Cleveland Clinic

Autoimmune Hemolytic Anemias

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Objectives

- Define immune hemolytic anemias
- Discuss:
  - Benign cold autoantibodies
  - Pathological cold autoantibodies
  - Warm autoantibodies
- Describe how autoantibodies interfere with blood bank testing
- Discuss different types of Drug-induced Hemolytic Anemias

Immune Hemolytic Anemia

- Shortened RBC survival mediated through the immune response

Three Categories

- Alloimmune
- Autoimmune
- Drug-Induced

Hemolytic Anemias

Alloimmune

- Patient produces antibodies to foreign or non-self RBC antigens
- Antigens introduced through transfusion, transplant, or pregnancy

Drug-Induced (12%)

- Patient produces antibody to drug which damages RBCs

Hemolytic Anemias

Autoimmune Hemolytic Anemia (AIHA)

- Antibodies produced against their own RBC antigens
- Antibodies bind to their own RBCs and most donors
- Most react with high frequency antigens
- Warm autoantibody (70%)
- Cold autoantibody (18%)

Lab Results for Autoantibodies

- Typical lab results
  - Positive DAT
  - Positive Autocontrol
  - All panel cells reactive
- Does not confirm diagnosis of AIHA
- Can have positive DATs with no evidence of hemolytic anemia
  - 0.1% of normal donors
  - 15% of hospitalized patients

	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	MTS
1	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	1+
2	+	+	0	0	+	+	+	+	+	+	+	0	+	+	0	+	+	+	1+
3	+	0	+	+	0	0	+	0	0	0	+	0	+	+	0	+	0	+	1+
4	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	0	1+
5	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	+	0	+	1+
6	0	0	+	+	+	0	+	0	+	+	0	0	+	+	+	+	0	+	1+
7	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	1+
8	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	1+
9	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	+	+	1+
10	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	1+
11	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	1+
Autocontrol																			2+

Compensated vs. Uncompensated Anemia

<u>Compensated Anemia:</u>

- May have mild decrease in Hgb and Hct

Destruction
=
Production

<u>Uncompensated Anemia:</u>

- Macrocytosis (young cell population)
- Spherocytosis (cell membrane damage)
- Reticulocyte count >3%
- Increased unconjugated bilirubin and LDH

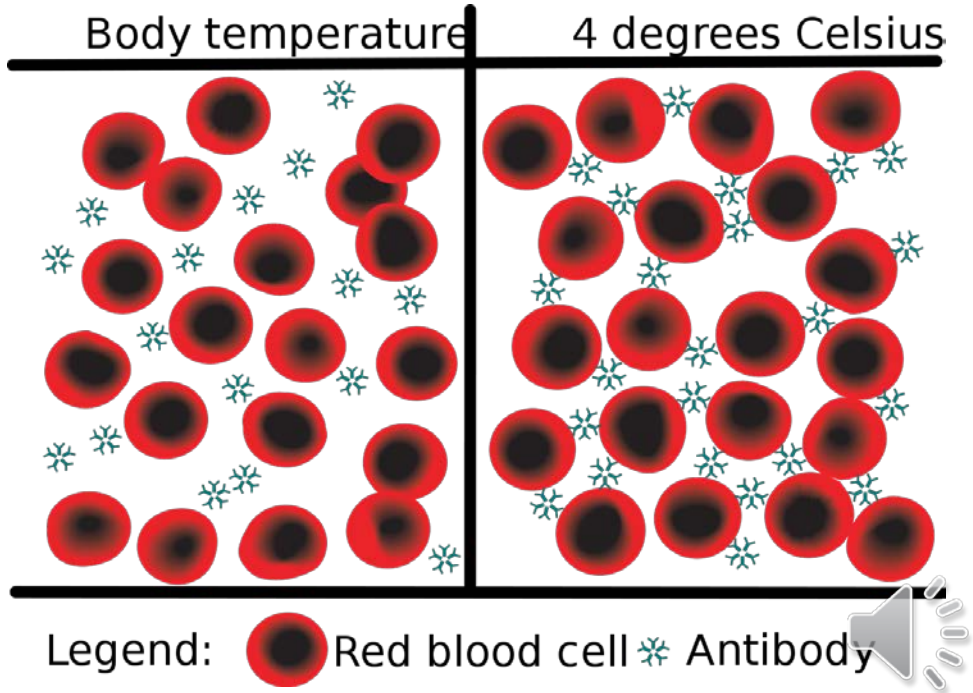
Destruction > Production

Types of Autoimmune Antibodies

Cold Autoantibodies	Warm Autoantibodies
Benign	Benign
Pathological • Cold hemagglutinin disease (CHD) • Secondary Cold AIHA • Paroxysmal cold hemaglobinuria	Pathological • Idiopathic • Secondary

Benign Cold Autoantibodies

- Most cold autoantibodies are benign
- Biggest problem: interference in testing
- Most individuals have autoantibodies reacting at 4°C
  - Not a problem
  - BB testing not usually done at these temps
- Autoantibodies reacting at 20-24°C:
  - More problematic
  - Begin interfering with BB testing



Cold Autoantibody Specificity

- Anti-I
  - Most common (even though adult cells are I positive)
  - Test against cord cells to help diagnose
    - Anti-I reacts with adult cells and not cord cells
  - Usually benign
- Anti-H, Anti-IH
  - Reacts like anti-I
  - Found mainly in A1 and A1B individuals (least amount of H)
  - React strongest with O cells (most H antigen) and weakest with A1B

Typical Reactivity of Cold Autoantibodies

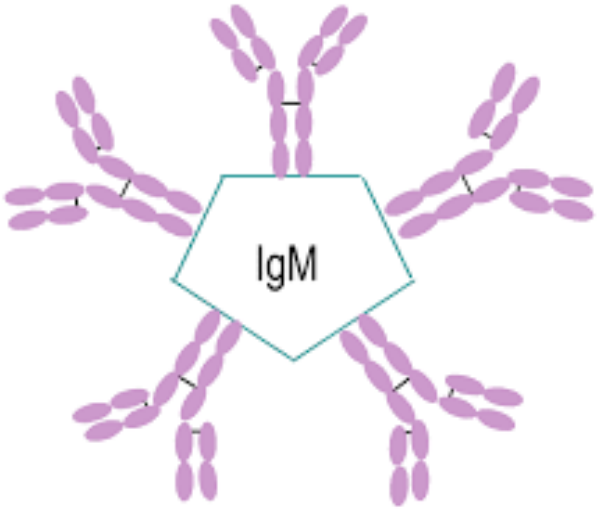
Test Phase	Group O Adult Red Cells
Saline 4°C	3+ - 4+
Saline 15°C-18°C	1+ - 2+
Saline RT (20°C-24°C)	0 – 1+
Saline 37°C	0
IgG Antiglobulin	0

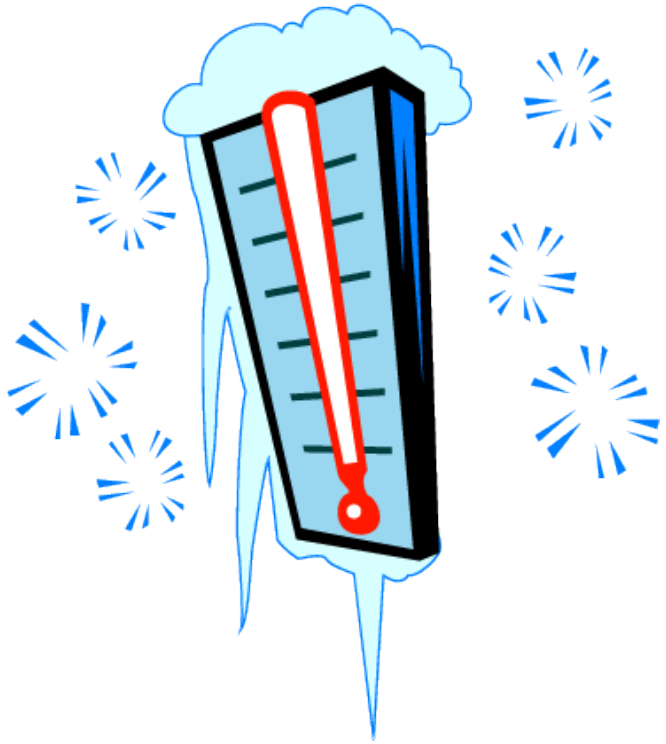
Pathological Cold Agglutinins

- Cold Hemagglutinin Disease (CHD) or Cold Agglutinin Syndrome (CAS)
  - Idiopathic
  - Secondary Cold AIHA
- Paroxysmal Cold Hemoglobinuria (PCH)

Cold Hemagglutinin Disease (CHD)

- 18% of cases of AIHA
- Cold autoantibody reacting between 4°C-30°C
- Usually IgM activating complement
- Causes moderate chronic hemolytic anemia



