Blood Banking

Part 2

MLS 404

HISTORY

YEAR	EVENTS
1492	Blood was taken from 3 young men and given to **Pope Innocent VIII**; all four died
1869	**Na phosphate** was recommended by Braxton Hicks as an anticoagulant for blood preservation
1901	**Karl Landsteiner** discovered the ABO blood groups
	Edward Lindemann carried out a vein-to-vein transfusion using multiple syringes and special cannula for puncturing the skin
	Unger designed his syringe-valve apparatus
1914	**Na citrate** was reported by Hustin as an anticoagulant for blood transfusions
1915	**Minimum amount of citrate** needed for anticoagulation was determined by Lewisohn
1916	**Citrate dextrose** was introduced by Rous and Turner

History

YEAR	EVENTS
	World War II stimulated blood preservation research because of the increased demand in blood products
1941	**Dr. Charles Drew** was appointed director of the 1st American Red Cross blood bank at Presbyterian Hospital
1943	**Acid-Citrate-Dextrose** was introduced by Loutit and Mollison
1947	On July, the **Journal of Clinical Investigation** devoted for blood preservation was published
1957	**Citrate-Phosphate-Dextrose** was introduced by Gibson

THINGS TO REMEMBER!

- **Blood collection unit**
  - Recruits, screens and draws blood donors, performs screening tests for blood units
- **Blood bank**
  - Hospital-based, performs compatibility testing and prepares blood components
- **Blood center**
  - Screens donors, draws donors, performs testing on donor blood and delivers appropriate components to hospital blood banks

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BLOOD GROUP ANTIGENS

- Refers to genetically encoded antigen system on erythrocytes, leukocytes, thrombocytes and plasma.

- Incidence
  - Low incidence: Private or Family Antigens
  - High incidence: Public Antigens

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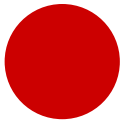
Blood Group Antibodies

- Autoantibody
- Alloantibody
  - Naturally occurring: stimulus is unknown
  - Immune antibodies: results from antigen exposure



ABO Blood Group System

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ABO Blood Group System

- **ISBT 001**
- Discovered by **Karl Landsteiner** : A, B and O
- **Sturle and Von Descatello**: AB
- MOST IMPORTANT BLOOD GROUP SYSTEM
- MOST IMMUNOGENIC yet, the simplest
- MOST COMMON CAUSE OF HDFN
- BGS which is the most common cause of HTR (second only to clerical errors)

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ABO Blood Group System

- With NATURALLY OCCURING ANTIBODIES
  - **IgM**
  - Cold-reacting antibodies
  - Reacts at room temperature
- ANTIGENS
  - found partly as **glycolipids**, but primarily as **glycoproteins**
  - may also occur in the secretion as **glycoproteins**

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LANDSTEINER LAW

1. Antigen on the RBC determines the blood group
2. The corresponding antibody is never found in the individual's serum
3. The opposite antibody is always present in the individual's serum

Must Know!

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GRADING OF AGGLUTINATION REACTIONS

GRADE	AGG.	DESCRIPTION
Neg	0%	**No aggregates**; *dark, turbid, homogenous supernatant*
Weak (+/-)		A few isolated aggregates; **mostly free-floating cells**; *supernatant appears red, dark or turbid*.
1+	25%	A **few small aggregates** just visible macroscopically; many free erythrocytes; *turbid reddish supernatant*.
2+	50%	**Medium-sized aggregates**; some free erythrocytes; *clear supernatant*.
3+	75%	**Several large aggregates**; some free erythrocytes; *clear supernatant*.
4+	100%	All erythrocytes are combined into **one solid aggregate**; *clear supernatant*.

Gel Technology Reaction Grading Chart

GRADING	DESCRIPTION
4+	Agglutinated cells form a cell layer **AT THE TOP** of the gel media.
3+	Agglutinated cells begin to disperse into gel media and are **CONCENTRATED NEAR THE TOP** of the microtube.
2+	Agglutinated cells disperse into the gel media and are observed **THROUGHOUT** the length of the microtube.
1+	Agglutinated cells disperse throughout the gel media and may **CONCENTRATE TOWARD THE BOTTOM** of the tube.
Negative	All cells pass through the gel media and form a **CELL BUTTON AT THE BOTTOM** of the microtube.
Mixed-field	Agglutinated cells form a layer **AT THE TOP** of the gel media. Unagglutinated cells pass to the **BOTTOM** of the microtube.

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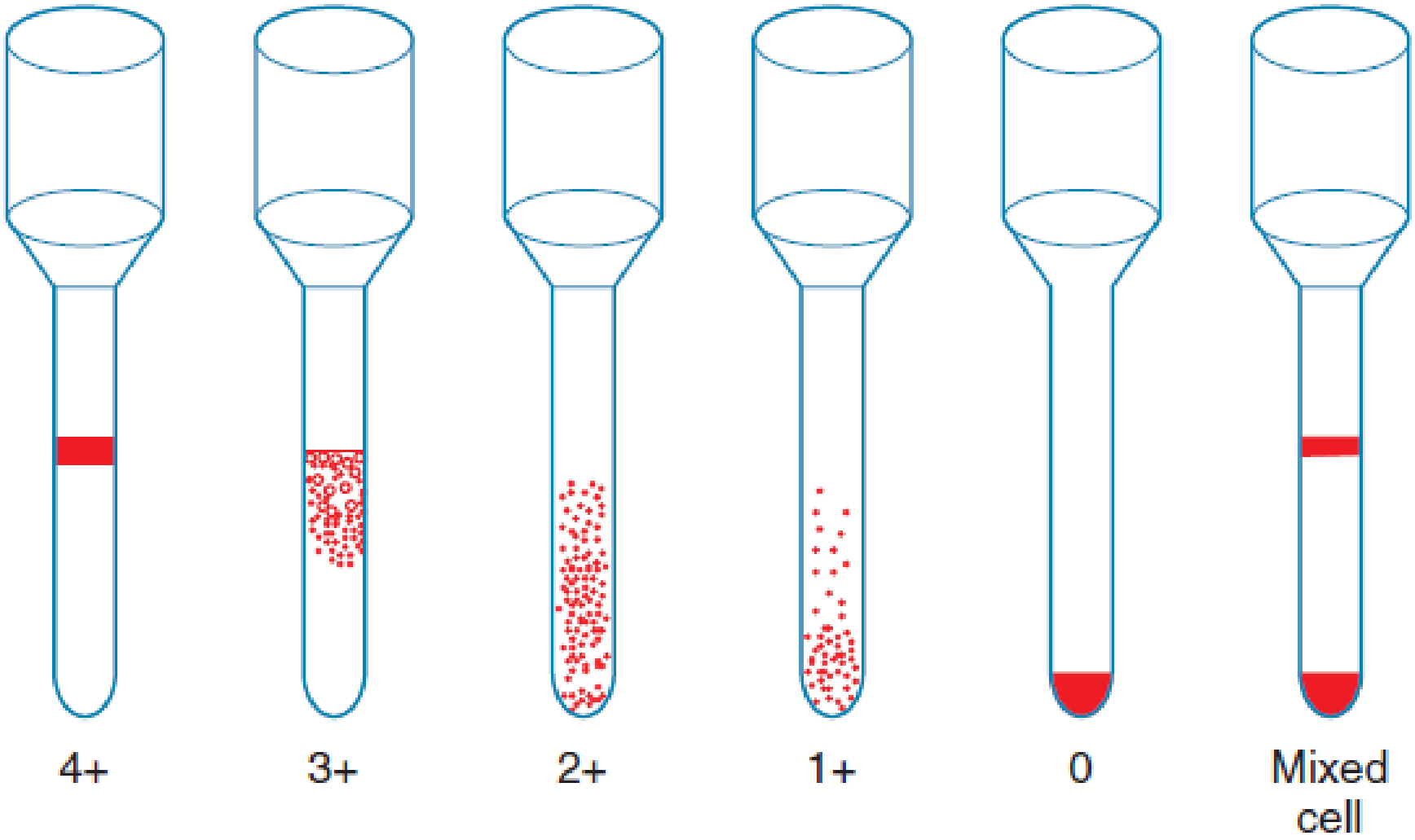

