



Cold Antibody **Blues**
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American Red Cross
Reference Laboratory

American Red Cross

Objectives

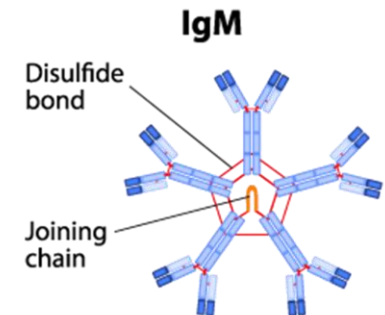


- Define Cold Antibodies
- Differentiate Cold alloantibodies vs Cold Autoantibodies
- Removing Cold Antibody Interference
- Risks associated with removing
- Case Studies
- Conclusion

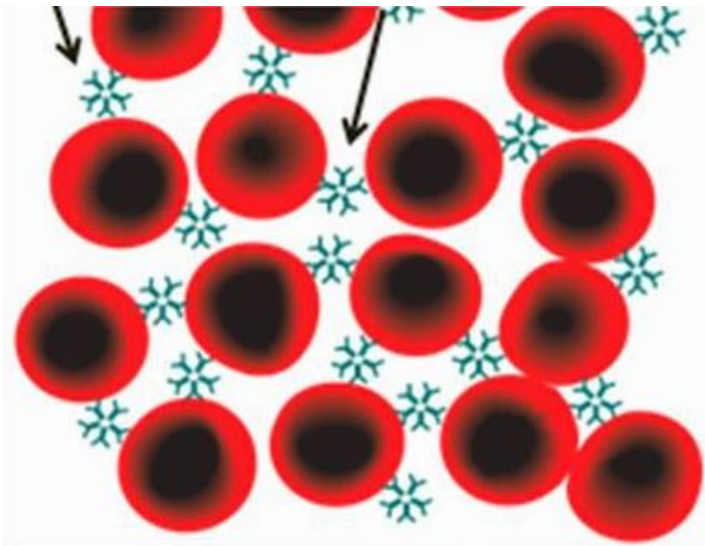
What is a Cold Antibody



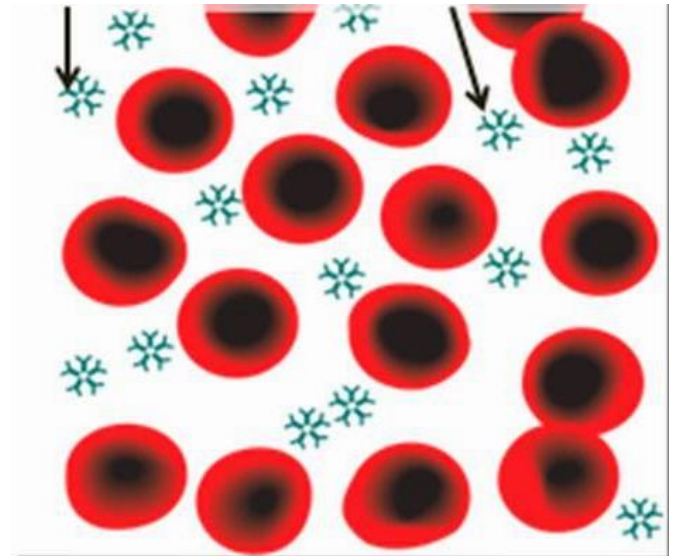
- An antibody that binds its target antigen best at levels below 37C
 - Thermal Range: 0C - 30C
 - Most don't react at 37C (reducing clinical significance)
- IgM in nature
- Often naturally occurring
- Most are benign
 - No HDFN, most don't cause HTRs
- Common Allos: Anti-Le^a, -Le^b, -I, -i, -M, -N, and -P₁
- Some are clinically significant
 - ABO antibodies (RT reactive)
 - Reaction at 37C may associate with disease