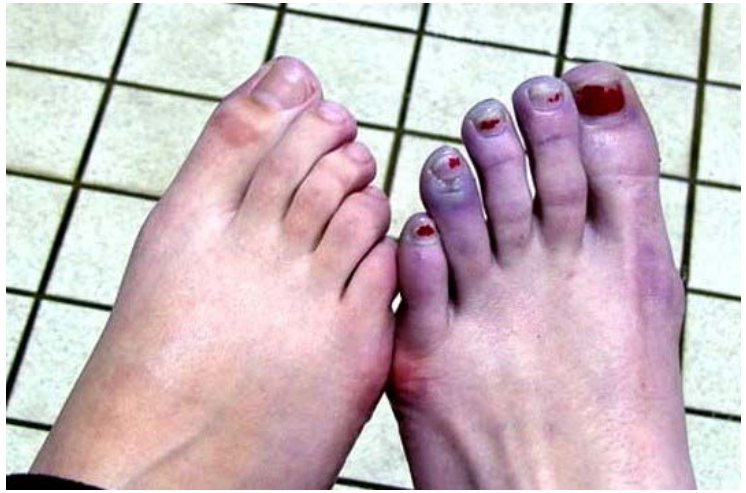


CHD Clinical Picture

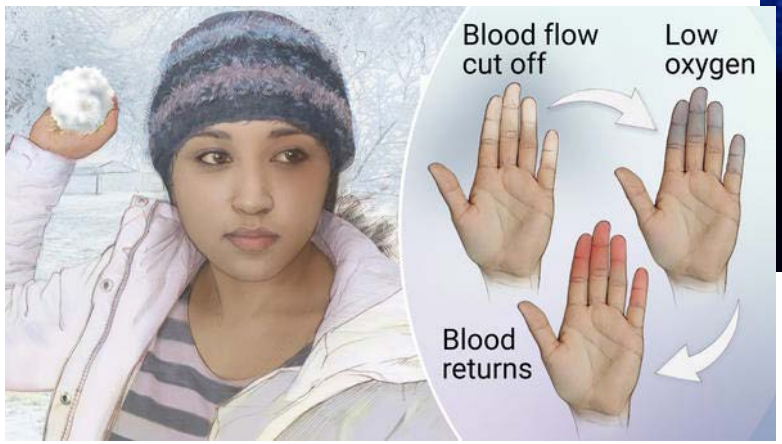
- Predominantly individuals > 50 years of age
- Usually anti-I specificity
- Lower the temp, the more antibody activity
- Extremities can be 28°C in the winter
- Seasonal- winter months precipitate signs and symptoms
- Live more comfortably in warmer climates

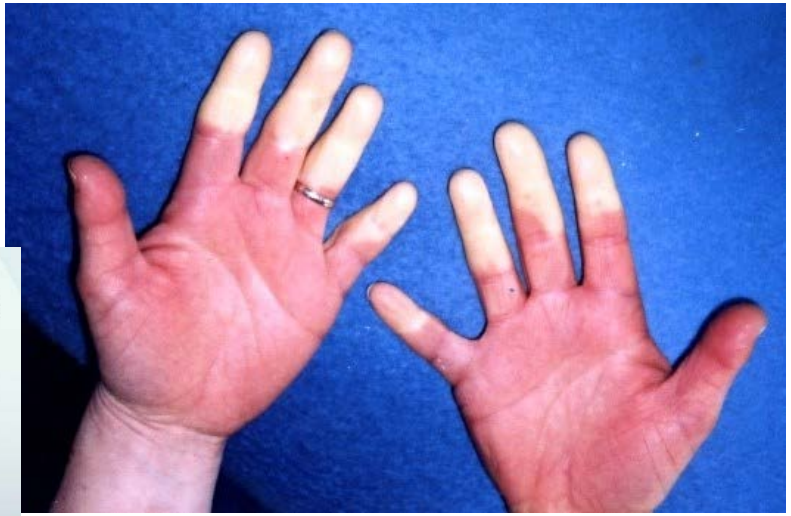
CHD Clinical Picture

- Acrocyanosis of hands, feet, ears, nose
- Numbness of extremities
- Possible hemoglobinuria
- Weakness
- Pallor
- Weight loss
- Jaundice
- Raynaud's disease



Acrocyanosis- vasospasm/constriction of small vessels due to cold



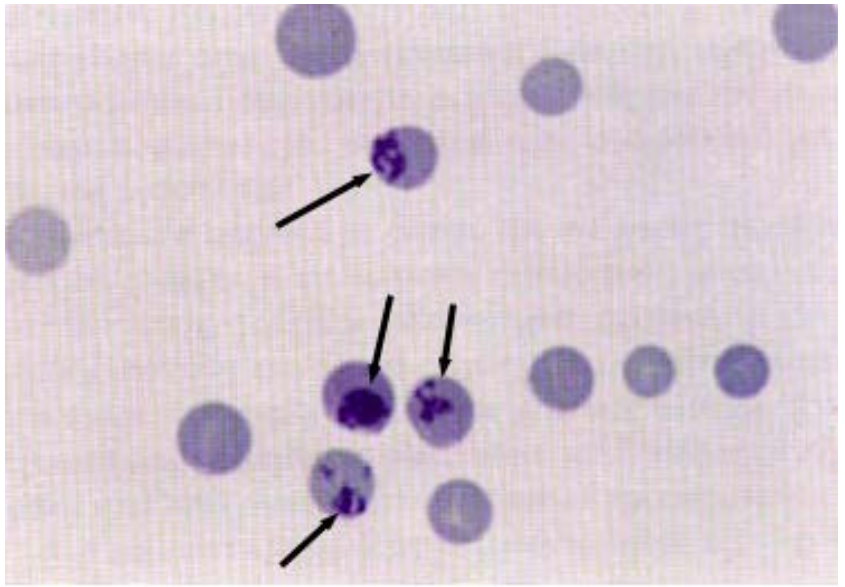


Raynaud's Disease- small arteries in fingers/toes narrow from cold limiting blood supply

CHD Lab Findings

- Reticulocytosis
- Positive DAT due to **complement only**
- Autoagglutination of anticoagulated whole blood samples

DAT Test	Result
IgG	0
C3b, C3d	4+



Reticulocytes
(New Methylene Blue)

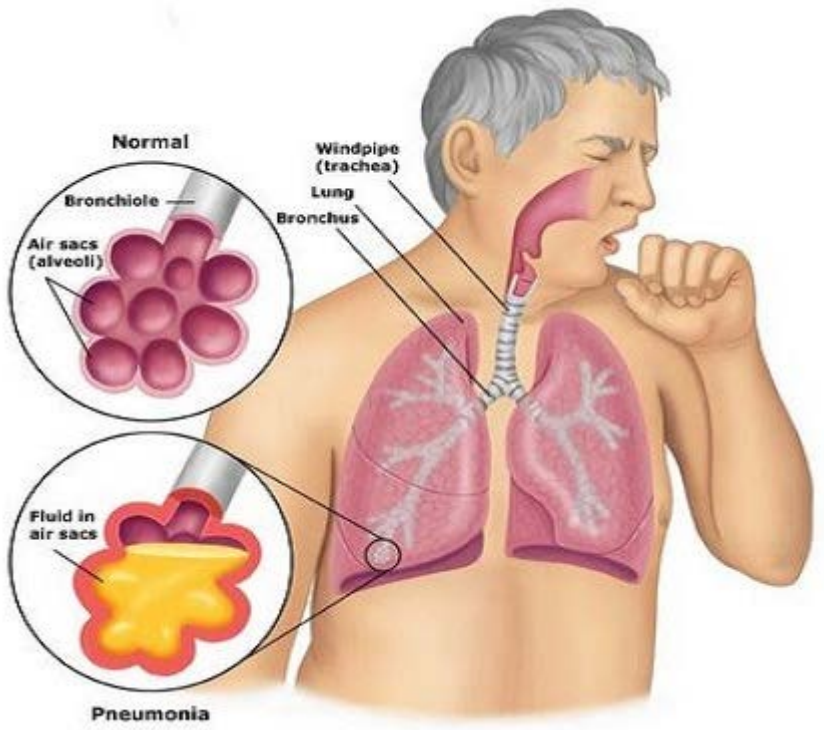
Reticulocystosis: increase in immature red cells

CHD Treatment

- Generally unnecessary
- Avoid cold and keep warm
- Move to warmer climates
- Can be issue for transfusion in surgeries requiring hypothermia
  - Blood warmers used

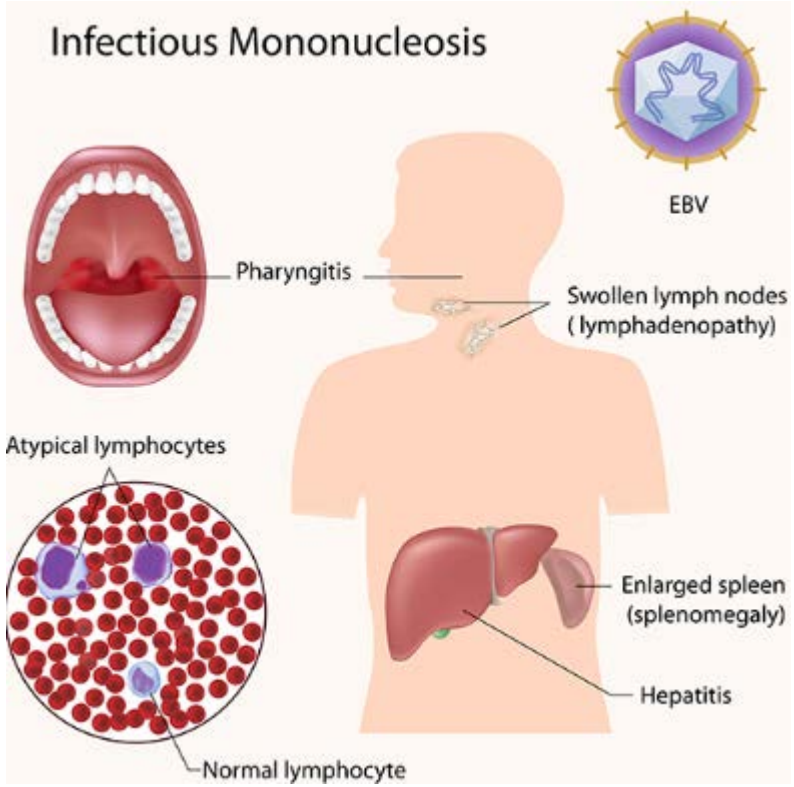
Secondary Cold AIHA

- CHD secondary to an infection
- Only lasts during infection
- *M. pneumonia*
  - 50% with infection have cold agglutinins
  - **Anti-I** specificity
  - Possibly antibody to mycoplasma is cross-reacting with RBCs
  - Symptoms: pallor, jaundice, hemolysis



