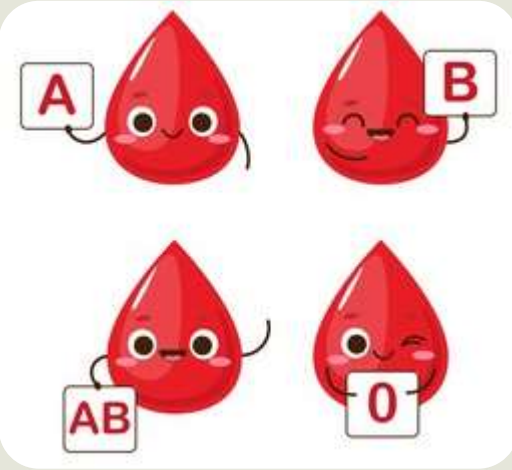
Blood Group Discrepancies



- What is ABO blood group?
- Type of blood group discrepancies
- Causes of blood group discrepancies
- Resolutions

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Introduction

What is ABO Blood Group?

- The **first** recognized blood group system in humans

- Classification of blood is based on the presence or absence of **inherited antigenic substances** on the surface of red blood cells (RBCs)

- These antigenic substances may be proteins, carbohydrates, glycoproteins or glycolipids depending on the blood group system

Introduction

Landsteiner's Rule

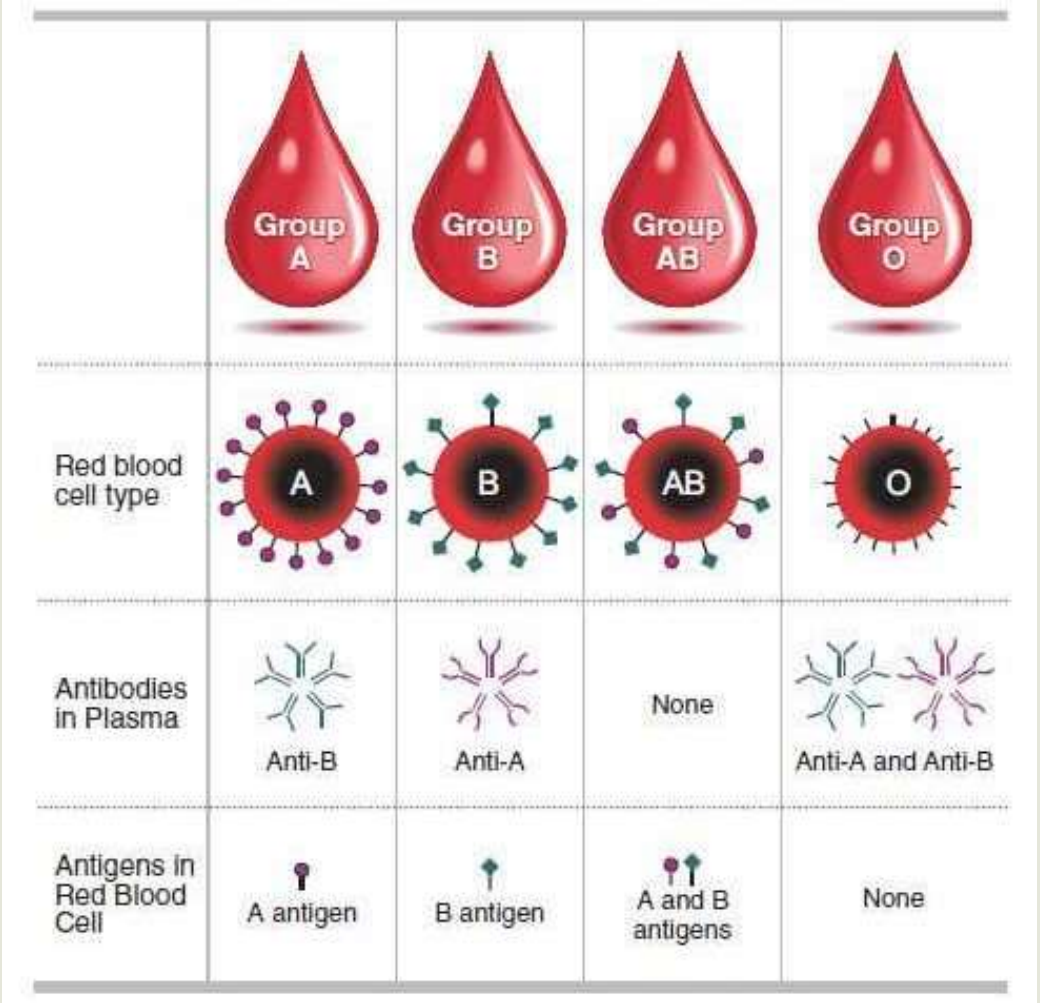
- If an **antigen** is present on an individual's red blood cells, the **corresponding antibody** will **NOT** be present in his/her plasma