ABO Forward Grouping

- Also known as:
  - Forward Typing
  - Cell Typing
  - Direct Typing
  - Red Cell Typing
- Used to **DETECT ANTIGENS** on RBC surface
- Uses commercial **ANTISERA** of known specificity
  - **Anti-A**: has **bromphenol blue** dye **(trypan blue)**
  - **Anti-B**: has **acriflavine** dye
  - **AHG: Ariavit tartrazine + Patent Blue V**

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ABO Forward Grouping

BLOOD GROUP	REACTION WITH ANTI-A	REACTION WITH ANTI-B
O	-	-
A	+	-
B	-	+
AB	+	+

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ABO Reverse Grouping

- Also known as:
  - Serum Typing
  - Reverse Typing
  - Indirect Typing
- Used to detect presence of **ANTIBODIES**
- Serves as a confirmatory procedure for cell typing
- Uses **KNOWN RED CELL SUSPENSION**
  - **2-5% red cell suspension**

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ABO Reverse Grouping

BLOOD GROUP	REACTION WITH A CELLS	REACTION WITH B CELLS
O	+	+
A	-	+
B	+	-
AB	-	-

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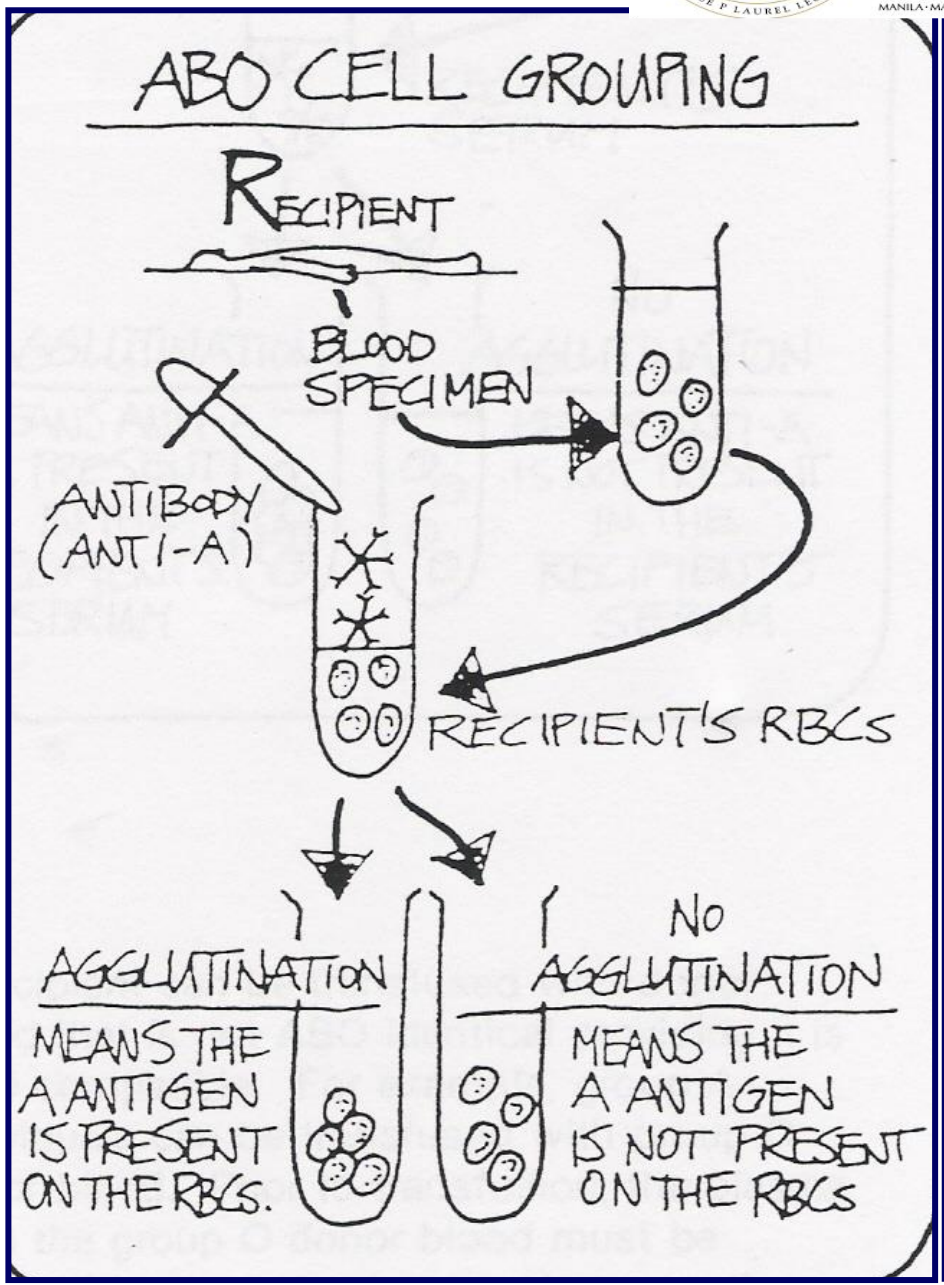