Secondary Cold AIHA

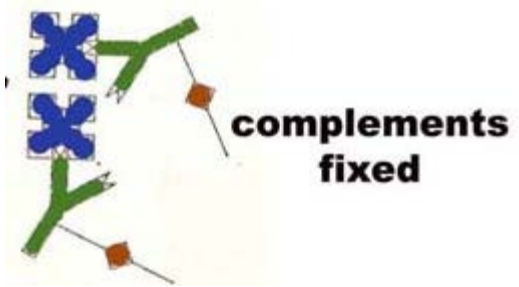
- Infectious mononucleosis
  - High titered IgM **anti-i**
  - Acute illness of sore throat and fever followed by weakness, anemia, and jaundice
  - Only a small number produce enough antibody to cause in vivo hemolysis



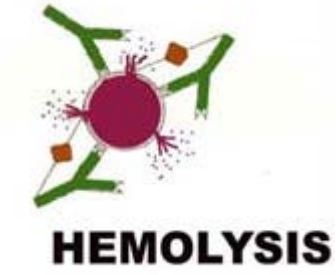
Paroxysmal Cold Hemoglobinuria (PCH)

- Least common AIHA (1-2% of AIHA)
- Usually in children with viral illnesses (measles, mumps, chickenpox, mono, flu)
- Biphasic autohemolysin
  - IgG antibody
  - Binds patient's RBCs at low temps and fixes complement
  - Hemolysis occurs when cells reach 37°C
  - **Anti-P specificity**

At low temps (extremities):



At 37°C (core body temp):



PCH Clinical Picture

- Episodes occur on exposure to cold
- Sudden onset fever, shaking/chills, abdominal cramps, back pain
- Hemoglobinemia, hemoglobinuria, bilirubinemia
- Severe and rapidly progressing anemia
- Can resolve in a few hours or persist for days

Normal Urine

Hemoglobinuria

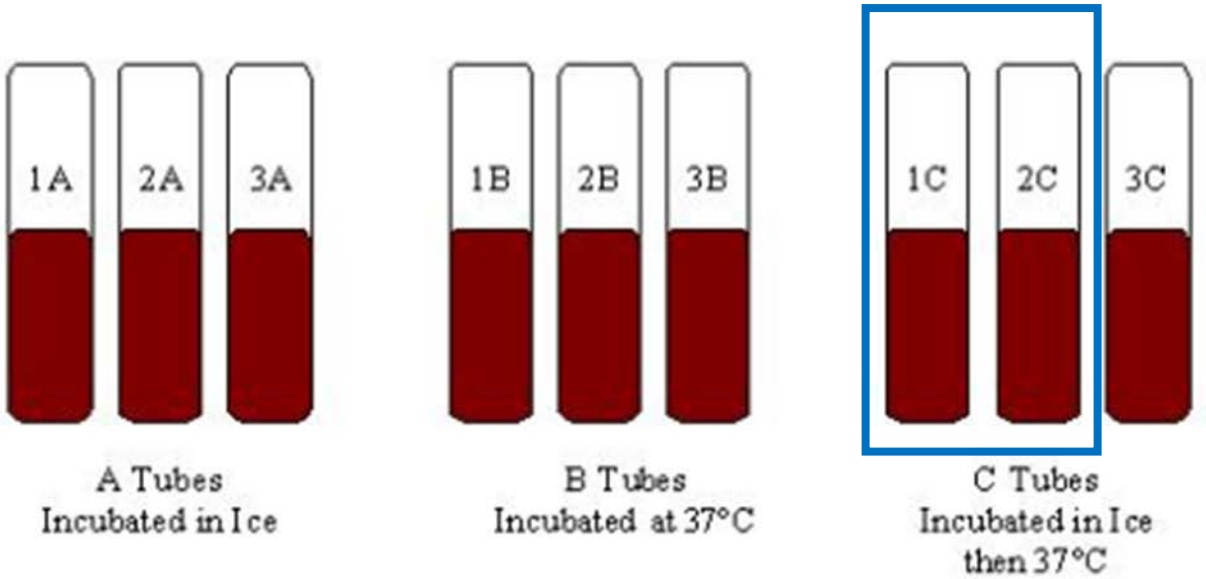
PCH Treatment

- Protect from cold exposure
- Usually resolves when illness is over
- Steroids/transfusion may be needed based on severity

PCH Testing

- DAT positive only with Complement
- Donath-Landsteiner Test
  - Test for bisphasic hemolysin

Donath-Landsteiner Test:



Tubes 1: Patient Serum
Tubes 2: Patient Serum + Normal Serum
Tubes 3: Normal Serum
All tubes contain P positive reagent red cells.

If biphasic hemolysin is present, Tubes 1C and/or 2C will be hemolyzed. All other tubes will not be hemolyzed.

PCH Testing

- Expected reactions for a positive Donath-Landsteiner Test:

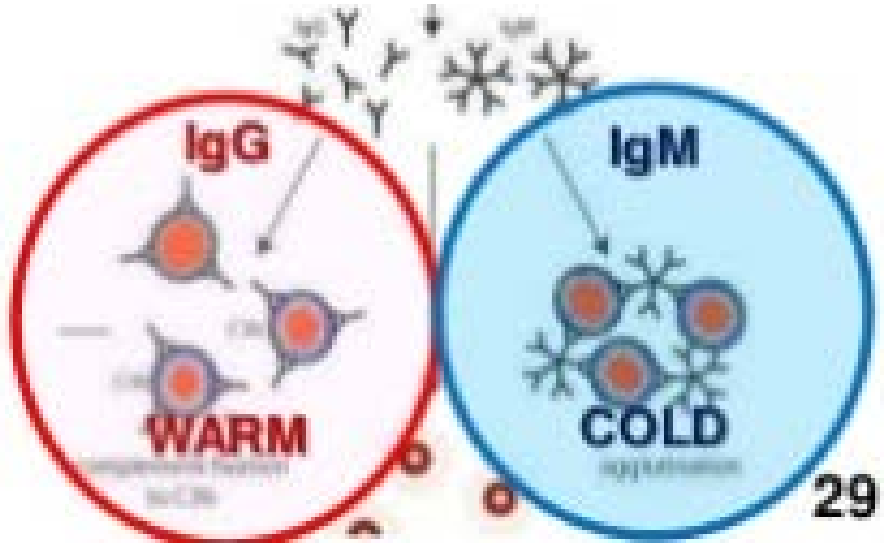
Incubation Phases	Tube 1 (patient serum)	Tube 2 (patient serum + normal serum)	Tube 3 (normal serum)
Ice bath followed by 37°C	+	+	0
Ice bath only	0	0	0
37° only	0	0	0

Comparing CHD and PCH

Factors	CHD	PCH
Patient population	**Elderly**/middle aged adults	**Children**/young adults
Pathogenesis	Idiopathic, lymphoproliferative disorder; following *M. pneumonia* infection	Following viral infection
Clinical features	Acrocyanosis; autoagglutination of blood at room temperature	Hemoglobinuria, acute attacks upon exposure to cold
Severity of hemolysis	**Chronic** and rarely severe	**Acute** and rapid
Site of hemolysis	Extravascular/intravascular	Intravascular
Autoantibody class	**IgM (anti-I/i)** monophasic	**IgG (anti-P** specificity; biphasic hemolysin)
DAT	3-4+ monospecific **C3 only**	3-4+ monospecific **C3 only**
Thermal Range	High (up to 30-31°C)	Moderate (<20°C)
Titer	High (>1,000)	Moderate (<64)
Donath-Landsteiner result	**Negative**	**Positive**
Treatment	Avoid cold	Supportive (disorder terminates when underlying disease resolves)

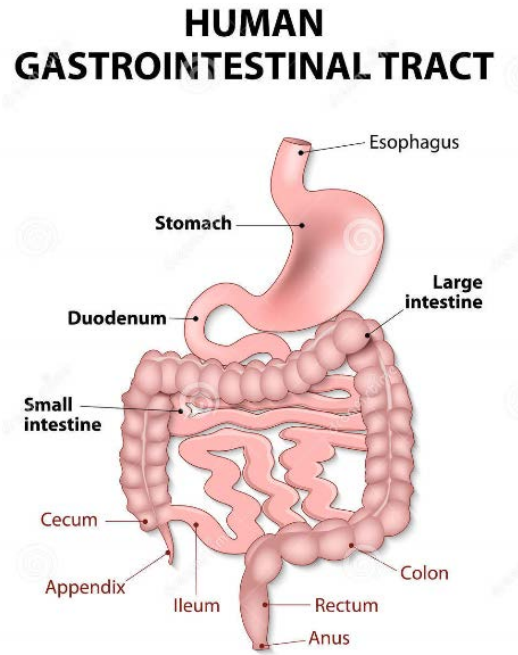
Warm Autoimmune Hemolytic Anemia (WAIHA)