IgM at 4C



IgM at 37C



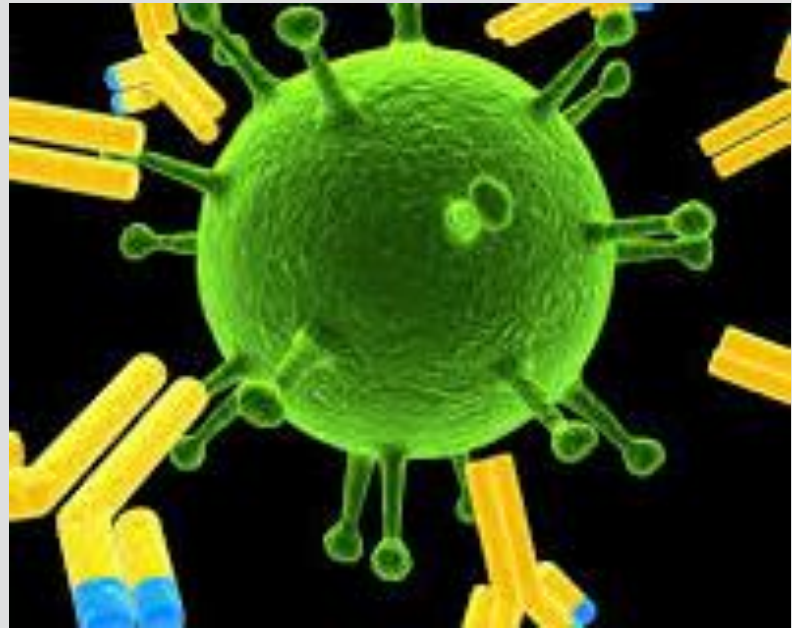
Clinical Significance



- How close to body temperature are the antibodies going to react?
 - If cold antibodies demonstrate a high thermal range (ex: 30C) discuss treatment options with Medical Director such as a blood warmer
- Cold Antibodies that react closer to body temperature and demonstrate high titer (>1000) are more likely to be clinically significant

Cold Alloantibodies

- Made against foreign red cells
- Autocontrol negative
- Common: Anti-Le^a, -Le^b, -M, -N, and -P₁
- Because they only react below body temperature:
 - Generally benign
 - Rarely cause RBC destruction
- Enjoy causing ABO discrepancies
 - Anti-M
- Panel:
 - Positive at IS
 - Negative or weaker at 37C / AHG



Cold Alloantibody Panel

Cell	Rh-ir	Rh-ir							Kell				Duffy				Kidd		P		Lutheran		MNSs				Lewis		Results					
		D	C	E	c	e	f	Cw	V	K	lc	Kp ^a	Kp ^b	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Lu ^a	Lu ^b	M	N	S	s	Le ^a	Le ^b	Cell	IS	AHG				
1	R ¹ K ¹	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	1	0	0	✓
2	R/R ¹	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	2	0	0	✓
3	R/R ²	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	0	3	0	0	✓		
4	R ⁰ r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	4	0	0	✓		
5	r ⁺ r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	5	2+	0	✓			
6	r ⁺ r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	+	0	6	0	0	✓			
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	7	2+	0	✓				
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	8	1+	0	✓				
9	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	9	1+	0	✓				
10	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	10	2+	0	✓				
11	rr	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	11	2+	0	✓				
AC																													AC	0	0	✓		

- Reactions only at IS, 37C incubation and AHG are negative
- Fits anti-M pattern perfectly, other colds are ruled out
- Anti-M identified

What is a Cold Autoantibody

- Antibody that targets against self antigens on RBCs
- Reacts best below body temperature
- DAT usually positive with C3, negative with IgG
- Auto-Anti-I very common
 - Allo-anti-I rare
- Panel:
 - All Panel cells equally positive at IS
 - Negative or weaker at 37C / AHG
 - AC most likely positive



Benign Cold Autoantibodies







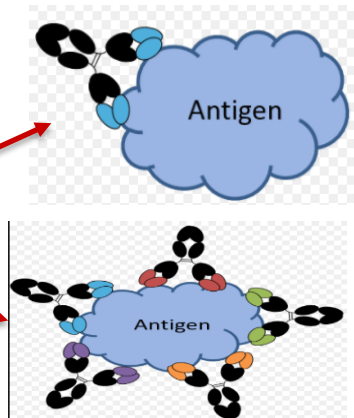
- Most CAs are not clinically significant
- Mask underlying clinical significant IgG antibodies, ABO discrepancies
- React at 4C, low thermal range
 - Pathologic Cold autos typically can bind to RBCs at 30-32C
- Low titer (<1:64)
- Usually polyclonal
 - Monoclonal associated with pathogenic cold auto-antibodies
- Common benign autoantibodies: anti-I, anti-IH