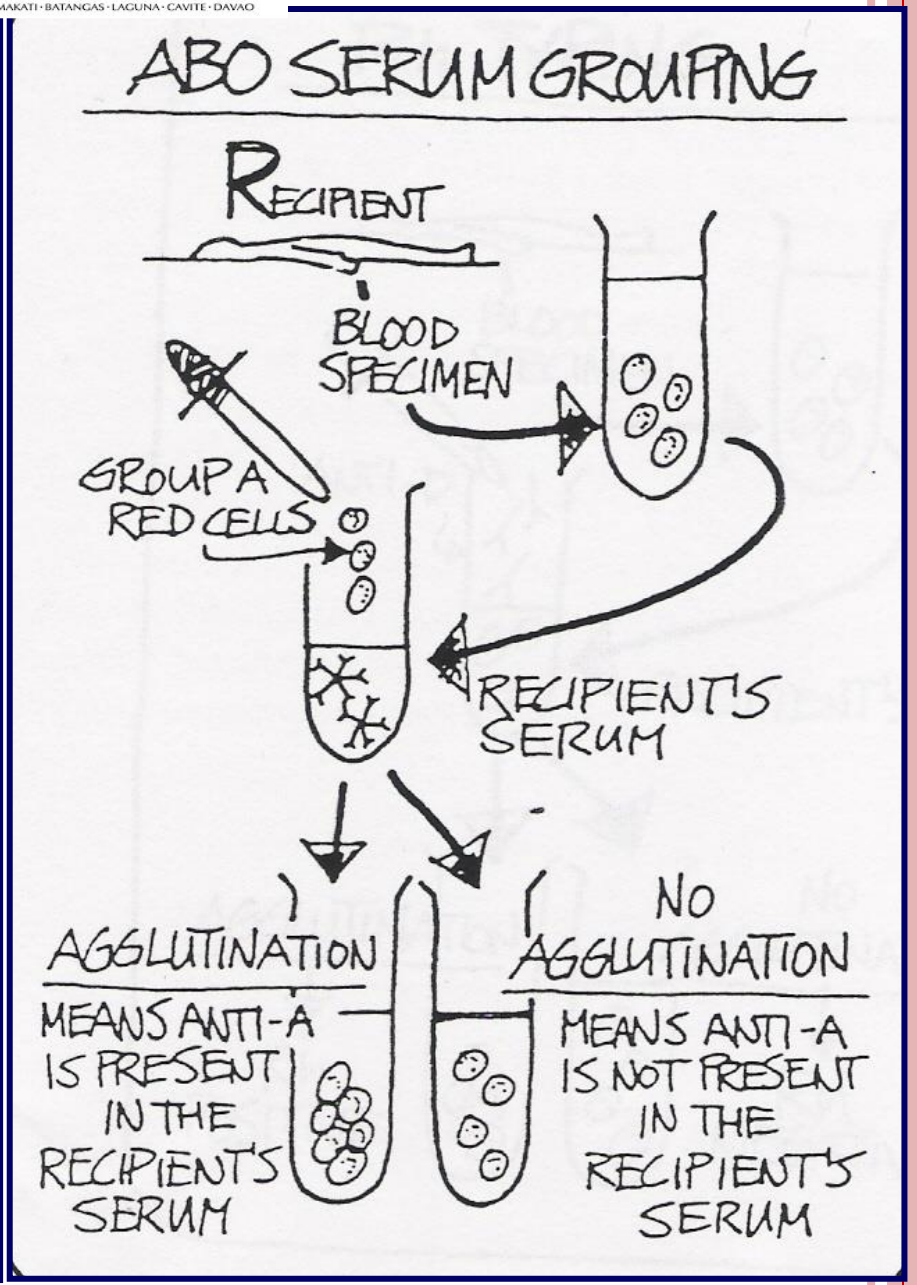

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ABO Nomenclatures

LANDSTEINER	JANSKY	MOSS
O	I	IV
A	II	II
B	III	III
AB	IV	I

MUST KNOW!

- **Genotype** – genes
- **Phenotype** – demonstrable antigen
- **Homozygous genotype** – identical alleles
- **Heterozygous genotype** – different alleles
- **Amorph** – GENE THAT DOES NOT PRODUCE ANY DETECTABLE TRAIT
- **Dominant gene** – always expressed
- **Recessive gene** – does not express itself in the presence of a dominant gene

Must Know!

- **Glycosyltransferases**
  - are ENZYMES that facilitate the transfer of carbohydrate (sugar) molecules onto carbohydrate precursor molecules

- **Immunodominant sugar**
  - is the sugar molecule that complete the antigenic determinant when combined with the precursor substance

MUST KNOW!

- **H genes (FUT1)** at a separate locus codes for the precursor substance on which the A and B gene products act
- ***H* (FUT1) and *Se* (FUT2)** loci are closely linked on **Chromosome 19**
- The products of the A and B genes are **enzymes** that act as a specific transferases

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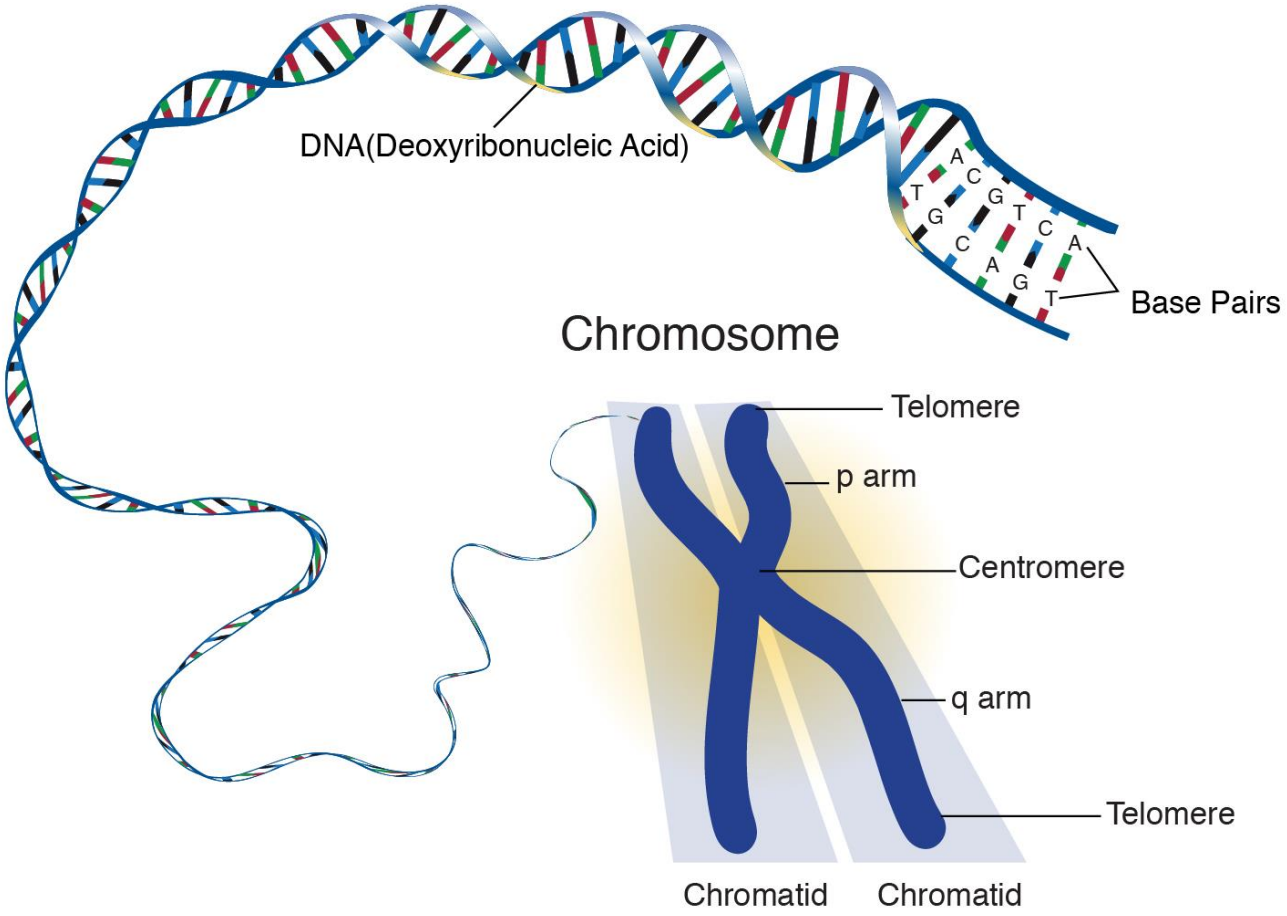