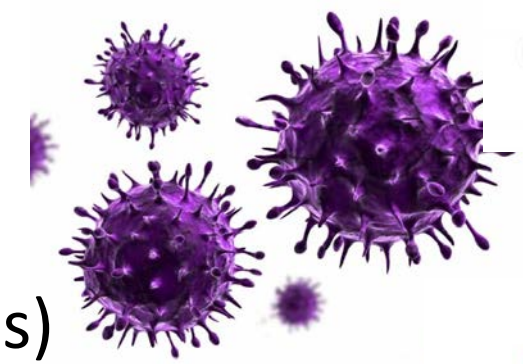
- 70% of autoantibodies
- React at 37°C
- Mostly IgG antibodies
- Some harmless – cannot distinguish serologically
- Most react with high incidence antigens
  - Can have antibody specificity
  - Very common with Rh's (especially anti-e)

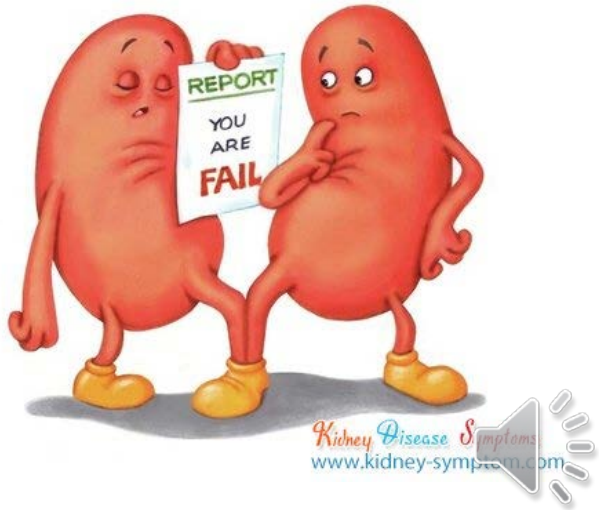


Diseases associated with WAIHA

- Sometimes sudden and unexplained
- Lymphomas/leukemias
- Collagen disease (lupus and rheumatoid arthritis)
- Infectious disease (viral)
- Immunologic disease
- Gastrointestinal disease (ulcerative colitis)
- Carcinoma
- Pregnancy
- Chronic renal failure
- Can be triggered by trauma or surgery

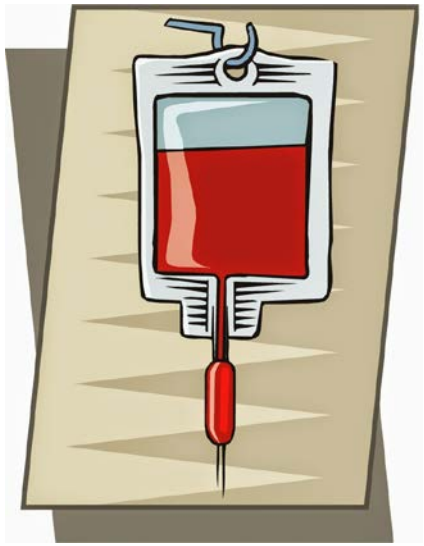


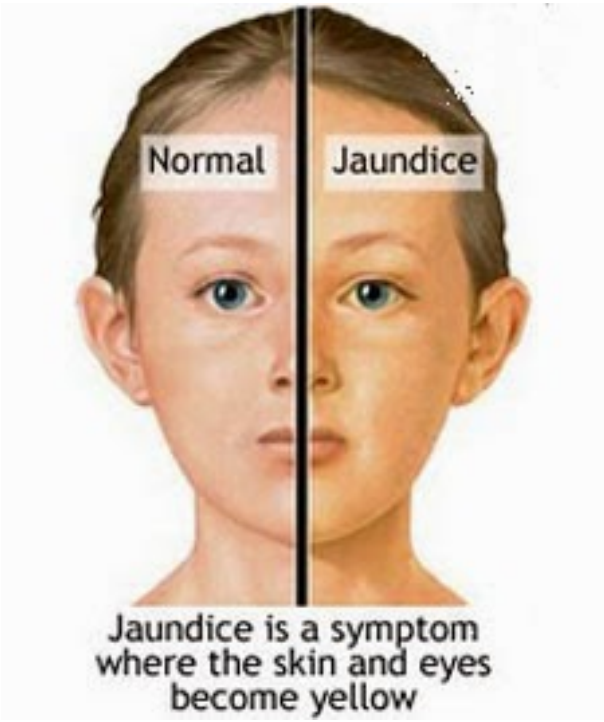




WAIHA Symptoms with Anemia

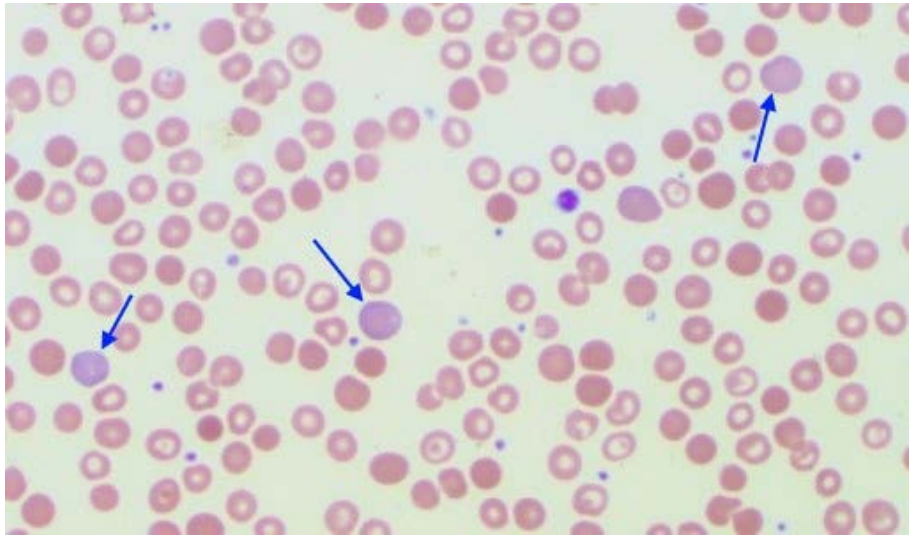
- Pallor
- Weakness
- Dizziness
- Dyspnea
- Jaundice
- Unexplained fever
- Significant number suffer from anemia requiring transfusion



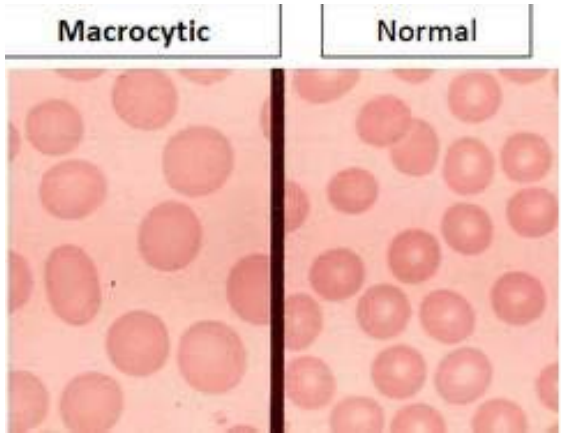


WAIHA Lab Findings

- Positive DAT
- Blood smear: polychromasia, macrocytosis, reticulocytosis
- Reticulocytopenia (lysing precursor cells)- associated with high mortality rate
- Increased bilirubin, urobilinogen
- Hemoglobinemia, hemoglobinuria
- Increased LDH



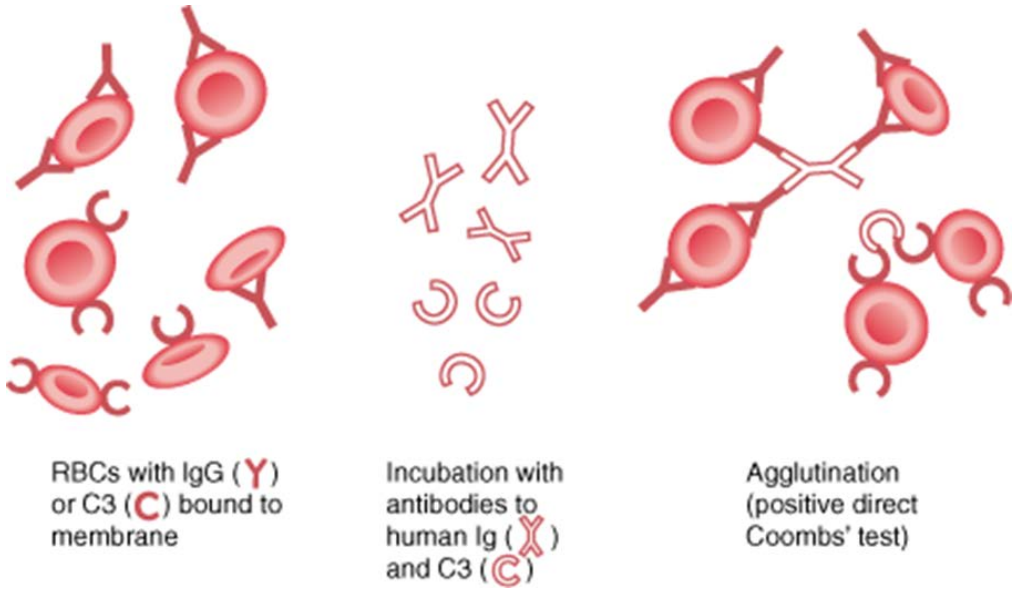
Polychromasia- high # of immature red cells