Cold Autoantibodies due to Disease State

- Primary:
 - Cold agglutinin disease (CAD)
- Secondary:
 - Infections
 - *Mycoplasma pneumoniae*, CMV, EBV
 - Lymphoma
 - IM, Waldenstroms
 - Drugs
 - Lenalidomide (Treats myelodysplastic syndrome, multiple myeloma)

Cold Agglutinin Disease (CAD)

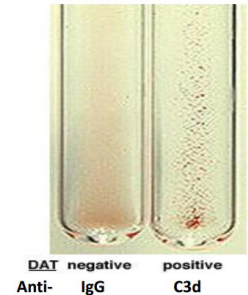
- Complement binding IgM antibody
 - IgM + RT/Cold exposure = Agglutination
- Avid complement fixation caused by the IgM
 - C3 is bound and fixed  causes Intravascular Hemolysis
 - High titer: >1000 @ 4C, reactive @ 30C 
 - RT / IS reactive
 - Pos DAT, C3 detected on RBC surface (not IgM)
 - Eluate nonreactive (only C3 coating red cells)
 - Most often anti-I
- Either Acute or Chronic
 - Antibody is monoclonal: Chronic Disease 
 - Antibody is polyclonal: Acute Disease 



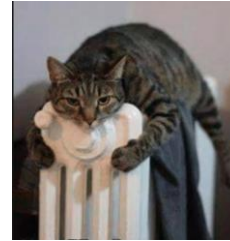
Cold Agglutinin Disease (CAD)



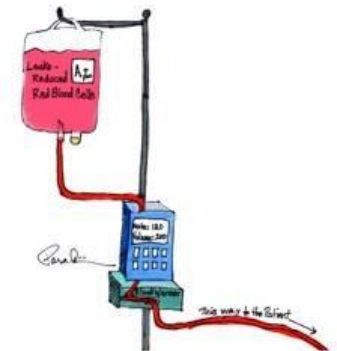
- Commonly seen in elderly patients
- RBCs agglutinate in blood vessels of extremities in cold conditions
- Common complaint is Acrocyanosis
 - Cold exposure = painful fingers and toes, purplish color
 - More symptomatic in colder months
- **Gold standard** Diagnostic Test: Positive DAT
 - C3 will be pos, IgG will be neg, C3 has been fixed by IgM
 - Others indicators: 1. LDH/Bili **↑** 2. Haptoglobin **↓** (Consumed) 3. Hgb/Hct **↓**
- Look for other diseases after Hemolytic Anemia diagnosis
 - Ex: Mycoplasma, Lymphoma



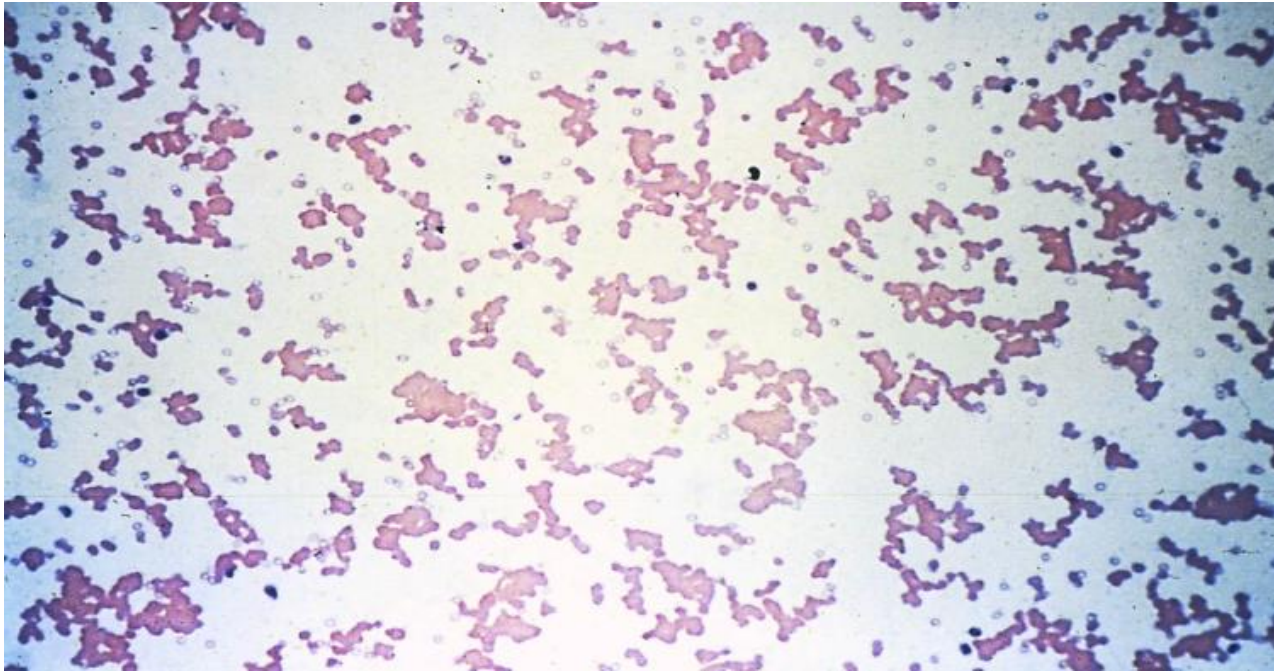
Cold Agglutinin Disease (CAD)