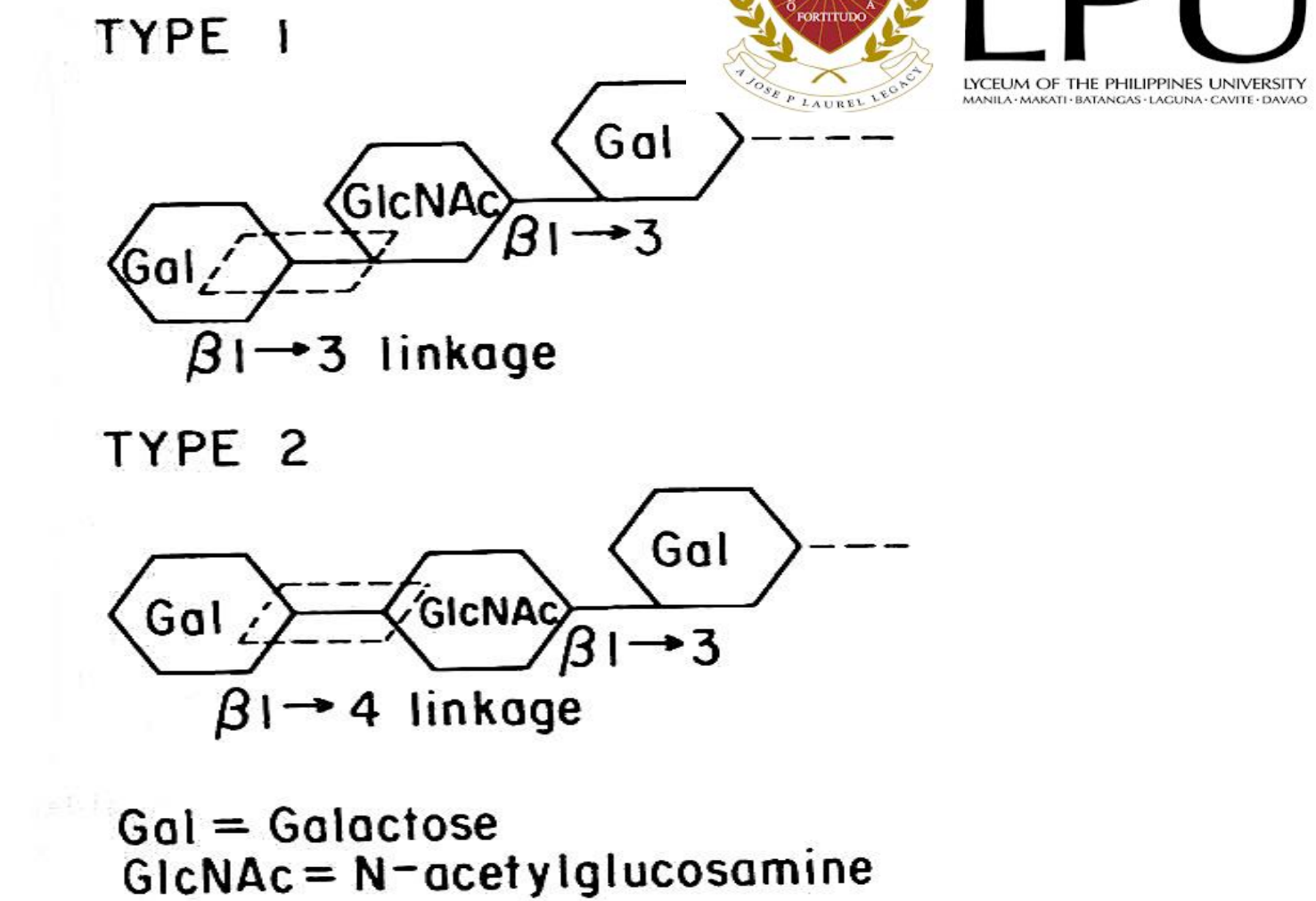
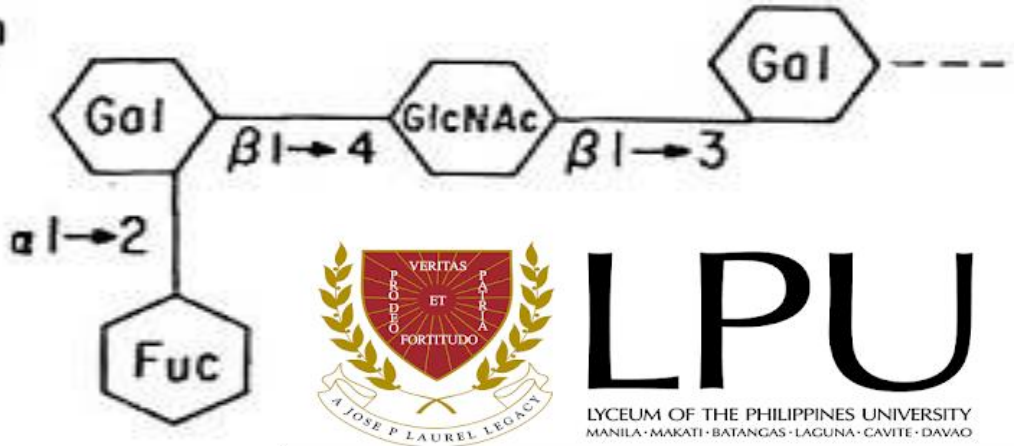
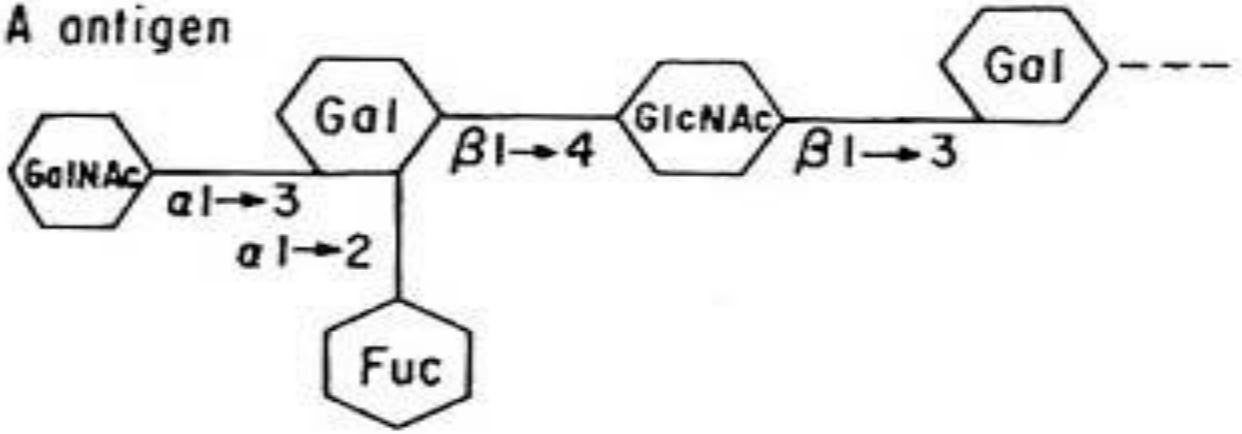


Figure 13-2. Type 1 and 2 oligosaccharide chains differ only in the linkage between the GlcNAc and the terminal Gal.