Macrocytosis- enlargement of RBCs

RBC Hemolysis in WAIHA

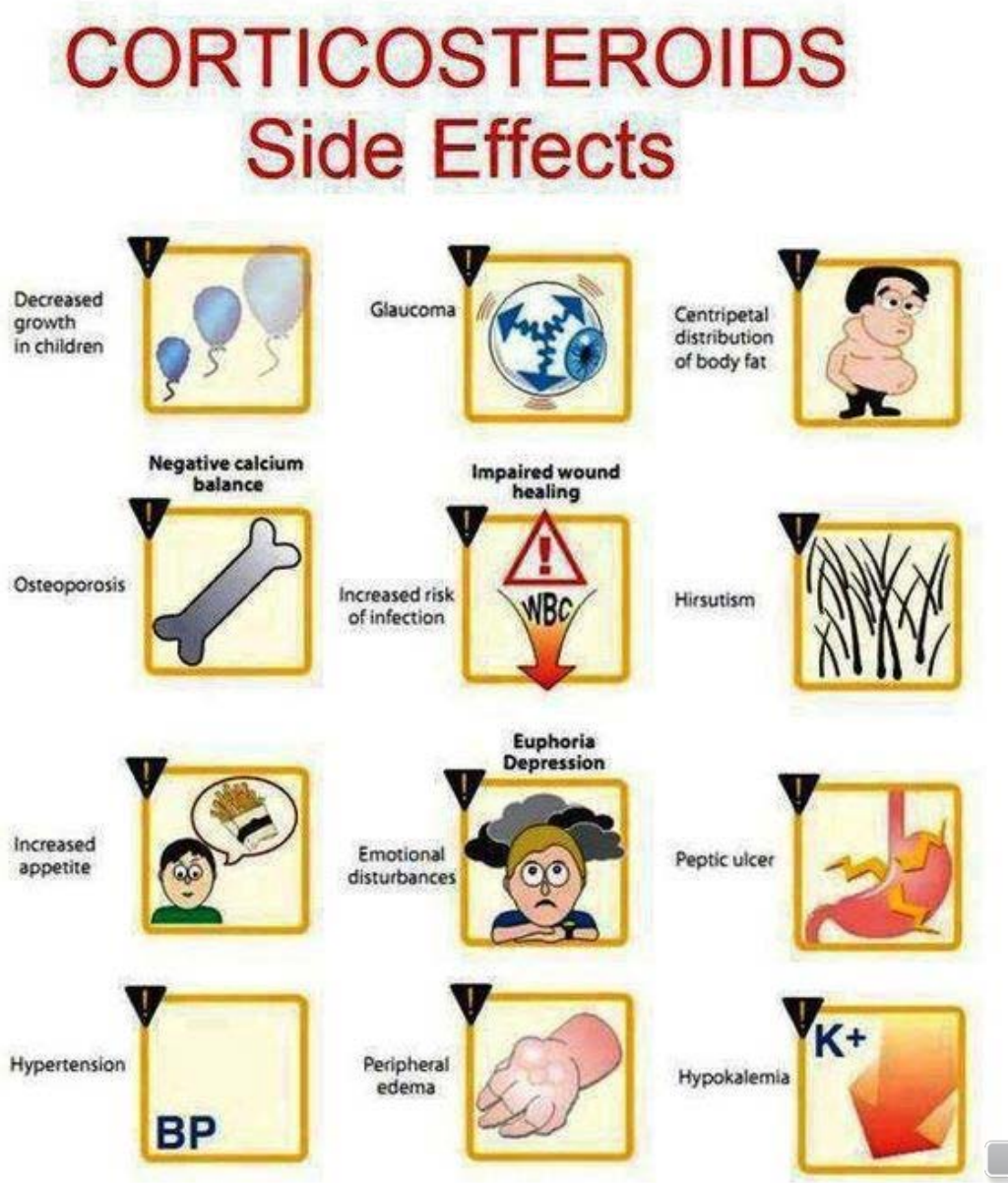
- Majority are IgG antibodies (<2% IgA or IgM)
  - 87% IgG1
  - IgG1 and IgG3 most common
  - IgG3 most destructive- best at binding complement
- Strength of DAT:
  - Correlates with presence of multiple IgG subclasses
  - Multiple IgG subclasses correlates with severity of hemolysis
- RBC destruction mostly extravascular (destroyed by the spleen)



DAT Positive Results in WAIHA	
IgG and C3	67%
IgG only	20%
C3 only	13%

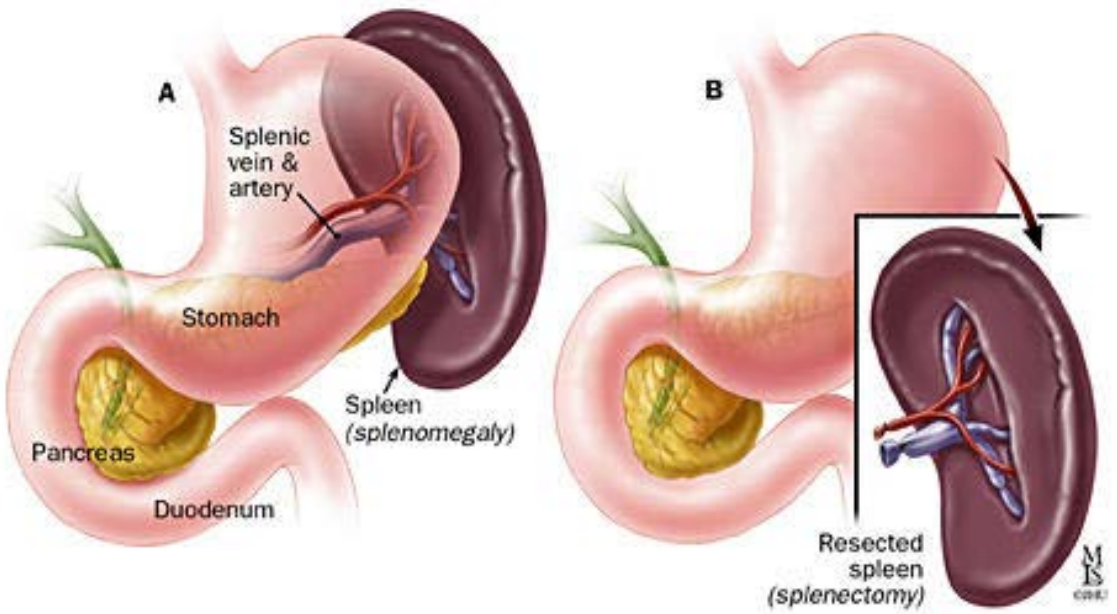
Treatment of WAIHA

- Avoid transfusion unless life threatening anemia- transfusion only accelerates hemolysis
- Corticosteroids/IVIG
  - Reduce antibody synthesis
  - Alter antibody activity
  - Alter macrophage receptors for IgG and C3 (reduce clearance of RBCs)
  - Benefits 50-65% of WAIHA cases



Treatment of WAIHA

- Splenectomy:
  - Decreases production of antibody
  - Removes potent site of RBC damage and destruction
  - Splenectomy used if:
    - Failure of steroid therapy
    - Need for continuous high-dose steroid maintenance
    - Complications of steroid therapy



- Immunosuppressive Drugs
  - Last approach
  - Interferes with antibody synthesis by destroying dividing cells

Mixed Type AIHA

- Combination of cold and warm AIHA- rare
- Positive with both IgG and C3
- Acute hemolysis- usually requires transfusion
- Adsorption performed to remove- first at 4°C then at 37°C

Comparing Warm and Cold AIHA

Factors	Warm AIHA	Cold AIHA
Optimal reaction temp	>32°C	<30°C
Immunoglobulin classification	IgG	IgM
Complement activation	May bind complement	Binds complement
Site of hemolysis	Usually extravascular (no cell lysis)	Extra/intravascular (cell lysis)
Frequency	70-75% of cases	16% of cases
Specificity	Frequently broad Rh specificity	Ii system (PCH autoanti-P)

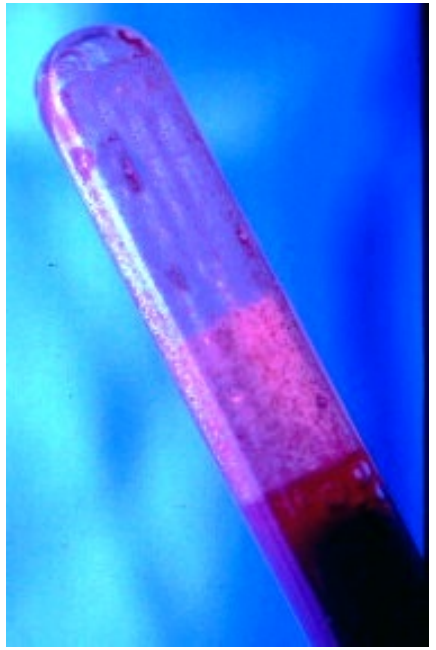
Autoantibody Interference in Testing

Test	Cold Autoantibodies	Warm Autoantibodies
ABORh	Yes	No
Antibody Screen/ID	Yes	Yes
Crossmatch	Yes (usually at immediate spin)	Yes (usually at AHG)

Autoantibody causes panreactivity

- Reacts with every cell performed
- Generally reacts stronger with the autocontrol

Interference with ABORh Typing



- Usually only cold autoantibodies
- Front type:
  - RBCs heavily coated with cold agglutinins
  - Agglutinate with anti-A, B, D and control regardless of type
  - Solution:
    - Wash patient RBCs with warm saline
    - Helpful to keep sample warm after drawing

A pos patient	Anti-A	Anti-B	Anti-D	Control
Serum-suspended RBCs	4+	1+	4+	1+
Warm-washed RBCs	4+	0	4+	0

Interference with ABORh Typing

- Usually only cold autoantibodies
- Reverse Type
  - Cold agglutinins in serum react at 20-24°C
  - Agglutinate all reagent cells- including A1 and B cells
  - Solution:
    - Prewarmed Testing
      - Warm serum and reagent cells separately
      - Incubate together at 37°C, then read the tubes
    - Autoadsorption- used if very strong autoantibody