- The Anti-A and Anti-B antibodies are naturally occurring which usually appear from the age of approximately 4-6 months of life

- Normally, the antibodies begin to decrease in later years: >65 years of age

Introduction

Antigen and Antibody present in ABO Blood Group



Definition of Blood Group Discrepancies

- Anomalous results in blood group testing, where forward and reverse grouping **does not tally** with each other or abnormal reactivity is present (i.e. Mixed Field)

- Can be due to:

1. Technical discrepancy
2. Clinical discrepancy

Definition of Blood Group Discrepancies

1. Technical Discrepancy

❑ Clerical errors

- Mislabeled specimen
- Patient misidentification
- Inaccurate interpretations recorded

❑ Reagent or Equipment Problems

- Using expired reagents
- Using uncalibrated centrifuge
- Contaminated or hemolyzed reagents

❑ Procedural Errors

- Reagents not added
- Not following manufacturer's instructions
- Incorrect concentration of RBC suspensions

Definition of Blood Group Discrepancies

2. Clinical Discrepancy

- Problems lie in the patients

- ABO blood group discrepancies are mainly divided into **4 major categories**:
  1. Group I discrepancy – Reverse grouping
  2. Group II discrepancy – Forward grouping
  3. Group III discrepancy – Protein or plasma abnormalities
  4. Group IV discrepancy – Miscellaneous problems

Group I Discrepancy

- Group I discrepancy mainly seen in **reverse grouping** which due to **weak reaction** or **missing antibodies**
- The reason for the weak reaction or missing antibodies is that the patient has depressed antibody production or cannot produced the ABO antibodies
- **Most commonly happened** among the other discrepancies

Reasons for Group I Discrepancy

❖ Reverse Grouping

Weak reaction or missing antibodies due to:

1. Age

- ❑ Newborns – Do not form antibody until 4-6 months of life
- ❑ Elderly – Weakened antibody activity

2. Diseases related

- ❑ Patients with leukemia (i.e. Chronic lymphocytic leukemia (CLL) or lymphoma have hypogammaglobulinemia which will produce little or no antibody
- ❑ Patients with bone marrow transplant
- ❑ Patients on immunosuppressive drugs

3. Dilutional Effect

- ❑ Plasma exchange / transfusion which dilute out patient antibodies

Resolutions for Group I Discrepancy

Reasons of discrepancy	Resolutions
Newborns	Only **forward grouping** is done till 4 months of age
Weakly or missing antibodies	• Enhance the reaction in reverse grouping • Methods: 1. Prolong the incubation of the patient's serum with reagent cells at **room temperature (~22 °C)** for **15 – 30 minutes** 2. If the antibody reaction still showing negative results, incubate the cells serum mixture at **lower temperature (~4 °C)** for **15 – 30 minutes**

Group II Discrepancy

- Group II discrepancy is associated with unexpected reaction in **forward grouping**, which due to **weak reaction** or **missing antigens**
- The **least common** type of discrepancies