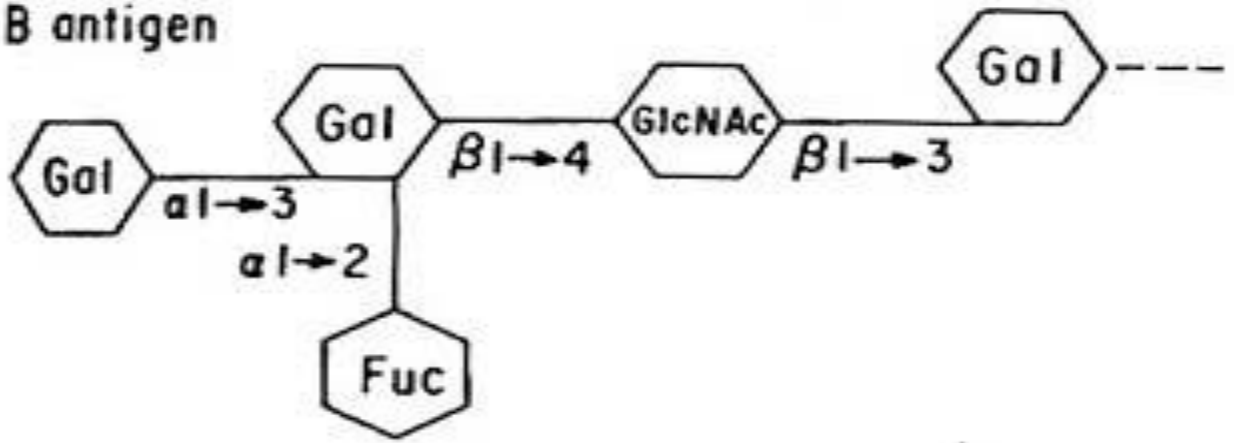
H antigen



A antigen



B antigen



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FORMATION OF ABH ANTIGENS

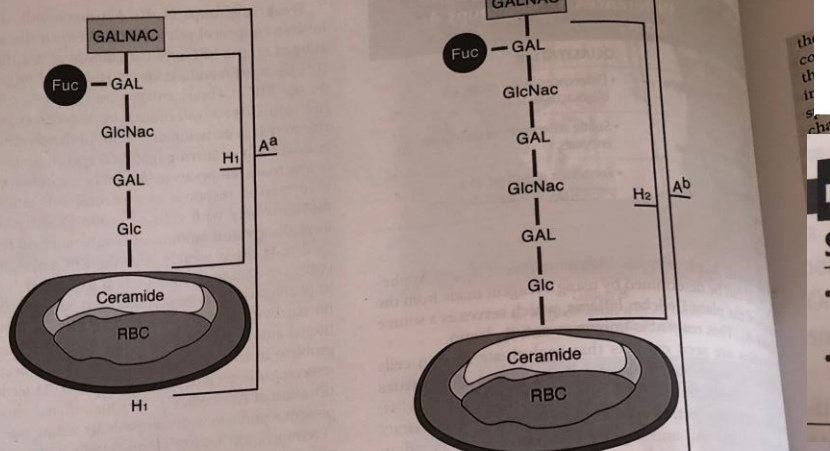
GENE	GLYCOSYLTRANSFERASE	IMMUNODOMINANT SUGAR	ACCEPTOR	ANTIGEN
H	L-fucosyltransferase	L-fucose	Precursor	H
O				H
A	N-acetylgalactosaminyl transferase	N-acetyl-D-galactosamine	H	A
B	D-galactosyltransferase	D-galactose	H	B
AB	N-acetylgalactosaminyl transferase D-galactosyltransferase	N-acetyl-D-galactosamine D-galactose	H	AB

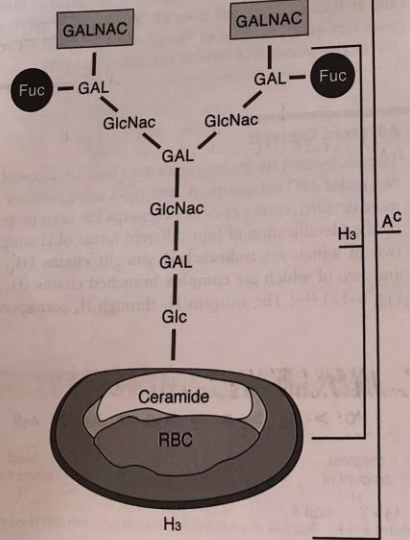
DIFFERENCES:

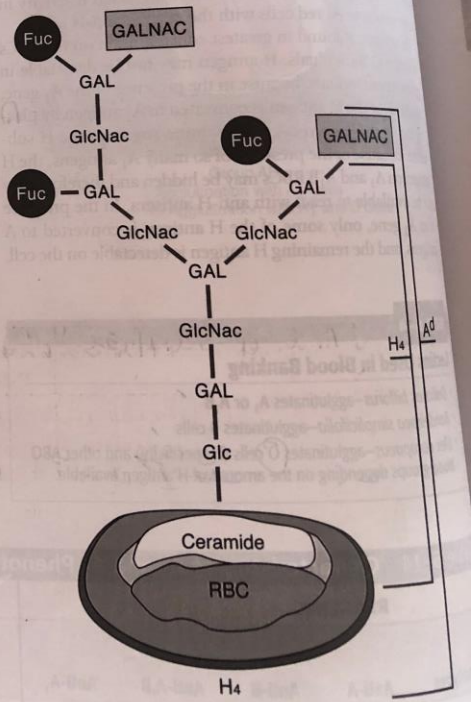
- **Type A**
  - 810,000 to 1, 170,000 A antigen sites
- **Type B**
  - 610,000 to 830,000 B antigen sites
- In **Type AB**:
  - 600,000 A antigen sites
  - 720,000 B antigen sites

Must Know!

- Amount of H antigens
  - $O > A_2 > B > A_2B > A_1 > A_1B$
- Group O: has the GREATEST amount of H antigens
- Group A_1B: hast the LEAST amount of H antigens







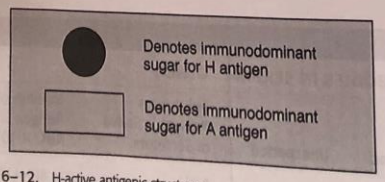


Figure 6–12. H-active antigenic structures.

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BOX 6–5

Structural Characteristics of A_1 and A_2 RBCs

- A_2 RBCs: Predominantly A^a and A^b and unconverted H_3 and H_4 antigen sites
- A_1 red cells: A^a, A^b, A^c, and A^d determinants and no unconverted H_3 and H_4 antigen sites

SECRETOR STATE

ABO GROUP SECRETORS	ABH SUBSTANCES IN SALIVA
O	H
A	A, H
B	B, H
AB	A, B, H

ABO Antigens

- **A PHENOTYPE**
  - **2 main subgroups:**
    - **A1** and **A2**, can be differentiated by using the lectin anti-A1 reagent made from ***Dolichos biflorus seeds***.
    - This reagent agglutinates RBCs with the A1 antigen, but not cells with the A2 antigen.
    - Approximately **80% of group A individuals are A1**
    - Approximately **20% are A2**
    - Anti-A1 can be found in 1% to 8% of group A2 individuals and in 22-35% of A2B individuals.
    - Several other subgroups of A exist, but are extremely rare. The **most common, A3**, shows mixed field agglutination with anti-A or anti-AB reagents.