A pos patient	A1 Cells	B cells
Serum	1+	4+
Prewarmed	0	1+

Interference with Antibody Detection and Identification

- Cold and warm autoantibodies
- Can hide real alloantibody reactivity
- Three ways to reduce cold autoantibody interference:
  - Prewarming technique
  - Cold autoadsorption
  - Cold alloadsorption
- Two ways to reduce warm autoantibody interference
  - Warm autoadsorption
  - Warm alloadsorption (triple adsorption)

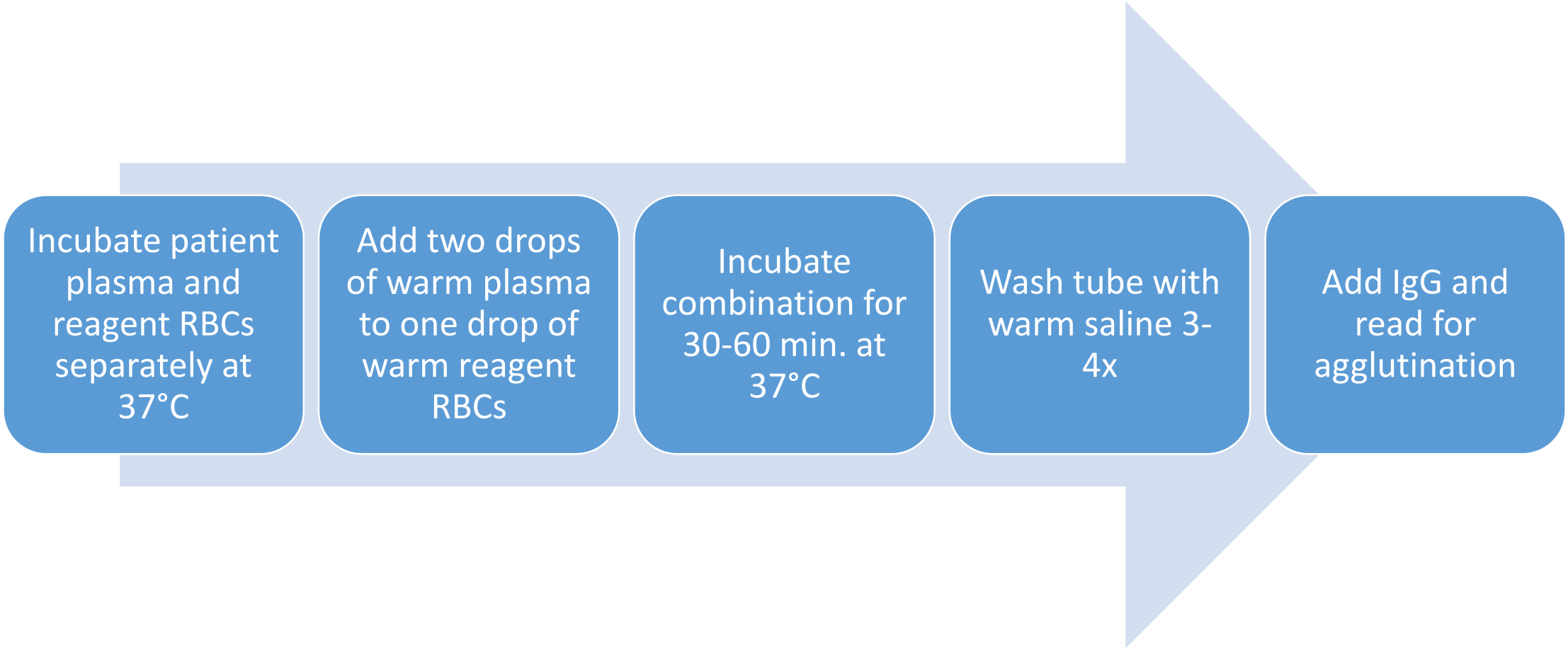
Before adsorption:

1 2 3

After adsorption:

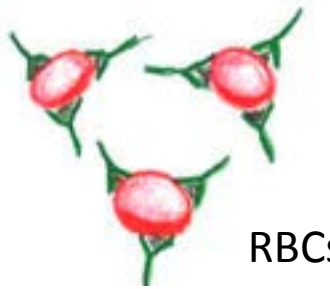
1 2 3

Prewarming


Incubate patient plasma and reagent RBCs separately at 37°C
Add two drops of warm plasma to one drop of warm reagent RBCs
Incubate combination for 30-60 min. at 37°C
Wash tube with warm saline 3-4x
Add IgG and read for agglutination

Cold and Warm Autoadsorption

Plasma



RBCs

Treat RBCs to remove bound autoantibody

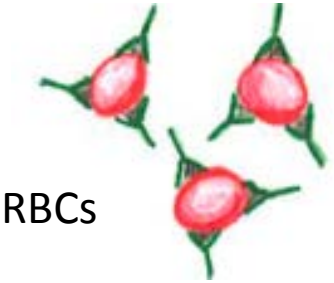
Mix patient plasma with treated patient RBCs and incubate at warm or cold temp

Centrifuge to separate plasma and RBCs

Autoantibodies are adsorbed onto RBCs and alloantibodies remain in plasma

Repeat until all autoantibody removed and then test plasma

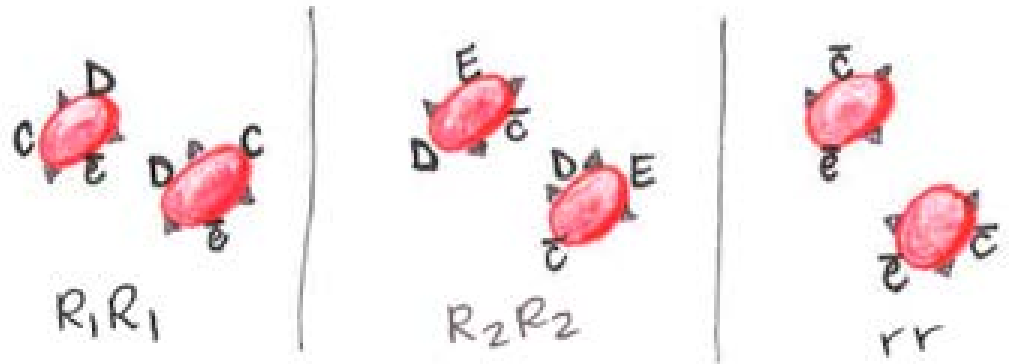
Plasma



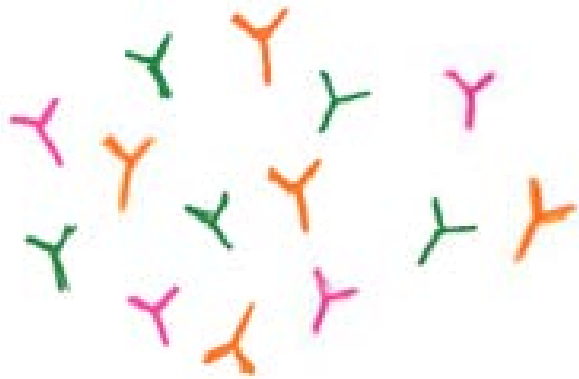
RBCs

Warm Alloadsorption (Triple Adsorption)

- Begin with three RBC reagents with known antigens in three separate tubes

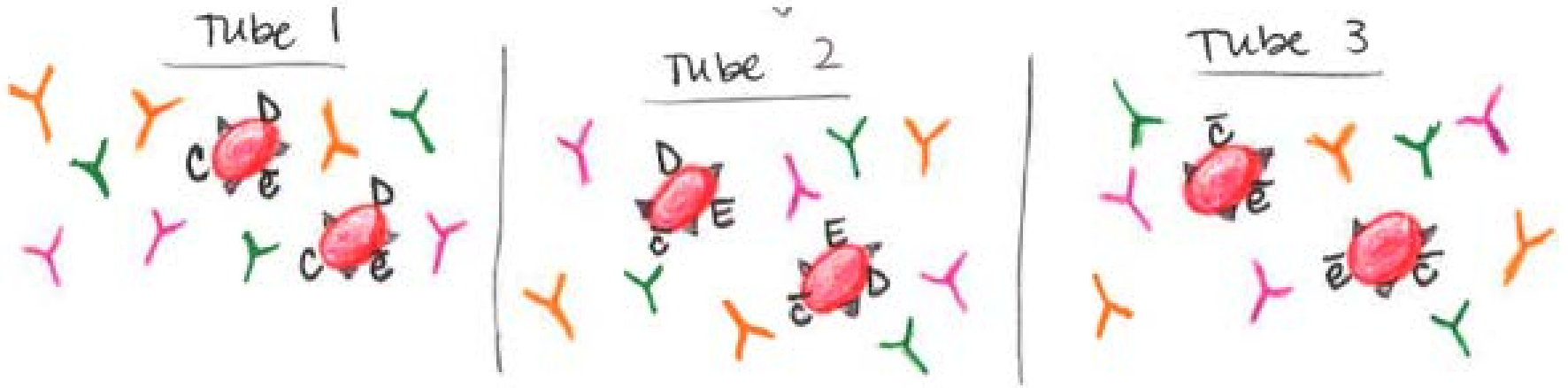


- Patient plasma may contain only autoantibody or autoantibody + alloantibodies
- In this case: green= autoantibody, pink= anti-E, orange= anti-c