- Prevention
 - Avoid Cold Exposure
- Affects 15% of population with Autoimmune Hemolytic Anemia
- Treatment:
 - Blood transfusion via a blood warmer
 - Can have HTR symptoms without
 - Keep warm



Cold Agglutinin Disease (CAD)



Cold Autoantibody Panel



Cell#	Rh-hr	Donor Number	Rh-hr											KELL				DUFFY		KIDD		S ₁ LEWIS		MNS				P		LUTHERAN		Special Antigen Typing	Pos Test Results		
			D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Xg ^a	Le ^a	Le ^b	S	s	M	N	P ₁	Lu ^a	Lu ^b					
1	R1wR1	322893	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	+	0	+	+	HLA+	IS	2+
2	R1R1	315560	+	+	0	0	+	0	0	0	0	+	0	+	+	+	+	+	+	0	0	+	0	0	+	0	0	0	0	0	0	+	HLA+	2+	0 ⁺
3	R2R2	105652	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	0	+S	0	+	+	+	HLA+	2+	0 ⁺	
4	Ror	312351	+	0	0	+	+	+	0	+	0	+	0	+	+	+	+	+	+	0	0	0	0	+	+	0	+	0	+	+	+	HLA+	2+	0 ⁺	
5	r ^r	107646	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	+	0	+	+	+	@, HLA+	2+	0 ⁺	
6	r ^r	319867	0	0	+	+	+	+	0	0	+	+	0	+	+	+	+	+	+	0	0	+	0	+	+	+	+	0	+	+	+	@	2+	0 ⁺	
7	rr	308735	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+	+	+	+	+	+	+	+	+	@, HLA+	2+	0 ⁺	
8	rr	114424	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	0	+	0	+	0	0	0	0	0	0	0	0	@	2+	0 ⁺	
9	rr	322463	0	0	0	+	+	+	0	0	0	+	0	+	+	+	+	+	0	0	+	0	+	0	0	0	0	0	0	0	0	HLA+	2+	0 ⁺	
10	rr	322633	0	0	0	+	+	+	0	0	0	+	0	+	+	+	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0	HLA+	2+	0 ⁺	
11	*R1R1	311485	+	+	0	0	+	0	0	0	+	0	+	+	+	+	+	+	+	0	0	0	0	+	+	0	0	0	0	0	0	+	HLA+	2+	0 ⁺
	Patient Cells	AC																																2+	0 ⁺
	Mode of Reactivity		37°C/Antiglobulin								Antiglobulin								Variable				Cold		Var.										

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment. "*" represents "Not Tested" for new donors.

- All cells pos at IS, including AC
- Cells negative when warmed at 37C, AHG added
- Cold Autoantibody suspected in this case, further investigation required to confirm

Cold Autoantibodies due to Disease State

- May cause Autoimmune Hemolytic Anemia (AIHA), as a result of Cold Autoantibodies, caused by disease
- Secondary:
 - Infections
 - *Mycoplasma pneumonia* (Auto-anti-I), CMV, EBV
 - Lymphoma
 - IM (Auto-anti-i), Waldenstroms
 - Drugs
 - Lenalidomide (Treats myelodysplastic syndrome, multiple myeloma)
- Paroxysmal Cold Hemoglobinuria