OTHER SUBGROUPS OF A

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PHENOTYPE	DESCRIPTION
A3	**MIXED-FIELD** pattern of agglutination with Anti-A and Anti-A,B reagents
Ax	**NOT AGGLUTINATED** by Anti-A but demonstrate **WEAK AGGLUTINATION** with Anti-A,B
Aend	**VERY WEAK MIXED-FIELD** pattern of agglutination with Anti-A and Anti-A,B reagents but very small percentage (≤10%) agglutinate)
Am	**UNAGGLUTINATED** by Anti-A and Anti-A,B, easily adsorbs and elutes Anti-A, **demonstrate A** substance in the saliva
Ay	**UNAGGLUTINATED** by Anti-A and Anti-A,B, adsorbs and elutes Anti-A with difficulty, with **small amount of A** substance in the saliva
Ael	**UNAGGLUTINATED** by Anti-A and Anti-A,B, adsorbs and elutes Anti-A, **secretor studies shows H only and no A** substance in the saliva

Other Subgroups of B

PHENOTYPE	DESCRIPTIONS
B3	**MIXED FIELD** and rapid agglutination with Anti-B
Bx	**WEAK AGGLUTINATION** with Anti-B and anti-A,B without mixed field agglutination
Bm	**UNAGGLUTINATED** by Anti-B and Anti-A,B, has H and B substance in the saliva
Bel	**UNAGGLUTINATED** by Anti-B and Anti-A,B, has H substance only in the saliva

BOMBAY PHENOTYPE

- First discovered in 1952 by **Bhende** in Bombay (New Dehli), India
- **Characteristics:**
  - A recessive mode of inheritance
  - Absence of H, A and B antigens
  - Presence of Anti-A, Anti-B and a potent Anti-H (reactive at body temp)
  - A, B, H nonsecretor
  - Absence of L-fucosyltransferase in serum and H antigens in the red cells
  - Presence of A or B enzymes in the serum
  - **Red cells DOES NOT react with Anti-H lectin (*Ulex europeus)***
  - Strong reactivity with anti-I

BOMBAY PHENOTYPES

- **Category 1: RBC H-Deficient, Nonsecretor; Bombay Phenotype (hh, sese)**
  - Classical Bombay Phenotype
  - Lacks all antigens in the red cell and secretions
  - homozygous recessive inheritance of h allele
- **Category 2: RBC H-Partially Deficient, Nonsecretor**
  - Have little amount of A and B antigens. The very little amount of H antigen is so well hidden
  - serum demonstrate A or B enzymes but NO H enzyme
  - weak anti-H present in serum
- **Category 3: RBC H-deficient, Secretor (hh, Se)**
  - Parabombay
  - Lacks the H gene but has the Secretor Gene
  - Has no detectable H antigens on rbcs but detectable on secretions
  - weak anti-H almost always present in the serum

Other Unusual, Rare Phenotypes

- **Cis-*AB* phenotype**
  - inheritance of THREE ABO genes instead of two (*A*, *B* and *O*)
  - A and B gene are inherited in *cis* position (normally, *A* and *B* are in trans position)
  - represents a problem in crossing-over
- **B[A] phenotype**
  - present of trace amounts of A antigen on B rbcs due to synthesis of A antigen by the B-gene enzyme

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RBCs with the cis-AB phenotype (a rare occurrence) express a weakly reactive A antigen (analogous to A_2 cells) and a weak B antigen.[4] The B antigen usually yields a weaker reaction with the anti-B from random donors, with mixed-field agglutination typical of subgroup B_3

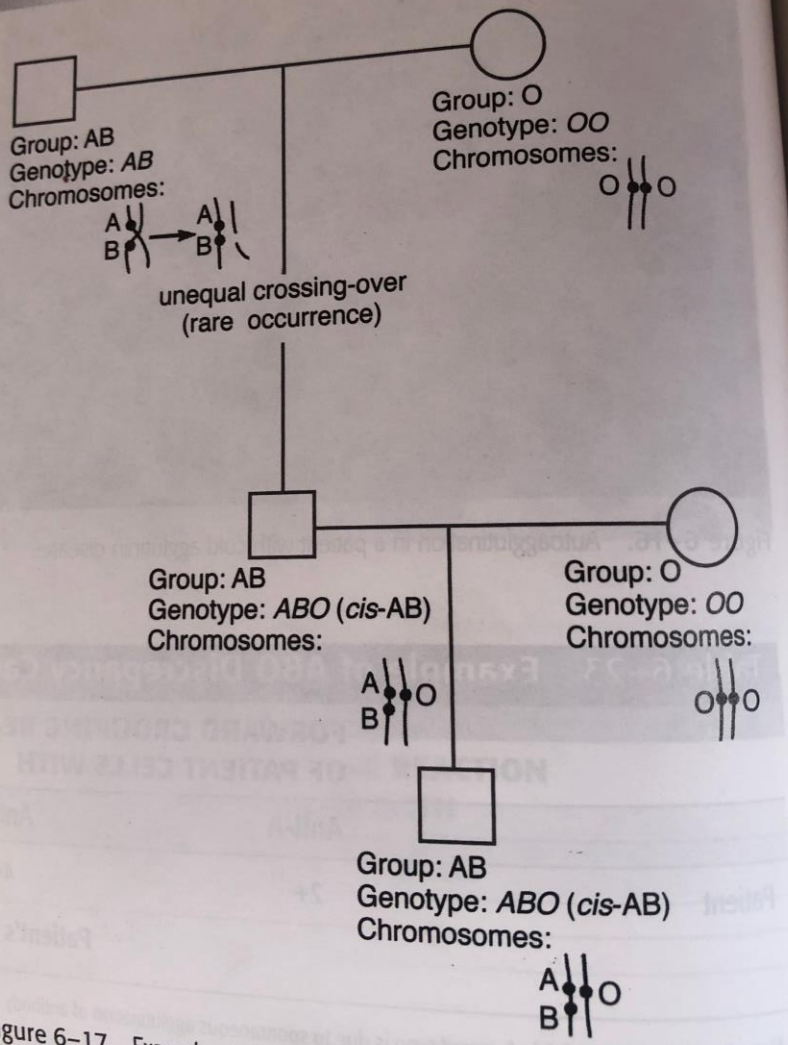

Figure 6–17. Example of cis-AB inheritance to unequal crossing-over. O = male; □ = female.