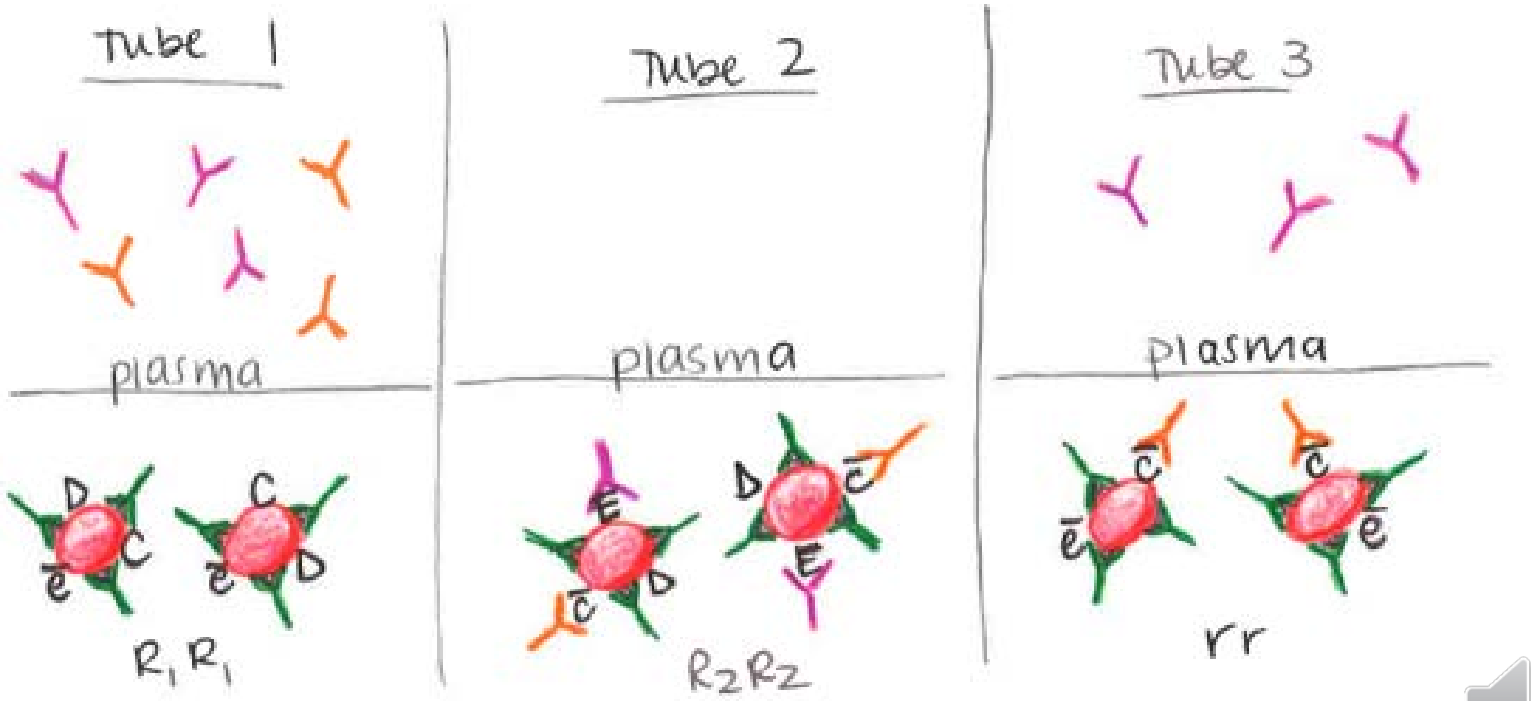
Warm Alloadsorption (Triple Adsorption)

- Add patient plasma to each of the three tubes and incubate at 37°C



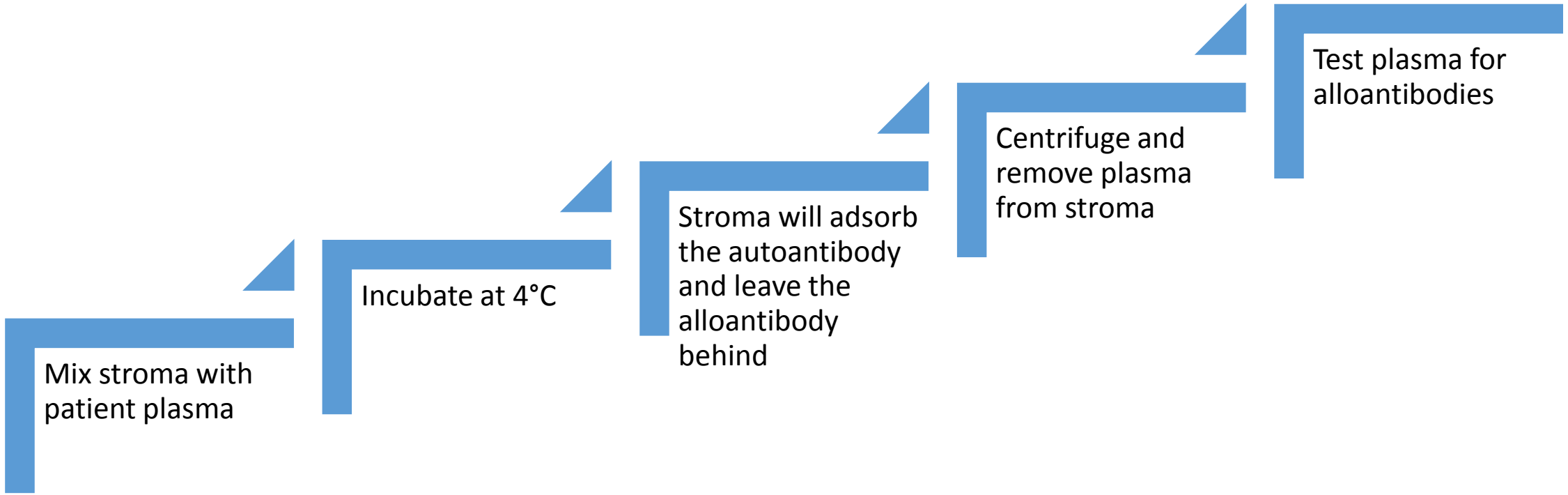
Warm Alloadsorption (Triple Adsorption)

- Centrifuge to separate plasma from reagent RBCs
- Repeat adsorption process 2-3x until all autoantibody is removed
- Test each tube of adsorbed plasma against a reagent screen to identify all alloantibodies



Cold Alloadsorption

- Use rabbit erythrocyte stroma instead of reagent red cells
- Stroma is rich in anti-I which is the most common cold autoantibody



Interference with Compatibility Testing

- Cold Autoantibodies:
  - Interfere at immediate spin readings
  - Once attachment of the cold auto occurs, it can react through IgG readings
  - Solution:
    - Perform prewarmed crossmatch
    - No immediate spin or 37°C reading

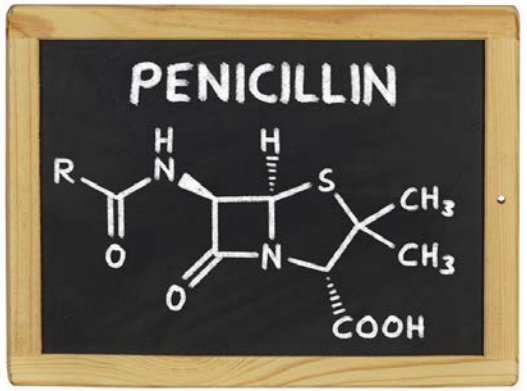
Interference with Compatibility Testing

- Warm Autoantibodies
  - Interfere at AHG readings and sometimes at 37 with LISS
  - Solution:
    - Give the least incompatible unit
    - Most times impossible to give blood negative for the autoantibody
    - Cells will likely be destroyed as rapidly as their own cells
    - If there is a specificity (anti-e) do not need to give negative for that antigen

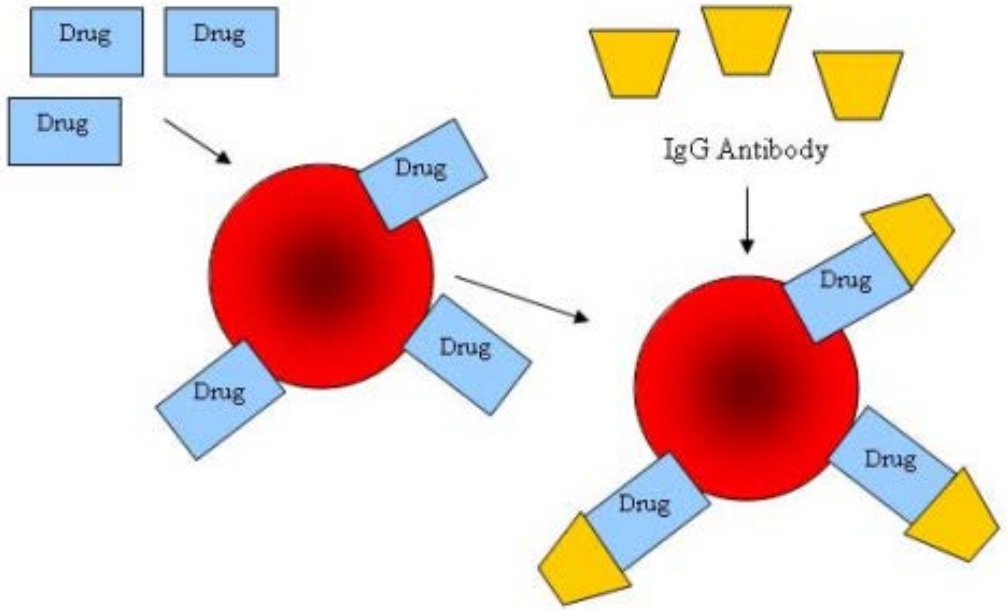
Drug-Induced Immune Hemolytic Anemia

- Less common
- Four mechanisms for drug-induced problems:
  - Drug Adsorption Mechanism
  - Drug-Dependent of Immune Complex Mechanism
  - Membrane Modification
  - Autoantibody Formation