Reasons for Group II Discrepancy

❖ Forward Grouping

Weak reaction or missing antigens due to:

1. Subgroups of A or B

❑ Subgroups of A:

- Group A red cells that react with both anti-A and anti-A1 are classified as A1
- Group A red cells that react with anti-A and not anti-A1 are classified as A2
- The production of both A1 and A2 antigens is a result of an inherited gene at the ABO locus and the immunodominant sugar on both A1 and A2 red blood cells which is N-acetyl-D-galactosamine

❑ Subgroups of B:

- Subgroups B3, Bx, Bm and Bcl
- Infrequent than subgroups of A

❑ Example of Subgroup A:

Anti-A	Anti-B	A1 Cells	B Cells	ABO Blood Group
2+	0	1+	4+	??
	A		A or B?	

Reasons for Group II Discrepancy

❖ Forward Grouping

Weak reaction or missing antigens due to:

2. <u>Acquired A or B antigens</u>

❑ Acquired B:

- Limited mainly to Group A1 individuals with:

  a) Lower gastrointestinal tract disease

  b) Colon / rectum cancer

  c) Intestinal obstruction

  d) Gram negative septicemia (i.e. E. coli)

❑ Example of Acquired B:

Red Cell		Serum		ABO Group
Anti-A	Anti-B	A1 cells	B cells	
4+	1+	0	4+	??

AB

A

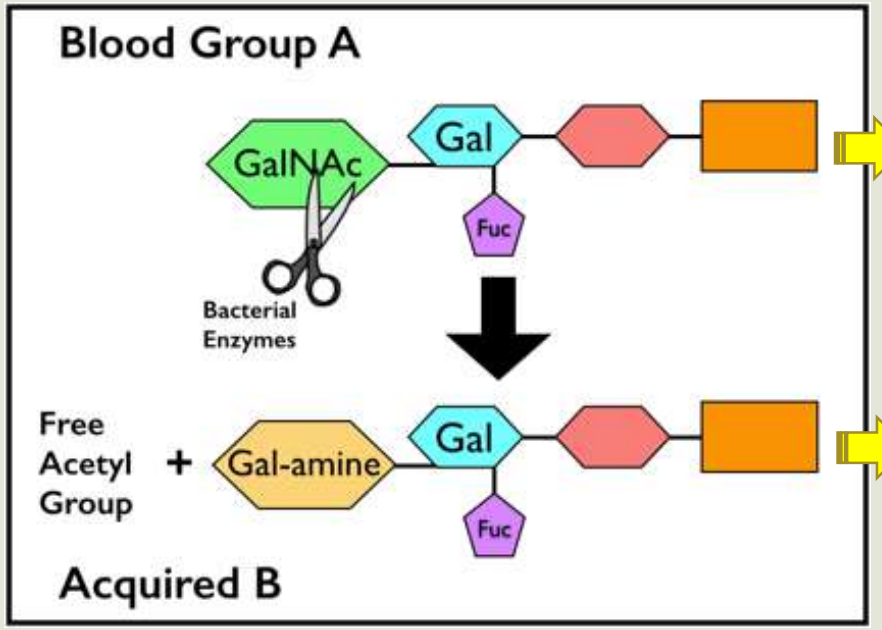
Reasons for Group II Discrepancy

❖ Forward Grouping

Weak reaction or missing antigens due to:

2. <u>Acquired A or B antigens</u>

❑ Acquired B:



Bacterial enzymes such as *Escherichia coli* cleave off the N-Acetyl of the Group A N-acetyl-galactosamine.

The remaining galactosamine becomes similar to the Group B galactose. So, Anti-B reagent will react with this B-like antigen causing agglutination.