Acquired Antigens

- **ACQUIRED A**
  - **In group O and B persons**
  - Associated with severe *Proteus mirabilis* infections
  - Tn-activated red cells
- **ACQUIRED B**
  - **In group A persons**
  - Associated with carcinoma of the colon or rectum, intestinal obstruction, massive lower GI tract infection and septicemia with *Proteus vulgaris*
  - May also be due to increased permeability of the intestinal wall and subsequent absorption of bacterial polysaccharide on RBCs: Escherichia coli O:86

ABO Antibodies

- **IgM**
  - Cold-reacting
  - Naturally-occurring
  - Does not cross the placenta
    - Group A: **anti-B**
    - Group B: **anti-A**
    - Group O: **anti-A, anti-B**
- Predominantly **IgG**
  - Warm-reacting
  - Immune antibodies
  - Can cross the placenta
    - Group O: **anti-A,B**

IMMUNE ANTIBODIES - produced due to prior exposure to antigens either via transfusion, organ transplantation or pregnancy

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ABO Discrepancies

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ABO Discrepancies

- ABO discrepancies are recognized when the reactions obtained in the forward type do not "match" the reactions obtained in the reverse type.
- When a discrepancy is observed one MUST determine if the problem is associated with the forward type, reverse type or both.
- **FALSE POSITIVE** means the reaction should have been **NEGATIVE** but agglutination occurred.
- **FALSE NEGATIVE** means the reactions should have been **POSITIVE** but no agglutination occurred.
- The key is to recognize which type of problem is occurring so that appropriate testing can be performed.

ABO Discrepancies

Common sources of technical errors resulting in ABO discrepancies:

- Inadequate identification of blood specimen, test tubes
- A mix up in samples
- Clerical error
- Cell suspension either too heavy or too light
- Failure to add reagent
- Failure to follow manufacturer's instruction
- Contaminated poor quality reagent
- Missed observation of haemolysis
- Uncalibrated centrifuge
- Unclean/ contaminated glassware

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ABO Discrepancies

- **Group I**: Weakly-reacting or Missing Antibodies- MOST COMMON
- **Group II**: Weakly-reacting or Missing Antigens- LEAST COMMON
- **Group III** : Protein or Plasma Abnormalities Leading to Rouleaux Formation
- **Group IV**: Miscellaneous

GROUP I-MOST FREQUENT

1. Newborns
2. Geriatrics
3. Chimerism
4. ABO subgroups
5. Px with leukemia → hypogammaglobulinemia
6. Px with lymphoma → hypogammaglobulinemia
7. Px taking immunosupressive drugs → hypogamma
8. Px with congenital agammaglobulinemia
9. Px with immunodeficiency diseases
10. Px who have undergone bone marrow transplantation → hypogammaglobulinemia

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GROUP I-MOST FREQUENT

- **RESOLUTION:**
  - Review patient's profile
  - Review patient's history
  - Enhance reaction by
  - Incubating the patient serum with reagent A1 and B cells at room temperature for approximately 15 to 30 minutes. If there's still no reaction, incubate further at 4°C for 15 minutes.
  - Autocontrol and O cells must be used to rule out naturally occurring cold autoantibodies OR
  - Adding one or two drops more plasma or serum to the test
  - OR Use Anti-A1 lectin, test serum against additional A1, A2 and O cells
  - Perform saliva studies or adsorption-elution

CHIMERISM

- Presence of two cell population
- **TRUE**
  (both cell population recognized as self)
  - Twins
  - Mosaicism (due to dispermy)
- **FALSE**
  (only original cell population recognized as self)
  - Blood transfusions
  - Transplanted bone marrow
  - Exchange transfusion
  - Feto-maternal bleeding

EXAMPLE

Cell Grouping			Serum Grouping		
Anti-A	Anti-B	Anti-A,B	A1 cells	B cells	O cells
3+	0	3+	0	0	0
Autocontrol = 0					

EXAMPLE

Cell Grouping			Serum Grouping		
Anti-A	Anti-B	Anti-A,B	A1 cells	B cells	O cells
0	0	0	0	0	0
Autocontrol = 0					

GROUP II- LEAST FREQUENT

1. Subgroups of A and/or subgroups of B
2. Weakening of antigens due to disease (leukemias, Hodgkin's disease)
3. "Acquired B" phenomenon
4. Excess amount of blood group specific soluble subtances (BGSS) secondary to stomach and intestinal cancer (neutralizes reagent Anti-A and Anti-B antisera)
5. Presence of antibodies to low incidence antigens in reagents anti-A and Anti-B
6. Chimerism (due to presence of two cell population, minor population less reactive to antisera)

GROUP II- LEAST FREQUENT

RESOLUTION:

- Verification of patient's clerical information.
- Review of patient's profile and medical history.
- Enhancement of the reaction by incubating the reagent red cells with patient's serum at room temperature for about 15-30 minutes.
- Further enhancement by incubating the samples and reagents at 4 degrees Celsius for 15 minutes.
- Autocontrol and O cells must be checked to rule out the presence of autoantibodies.

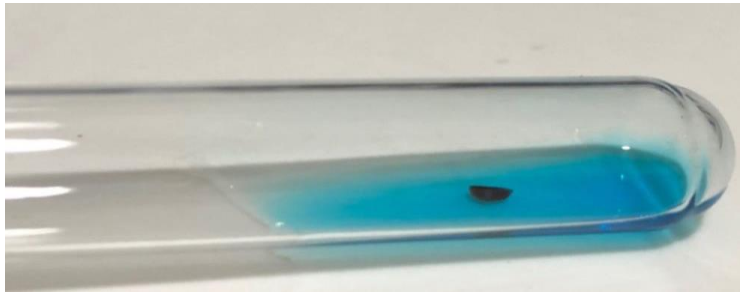
GROUP II- LEAST FREQUENT

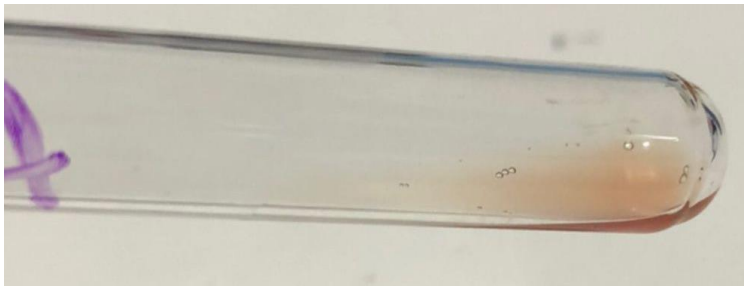
RESOLUTION:

- For acquired B phenomenon, the following must be considered:
  - acidified antiserum and patient's own anti-B will not agglutinate acquired B cells
  -monoclonal anti-B ES4 clone should not be used
  -reacetylation of acquired B cells using acetic anhydride
    -secretor studies may be performed
- For the presence of excess BGSS, patient red cells must be washed.
- Forward typing may be repeated using antisera with different lot number.

EXAMPLE

Cell Grouping			Serum Grouping		
Anti-A	Anti-B	Anti-A,B	A1 cells	B cells	O cells
4+	2+	3+	0	4+	0
Autocontrol = 0					





FORWARD GROUPING:
ANTI A=4+
ANTI B=1+
ANTI-D= 4+

REVERSE GROUPING:
KA= 0
KB=3+

Group III

1. Elevated levels of globulin from certain diseases states
   - Multiple myeloma, Waldenstrom's macroglobulinemia, Plasma cell dyscrasias and advanced hodgkin;s lymphoma
2. Elevated fibrinogen levels
3. Plasma expanders such as dextran and polyvinylpyrrolidone
4. Wharton's Jelly

GROUP III

RESOLUTION:

- Verification of patient's clerical information.
- Review of patient's profile and medical history.
- Wash red cells 2-3 times and cord blood cells 6-8 times.
- Perform saline replacement technique to differentiate rouleaux from true agglutination.