Cold Autoantibodies due to Disease State

- Paroxysmal Cold Hemoglobinuria (PCH)
 - Exception to the Cold Autoantibody Rule
 - Auto-anti-P seen most frequent
 - DAT positive, C3 bound to RBCs (like in CAD)
 - “Biphasic” **IgG** that reacts against P antigens
 - IgG binds to RBC at cold temp, complement is attached,
 - When RBCs are warmed they lyse with complement
 - Antibody then dissociates, complement remains
 - Result is Intravascular hemolysis
 - Formerly associated with syphilis
 - Now associated with viral infections in children
 - Measles, mumps, influenza



Cold Autoantibody Workup Options



- Unmasking the Cold antibody
 - Prewarm
 - RESt adsorption
 - Cold Auto-Screen
 - Cold Autoadsorption or allogeneic adsorption
 - Cannot perform Autoadsorption if transfused within past 3 months
- Crossmatching
 - Prewarm technique, adsorptions ok
 - RESt used only for ABID, Not used for compatibility testing

Prewarming

- Purpose: to disable cold antibodies (allo and auto) in the potential presence of clinically significant antibodies
- Prewarming incubates the sample at 37C
- Pros:
 - Eliminates unwanted/insignificant reactions in workup
- Cons:
 - Can weaken significant antibodies or be missed completely
 - Ab Enhancement (PEG/LISS) does not ensure detection when prewarmed
 - May be inappropriately used
- Use only to confirm cold antibodies
 - Do not use solely to make reactions go away



Rabbit Erythrocyte Stroma “RESt”



- Cold autoantibodies mask significant alloantibodies
- Treated rabbit cells adsorb cold antibodies from pt plasma
- Goal is to remove interference by cold antibodies, to make rule-outs possible
 - Auto-anti-I most common
- Like Prewarm, can adsorb clinically significant antibodies as well
 - Anti-D, -E, -Vel and -M reportedly adsorbed by RESt
- Risk of performing technique must be considered
- Do not use for ABO testing, anti-B can be partially or completely adsorbed

RESt Panel

Supplier Lot #	Donor/ Vial#	RhHr										Kell				Duf		Kid		Lew		P		MN			Lut		X	Additional Antigens	Patient's Plasma Test Results			
		D	C	c	E	e	f	V	w	K	k	K	k	J	j	F	f	J	j	L	l	P	M	N	S	s	L	l			U	u	G	g
1 Immucor 41538	B6447 1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	0	+	+	0	0	+	+	Lu:14	0	0
2 Immucor 41538	C6345 2	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	0	0	0	
3 Immucor 41538	G1563 3	0	0	+	0	+	0	0	+	+	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	0		
4 Immucor 42547	B6997 1	+	+	0	0	+	0	0	+	+	+	+	0	+	+	+	+	0	+	0	w	0	+	+	0	0	+	0	0	0				
																															0	0		



- Cold antibodies adsorbed from plasma
- Cells negative at all phases, all antibodies ruled out

Cold-Auto Screen



- Performed if cold antibody is suspected without history
- Incubate patient plasma and red cells at 4C 15-30 min
- Positive result indicates cold antibodies activated onto all red cells
- Always use auto control
 - Will be positive in Cold Auto-antibody cases
- Used as a tool for workup, does not confirm Cold Antibody

Cold-Auto Screen



Cell #	Rh-hr	Donor	Rh-hr							Kell				Duffy		Kidd		Lewis		MNS				P		Lutheran		Additional Antigens				TEST RESULTS		Cell #											
			D	C	E	c	e	f	*V	C*	K	k	Kp ^a	Kp ^b	*Js ^a	*Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P1	Lu ^a	Lu ^b	Xg ^a	W ^a	Sa ^a	T ^a		4C										
1	R ₁ ^w R ₁	8303180714016	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	0	+	+	0					4C								1	
2	R ₂ R ₂	2768020697758	+	0	+	+	0	0	NT	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	0	0	+	+	0					3+								2	
3	rr	2768020941246	0	0	0	+	+	+	NT	0	+	+	0	+	0	+	+	+	0	+	+	0	+	0	0	+	0	+	+	+	0					3+								3	
4	Patient Cells - Auto Control																																				3+								