Reasons for Group II Discrepancy

❖ Forward Grouping

Weak reaction or missing antigens due to:

3. Diseases related

❑ Leukemia and lymphoma patients:

- Leukemia may yield weakened A or B antigens
- Example: Hodgkin's disease has been reported in some cases to mimic the depression of antigens

4. Excess antigen of blood group soluble substances

❑ Patients usually come with large amounts of soluble A or B antigen which will inhibit anti-A or anti-B typing reagent

❑ Happened in patients with certain types of cancer

Resolutions for Group II Discrepancy

Reasons of discrepancy	Resolutions
Subgroups of A or B	• Repeating blood grouping by using **washed cells** • Use anti AB antisera and anti-A1 lectin (Dolichos Biflorus) to test on patient's sample • Absorption Elution technique
Acquired A or B antigens	• Perform **auto control test** • Methods: 1. Test patient plasma with patient red blood cells. 2. Patients who are truly group A, without any true B antigen, will demonstrate a **negative** auto control. 3. Since there is no B antigen present, there is only anti-B found in patient's plasma. No agglutination should be observed. • Test with acidified Anti-B antisera

Resolutions for Group II Discrepancy

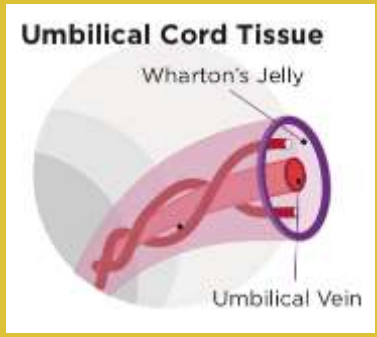
Reasons of discrepancy	Resolutions
Diseases related	• Repeat blood grouping by washing and re-suspending red cells in saline, following by repeat forward typing with anti-A and anti-B reagent

Group III Discrepancy

- Group III discrepancy can be happened between **forward and reverse groupings** which caused by **protein** or **plasma abnormalities**

- Examples:

1. Elevated levels of plasma globulins as seen in cases of Multiple Myeloma, Waldenstrom's macroglobulinemia and Hodgkin's lymphoma
2. Elevated levels of fibrinogen
3. Use of plasma expanders such as dextran
4. Wharton's jelly in cord blood samples



Reasons for Group III Discrepancy

❖ Protein or plasma abnormalities

1. Rouleaux formation

- ❑ This can be happened in Multiple Myeloma, Waldenstrom's macroglobulinemia and patients using dextran
- ❑ Can cause both extra antigens and extra antibodies
- ❑ **"Stack of coins"** appearance
- ❑ May falsely appear as agglutination due to increase of serum proteins (globulins)

2. Wharton's Jelly

- ❑ Defined as a **gelatinous substance** derived from connective tissue that is found in **cord blood** and may cause false agglutination, giving false positive results

Resolutions for Group III Discrepancy

Reasons of discrepancy	Resolutions
Rouleaux Formation	• If the forward grouping is affected, **wash the red cells** with normal saline 6 – 8 times to remove excess protein and confirm with microscopic examination. • If the reverse grouping is affected, perform **saline replacement technique**. • Methods: 1. Mix reagent cells and patient serum and centrifuge to allow antigen and antibody to react. 2. Remove patient serum and replace by an equal volume of saline. Mix well. 3. Centrifuge and reexamine for true agglutination.

Resolutions for Group III Discrepancy

Reasons of discrepancy	Resolutions
Wharton's Jelly	• Wharton's Jelly **coats newborn cord cells** and the patient blood type may appear as **group AB**. • Since we only perform forward typing in cord blood samples, we can wash the red cells 6 – 8 times and re-test again.

