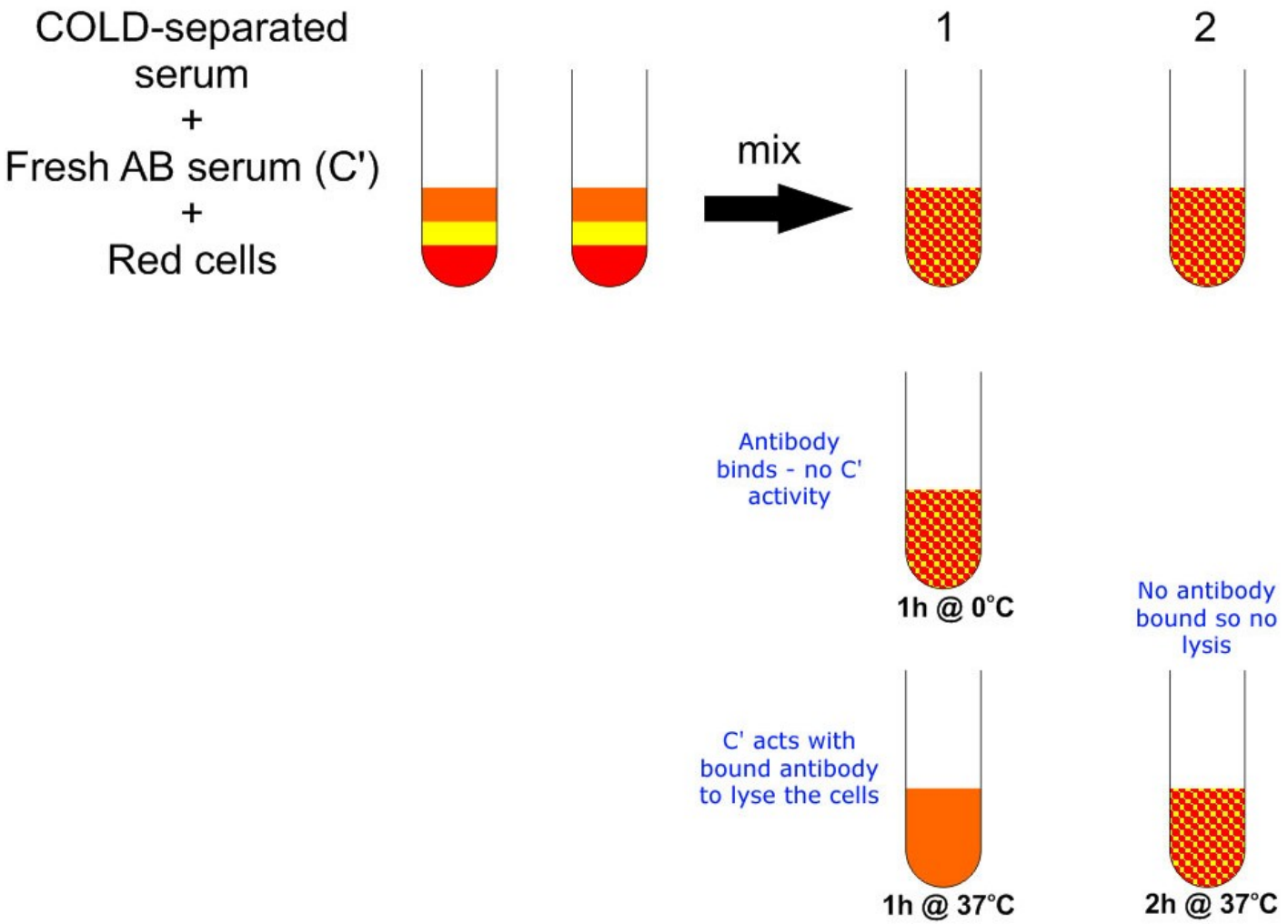
The D-L Test

- 2 sets of dilutions of patient's warm separated serum in complement rich fresh serum
- One set incubated at 0˚C for 1hr and then placed at 37˚C for 1hr, other set 2hrs at 37˚C.
- Only cooled and warmed tubes should be positive.
- SEE NEXT SLIDES

The Donath-Landsteiner Test



NHS
Blood and Transplant



NHS
Blood and Transplant

Treatment - Children

- Keep the patient warm
- Acute condition often requiring transfusion
- Fatalities are very rare
- Any advantage using very rare pp red cells for transfusion? – Maybe (exceptional cases)
- Condition usually resolves in 1-3 weeks

NHS Blood and Transplant

Treatment - Adults

- Avoid cold
- Chronic condition
- Need for transfusion less likely, but occasional episodes of haemolysis
- Donath-Landsteiner test can remain positive for many years

NHS Blood and Transplant

Drug-Induced IHA

COMPLICATED!

NHS Blood and Transplant

Investigation

- Lengthy and comples
- Do your homework first!
  - Full clinical/drug history
  - Search scientific literature
- Many drugs are capable of binding to circulating cells.
- Can lead to immune response.
- Antibodies either formed to drug itself, or to drug plus membrane components

NHS Blood and Transplant

Mechanisms

1. Drug-dependent: antibodies reactive with drug-coated red cells hapten type or drug adsorption
2. Other drug-dependent antibodies: immune complex mechanism
3. Drug-independent antibodies: autoantibody production: Methyl dopa type
4. Non-immunologic: protein adsorption

NHS Blood and Transplant

Penicillin

- Approximately 3% of patients receiving high dose penicillin intravenously develop a positive DAT
- Occasionally they develop haemolytic anaemia
- Haemolysis develops gradually and can become life-threatening.
- Stopping the drug stops the process, BUT it may take weeks for hemolysis to stop completely.

NHS Blood and Transplant

Cephalosporins

- Approximately 4% receiving 1st or 2nd generation cephalosporins develop a positive DAT
- Prevalence and severity of cephalosporin-induced immune haemolysis is on the increase
- Discontinue the drug and the process stops.
  - May take weeks for hemolysis to stop completely
- Main offenders Cefotetan and Ceftriaxone

NHS
Blood and Transplant

THE END