Drug Adsorption (Hapten) Mechanism

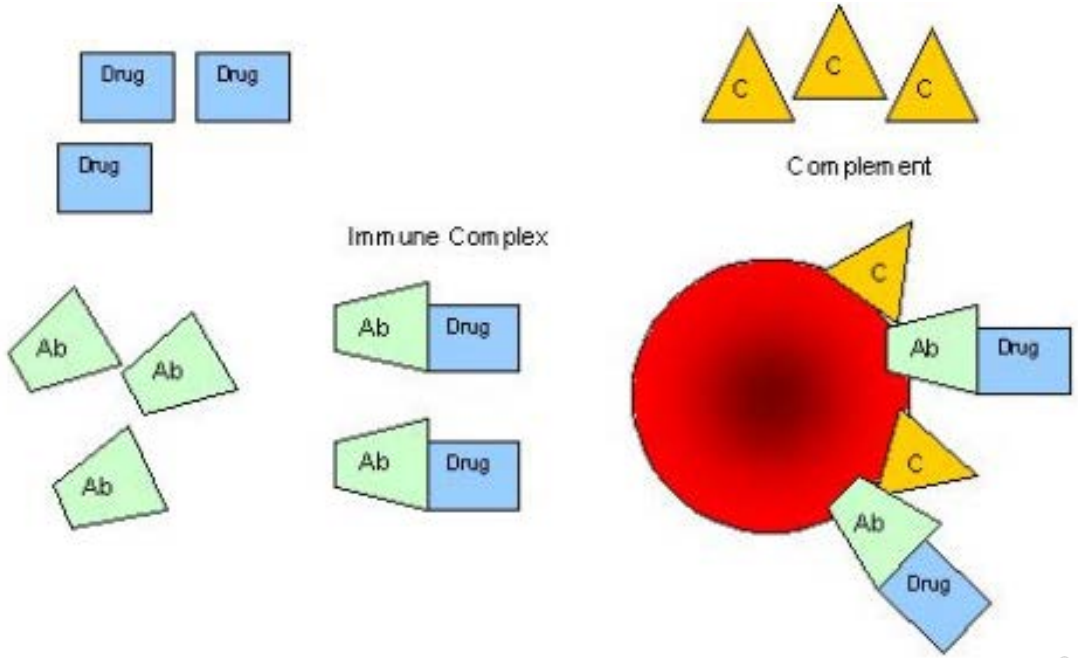


- Drug binds to proteins of RBC membrane
- Antibody is formed to the drug
- Ex. Penicillin, cephalosporins
- Need massive doses of drugs
- Usually IgG only
- Extravascular destruction- slowly developing anemia
- Patient improves when the drug is stopped



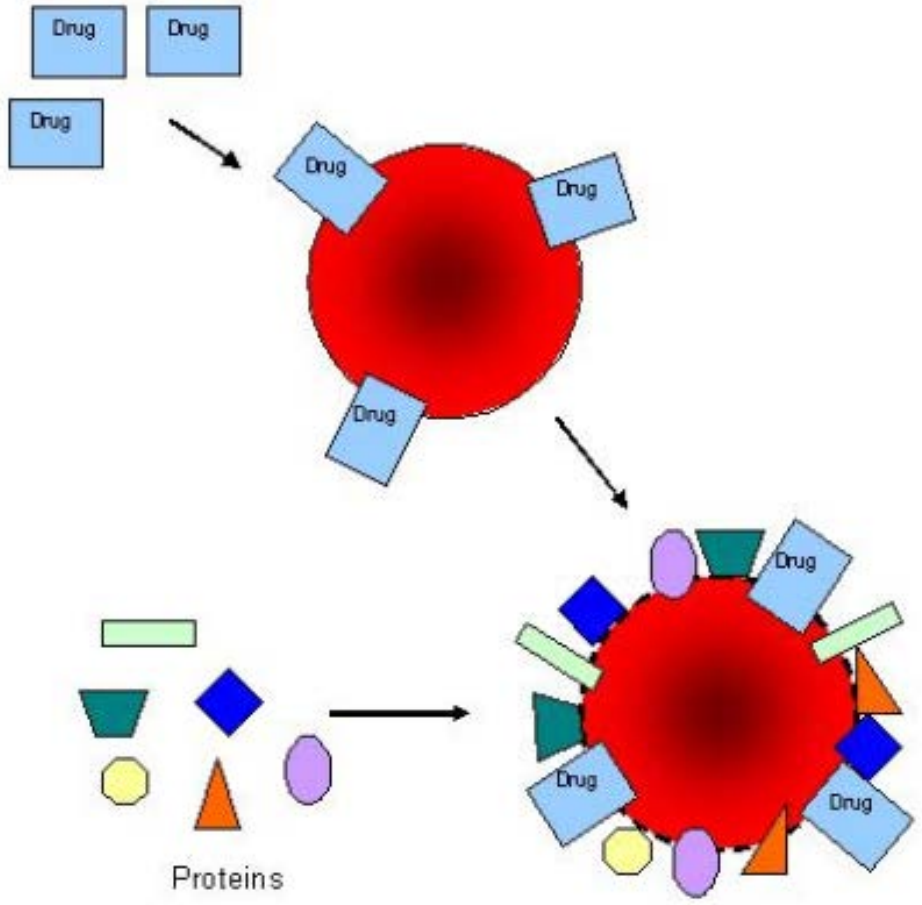
Drug-Dependent or Immune Complex Mechanism

- “Innocent bystander”
- Usually IgM binds with drug forming complex
- Complex loosely attaches to RBC membrane
- Complex initiates complement cascade- intravascular hemolysis
- Only small doses needed
- DAT- IgG negative
- Antibody screen negative
- Treatment- discontinue drug
- Ex. Quinidine, phenacetin



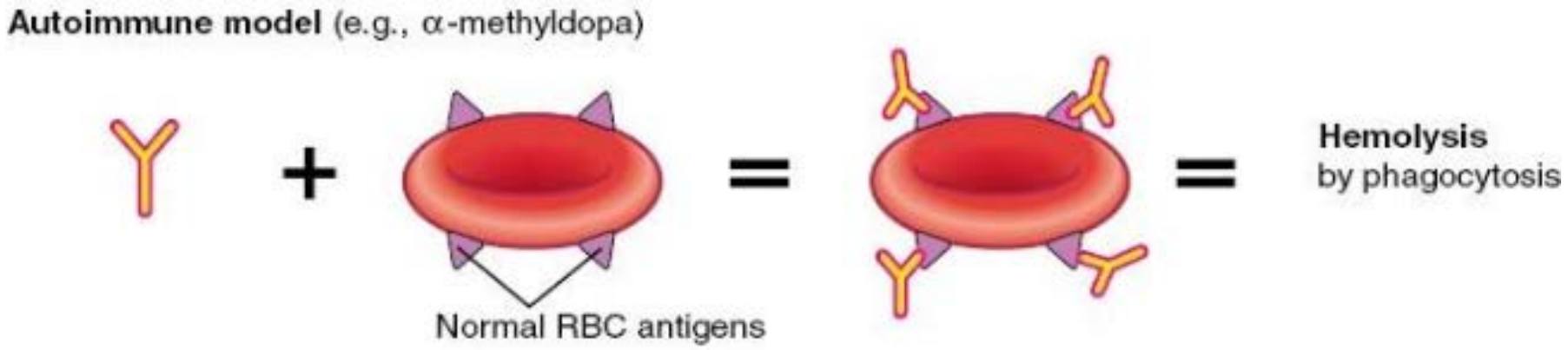
Membrane Modification

- Nonimmunologic Protein Adsorption
- Drug modifies RBC membrane so plasma proteins (IgG, IgM, IgA and complement) can bind to the membrane
- Uptake of immunoglobulins is not a specific antigen-antibody interaction
- Ex. Cephalosporins



Autoantibody Formation

- Alpha-methyldopa (Aldomet) induces production of autoantibody recognizing RBC antigens
- Only mechanism that will produce a positive eluate



Summary of Drug-Induced Hemolytic Anemias

Mechanism	Drugs	Ig Class	DAT Results	Eluate
Drug Adsorption	Penicillin Cephalosporin	IgG	Strongly positive	Negative
Immune Complex	Quinidine Phenacetin	IgM (sometimes IgG)	Positive- often C3 only	Negative
Membrane Modification	Cephalosporin	Many plasma proteins	Positive	Negative
Methyldopa induced	Methyldopa (Aldomet)	IgG	Strongly positive	Positive

Summary of Autoantibodies

Characteristics	Warm Reactive Autoantibody	Cold Reactive Autoantibody	Paroxysmal Cold Hemoglobinuria	Drug-Related Autoantibody
Immunoglobulin characteristics	Polyclonal IgG- occasionally IgM and IgA may be present	Polyclonal IgM (infection) Monoclonal IgM (cold agglutin disease)	Polyclonal IgG	Polyclonal IgG
Complement Activity	Variable	Always	Always	Depends on mechanism of drug, antibody, and RBC interaction
Thermal reactivity	20-37°C	4-32°C	4-20°C biphasic hemolysin	20-37°C
Titer of free antibody	Low to moderate (<32)	High (>1,000)	Moderate to low (<64)	Depends on mechanism of drug
Most common specificity	Anti-Rh; anti-LW; anti-U; high frequency antigens	Anit-I, anti-i, anti-Pr	Anti-P	Anti-e-like, methyldopa antidrug
Site of RBC destruction	Spleen and some liver	Liver; rarely intravascular	Intravascular	Intravascular and spleen

Cleveland Clinic

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