Group IV Discrepancy

- Group IV discrepancy occurs in between **forward and reverse grouping** due to **miscellaneous problems**

- Examples:

1. Polyagglutination
2. Patients with cold allo or auto antibodies
3. A2 or A2B individual with anti-A1 antibodies
4. Naturally occurring or irregular antibodies reacting at room temperature

Reasons for Group IV Discrepancy

❖ Miscellaneous Problems

1. Polyagglutination

- ❑ Defined as agglutination of red blood cells with human antisera no matter what blood type
- ❑ Due to bacterial or viral infections, causing expression of hidden T antigens react with antisera

2. Cold antibodies

- ❑ Cold antibodies can be divided into allo- or auto-
- ❑ Sometimes, patients will develop cold reacting allo- or auto-antibodies that appear as "extra" antibodies on reverse grouping
- ❑ Alloantibodies are made against foreign red cells
- ❑ Examples of alloantibodies:

- Anti-I, H, M, N, P and Lewis

Reasons for Group IV Discrepancy

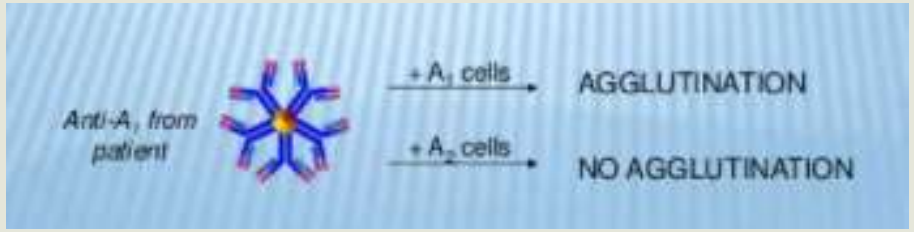
❖ Miscellaneous Problems

2. Cold Antibodies

- ❑ Autoantibodies are made against ones own red cells
- ❑ Examples of autoantibodies:
- IgM which can cause false-positive results in both cells and serum grouping

3. Anti-A1 Antibodies

- ❑ Sometimes, A2 or A2B individuals will develop an anti-A1 antibody
- ❑ A2 or A2B individuals have less antigen sites than A1 individuals
- ❑ The antibody is a naturally occurring IgM, it will only react with A1 cells but not A2 cells



Resolutions for Group IV Discrepancy

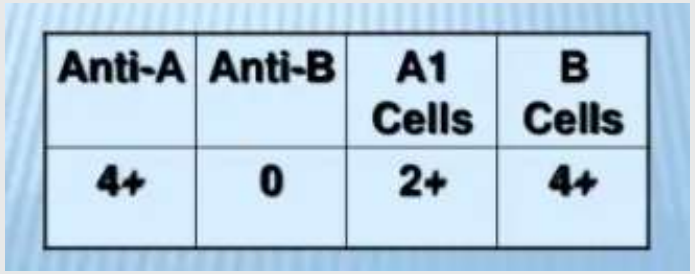
Reasons of discrepancy	Resolutions
Polyagglutination	• Test with **auto control** ⇨ **Negative** • Test with **Direct Antiglobulin Test (DAT)** ⇨ **Negative** • Can be resolved by using monoclonal antibodies typing reagents such as (*Glycine soja, Arachis hypogea*).
Cold Antibodies	• Test with **auto control** ⇨ **Positive** • If alloantibodies present, can proceed for **Antibody Identification** tests.

Resolutions for Group IV Discrepancy

Reasons of discrepancy	Resolutions
Cold Antibodies	• If autoantibodies present, **pre-warm** both the patients serum and reagent cells at **37°C**. • Pre-wash the red cells with warmed saline prior testing to disperse the agglutination. • If the agglutination still present, can use **2-mercaptoethanol (2-ME)** or **dithiothreitol (DTT)** to destroy the IgM bonds. • Methods: 1. Prepare equal quantity of 0.01 M DTT and washed red cells. 2. Incubate the samples at 37°C for 15 minutes. 3. Perform blood grouping again.

Resolutions for Group IV Discrepancy

Reasons of discrepancy	Resolutions
Anti-A1 Antibodies	• Typing patient's RBCs with Anti-A1 lectin (Dolichos Biflorus). • Repeat reverse grouping with A2 cells instead of A1 cells. • Both results should yield **NO agglutination**.



Anti-A	Anti-B	A1 Cells	B Cells
4+	0	2+	4+