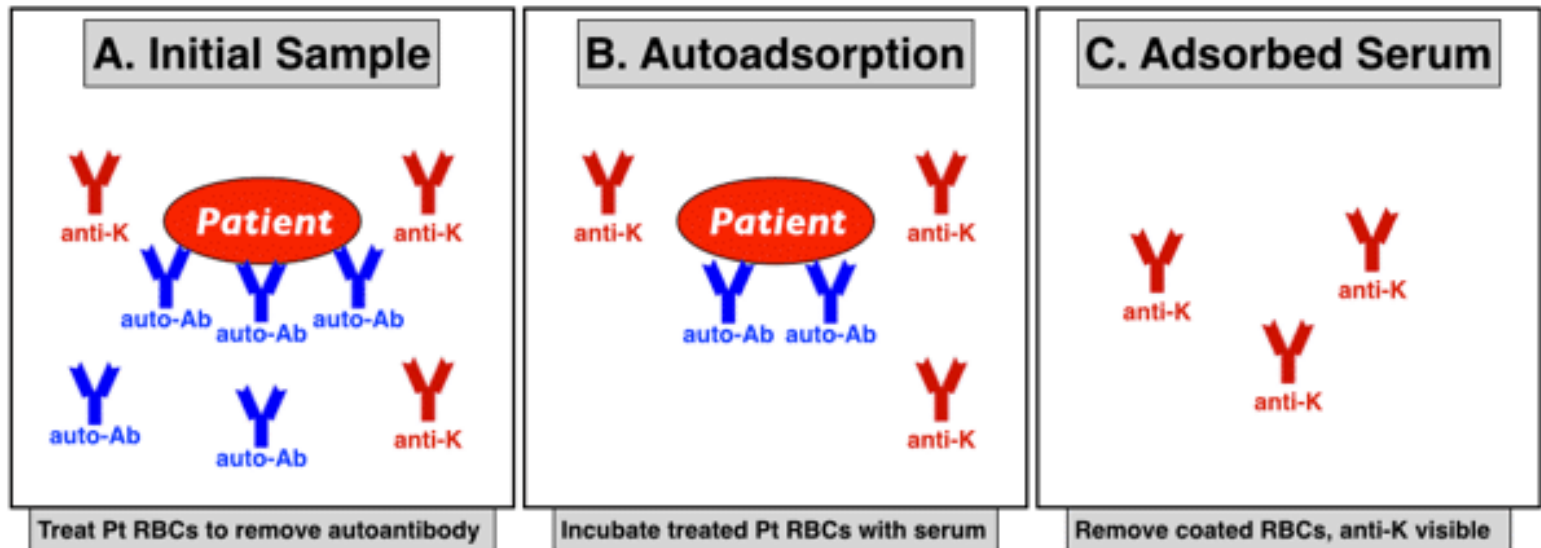
- Screen cell set incubated at 4C, creating cold environment “suspected” Autoantibody likes
- Autoantibody is activated, All cells including Auto Control are positive
- Test used as a tool in suspecting Cold Autoantibody cases, further investigation required to confirm

Cold-Autoadsorption



- “Gold Standard” for confirming Cold Autoantibodies
- Procedure binds Autoantibodies currently in plasma to patients own red cells
- Purpose is to have plasma to be free of Cold Autoantibodies and test plasma for what is left behind
 - Eliminates Autoantibody Interference
 - Minimal risk for clinically significant antibodies being missed
- Can take up to 6 adsorptions to fully remove Cold Autoantibody depending on strength & panel reactions
 - Each case is unique

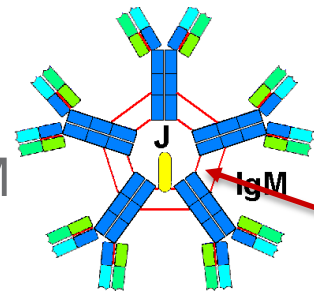
Cold-Autoadsorption



ABO RH Interference



- RBCs may be heavily coated with cold antibody, may react at RT
- Causes false positive reaction on front type (anti-A and anti-B)
- If suspected, Group O rbc's can test vs patient plasma
 - Both tubes likely to be positive if cold autoantibody is present
- Resolutions
 - Wash red cells with warm saline
 - Treat plasma with DTT (reduces disulfide bonds on IgM molecules, cannot agglutinate red cells)