EXAMPLE

Cell Grouping			Serum Grouping		
Anti-A	Anti-B	Anti-A,B	A1 cells	B cells	O cells
4+	4+	3+	2+	2+	2+
Autocontrol = 0					

GROUP IV

- caused by <u>miscellaneous problems</u>

1. Polyagglutination
2. Cold reactive antibodies (spontaneous agglutination regardless of the antisera specificity)
3. Warm autoantibodies
4. Unexpected ABO isoagglutinins
    - Anti-A1 in A2 and A2B individuals
    - Anti-H in A1 and A1B individuals
    - Anti-A or Anti-B in weak subgroups

GROUP IV

5. Unexpected non-ABO alloantibodies (reagent cells do not only contain A and B antigens!)

6. Patient has antibody to acriflavine (present in Anti-B antiserum). Anti-acriflavin and acriflavin form a complex that spontaneously agglutinates

7. RBC's with the cis "AB Phenotype"

POLYAGGLUTINATION

- RBC agglutinates with ABO compatible sera due to altered membrane component
- **TYPES:**
  - **A. ACQUIRED POLYAGGLUTINATION**
    - **a) Microbial-associated: -Hubner-Thomas-Friedenrich- Phenomenon**
      1. **T-polyagglutination**
         - T-cryptic antigen
         - Organism with neuraminidase
           - *V. cholerae*
           - *Influenza* virus
           - *Pneumococci*
           - *E. coli*
           - *Clostridium*

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2. **TK-polyagglutination**
   - Organisms with B-endogalactosidase
     - *Bacteroides fragilis*
     - *Serratia marcesens*
     - *Candida albicans*
3. **Acquired B phenomenon**
   - Organisms with D-acetylase
     - *E. coli* O86
     - *P. vulgaris* OS19
     - *Clostridium tertium*
4. **Th-polyagglutination**
5. **VA-polyagglutination**

b) **Non-microbial associated Polyagglutinatio**n
   - Tn polyagglutination-acquired A phenomenon

B. **INHERITED POLYAGGLUTINATION**
   - **CAD: Cazal Autosomal Dominance**
     - Acquired A
     - Associated with SID antigen
   - **HEMPAS: Hereditary Erythroblastic** Multinuclearity with Positive Acidified Serum Test
     - Related with I antigen on CDA type II normoblast
   - Hemoglobin M-Hyde Park
   - NOR

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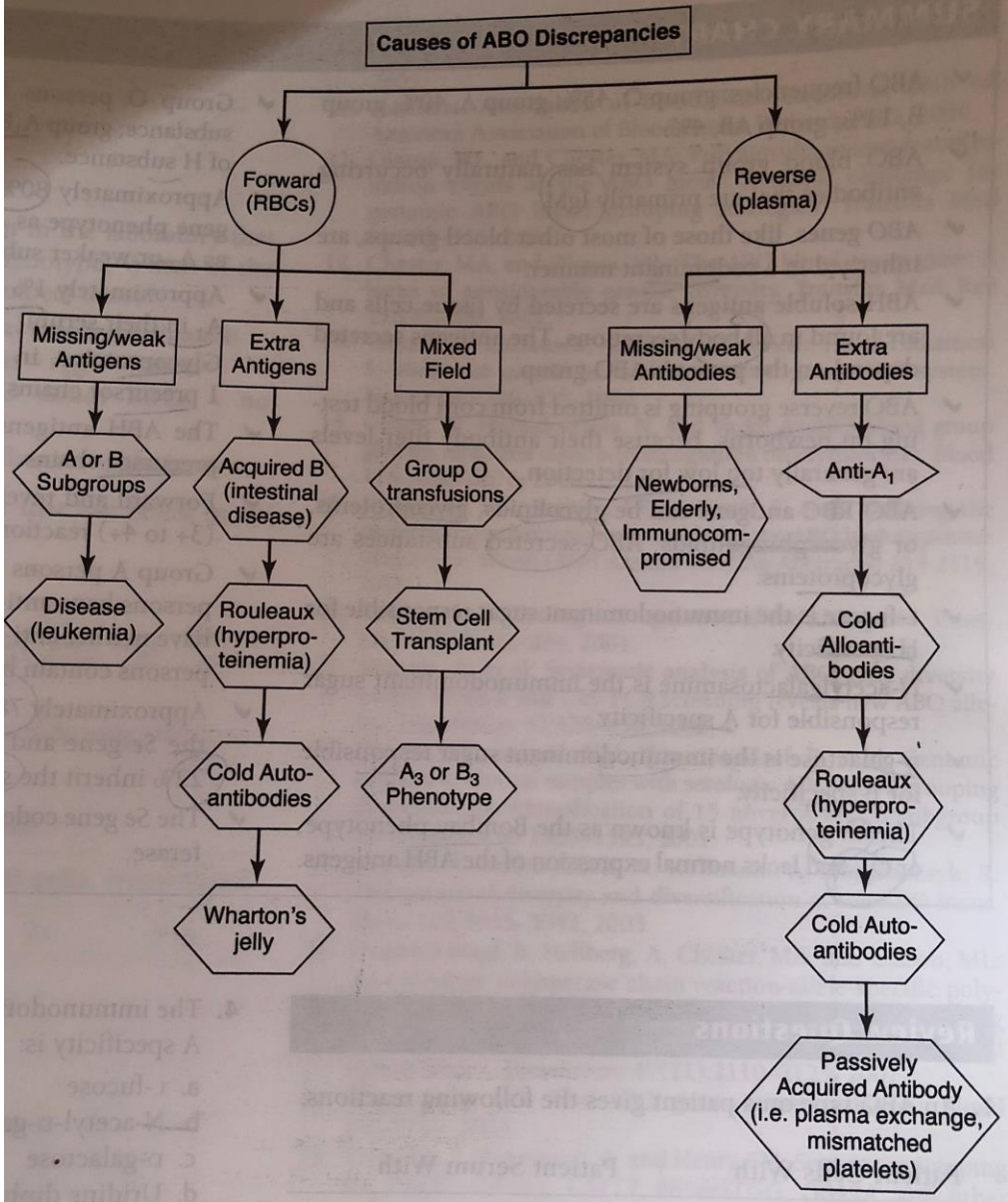
- **RESOLUTION**
  - For polyagglutination, perform lectin studies
  - For cold reacting antibodies, incubate at 37 C, then wash 3x with NSS heated to 37 degrees C then repeat blood typing
    - if there is still a problem, then use DTT or 2-ME
  - For warm reacting antibodies, perform elution at 45 degrees C
  - For antibodies other than Anti-A and Anti-B, perform Antibody Screening and Identification then repeat typing

EXAMPLE

Cell Grouping			Serum Grouping		
Anti-A	Anti-B	Anti-A,B	A1 cells	B cells	O cells
4+	4+	3+	1+	0	0
Autocontrol = 0					



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