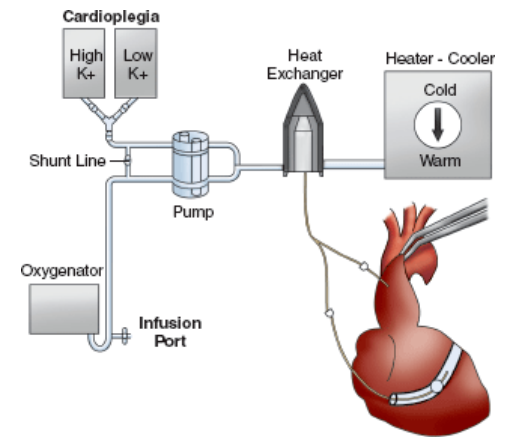
Crossmatching



- Prewarm technique, adsorptions ok
 - RESt used only for ABID, Not used for compatibility testing
- Neat and adsorbed plasma both tested
 - Testing plasma with and without Cold Autoantibody present
- Report Compatible/Incompatible Crossmatches according to Lab Director Discretion and Lab specific procedures

Cardioplegia

- Dextrose and potassium solution
- Inhibits myocardial muscle contraction during surgery (ex: CABG)
- May affect patients with cold antibodies if solution creates low enough environment for antibodies to react
- Depends on antibody strength, what temp they prefer to react at
 - Thermic range will vary (0C-30C)





Cold Case Study 1



- 73 year old woman
- Coronary artery bypass surgery
 - Cardioplegia to be used during surgery
- Anti-M identified, nonreactive at 37C prewarm
- Compatible AHG Crossmatch
- Compatible units issued
- No recommendation on management of cardioplegia

Cold Case Study 1



- Surgery begins, cold blood cardioplegia initiated
- RBC agglutination immediately observed in cardioplegia line
- Cardioplegia stopped, patient warmed, RBC agglutinates intercepted before reach patient, no clinical reaction noted
- Warm cardioplegia used, surgery complete at 7 hours, no further incident
- TR Workup:
 - Neg DAT, no hemolysis, no history of cold agglutinin disease
- Ref Lab Workup:
 - Titer of 512 at 4C with M-neg RBCs, non reactive at 30C
- Findings consistent with nonpathogenic Cold Antibody (CA)

Cold Case Study 1 Takeaways



- 512 titer at 4C explains the agglutination with the cold cardioplegia during surgery
- Warming the patient during surgery disables the cold antibodies ability to agglutinate red cells (same as with CAD)
- Necessary to inform Clinical team of cold antibody presence
- Patients with history of cold antibodies should avoid hypothermic conditions during surgery
- Cardioplegia line should always be monitored during surgery

Cold Case Study 2



- 73 year old man
- Admitted for CABG
- Antibody Screen Positive
- Pos panels at IS and 37C, Negative @ AHG with PEG & LISS
- Pos DAT (Poly & C3)
- Neg 2 hour settle @ 37C
- 2 XMs performed: compatible in PEG

Cold Case Study 2



- Cold Autoantibody ID'd
- Undergoing Plasmapheresis in Femoral Vein to mitigate the cold-auto
 - Warm albumin used
 - Via waterbath
- Plasmapheresis did not remove the cold-auto reactivity
- Communication to clinical team occurred
 - Blood warmer used
 - Heated room and patient
- No issues noted in OR during the cardiac surgery

Final Cold Case



- 20 year old female
- Right upper quadrant pain, throat irritation, 1 min loss of consciousness
- Febrile at 38.8C, jaundiced and right upper quadrant tenderness
- Initial Lab Results:
 - Increased: ESR, MCV (Macrocytic), Retics (35%), total bilirubin
 - Decreased: Hgb (9.7 g/dl), haptoglobin
 - Positive DAT, Monospot Test
 - Cold Autoantibody ID'd, EBV diagnosed in high titers
- Clinical team notes progressively anemic
- Patient admitted, treated, released after hematocrit stabilized


Final Cold Case Takeaways



- Infectious Mononucleosis diagnosed
 - Cold reactive autoantibodies formed secondary to disease
 - Patient developed Autoimmune Hemolytic Anemia due to Cold autoantibodies produced due to IM
 - Jaundice experienced due to the AIHA caused by Cold Autoantibodies
 - increased bilirubin and decreased haptoglobin
 - 0.5-3% of IM patients experience AIHA (due to Cold Autoantibodies)
 - Usually anti-I
-

Conclusion



- IgM in nature, wide thermal range
- Cold agglutinins are rarely clinically significant
- Can be result of disease state (CAD, PCH)
 - Pos Poly DAT, pos C3, IgG neg
- Cause Interference in ABO, Screens and Panels
- Workup Options
 - Prewarm, RESt, Cold Agglutinin Screen, Autoadsorption 
- Susceptible patients require special needs during procedures

Questions?





**American
Red Cross**