<table>
<thead>
<tr>
<th>Forward Grouping</th>
<th>Reverse Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Px RBC (Ag)</td>
<td>Px Serum (Ab)</td>
</tr>
<tr>
<td>Commercial Antiserum Reagent (Ab)</td>
<td>Known RBC reagent (Ag)</td>
</tr>
<tr>
<td>(Anti-A &amp; Anti-B)</td>
<td>-Human Source</td>
</tr>
<tr>
<td>-Monoclonal Ab</td>
<td>-4-5% red cell suspension</td>
</tr>
<tr>
<td>-Highly specific</td>
<td>-1 drop</td>
</tr>
<tr>
<td>-igM</td>
<td>-2+ to 4+ reaction</td>
</tr>
<tr>
<td>-Anti-A (Clear blue)</td>
<td></td>
</tr>
<tr>
<td>-Anti-B (Clear yellow; acriflavine dye)</td>
<td></td>
</tr>
<tr>
<td>-3+ to 4+ reaction</td>
<td></td>
</tr>
<tr>
<td>-1-2 drops</td>
<td></td>
</tr>
</tbody>
</table>

**ABO BLOOD GROUP SYSTEM**

**Causes of Fatal HTR due to ABO incompatible blood transfusion:**
1. Recipient ID error at transfusion time (NURSE’S)
2. Patient sample label switched (PHLEB’S)
3. Sample collected from incorrect patient (PHLEB’S)
4. Patient sample mistyped (LAB’S)

**Gene/Ag AB: 600k chain and confer blood group specificity**

- **ABH Antigen production**
  - Birth: too low for detection
  - 3 – 6 months: invalid (some/all may be maternal IgG that have crossed placenta)
  - 5 - 10 years: peaks
  - Later in life: declined

**Inheritance of the ABO blood group**
- **O gene:** amorph (no detectable antigen)
- **A and B phenotypes:** autosomal recessive

**Determination of exact blood group genotype:** Family studies / Molecular Assays

**FORMATION OF A, B, and H Red Cell Antigen**
- 3 separate loci: ABO, H, Se → produce specific glycosyltransferase
  → Add sugars to a basic precursor substance

**H Antigen**
- Precursor structure on A and B Antigen
- Located on chromosome 19
- Linked with Se genes
- Not part of ABO system
- Its inheritance influences A and B Ag expression
- Must be inherited to form ABO antigen on RBC

**Se Genes**
- Located on Chromosome 19
- Not part of ABO system
- Its inheritance influences A and B Ag expression
- Must be inherited to form ABO antigen in secretions

**RBC:**
- **TYPE 2 (terminal galactose)** → beta 1→4 → N-acetylgalcosamine
  → oligosaccharide chain → ABH Antigen
- **TYPE 1 (galactose)** → beta 1→3 → N-acetylgalcosamine

**ABH Antigen production**
- Fetal life: developed
- Gestational period: weak strength
- Newborn: 25-50% (weak reaction)
- 2-4 years: fully developed
- Life: remains constant

**INTERACTION OF Hh and ABO Genes**
- **Blood Group O**
  - HH/HH + 2 O genes
- H gene → α-2-L-fucosyltransferase → fucosyl – Oligosaccharide (terminal galactose)
- **Immunodominant sugar:** sugars that occupy terminal position of the precursor chain and confer blood group specificity

<table>
<thead>
<tr>
<th>Gene/Ag</th>
<th>Glucosyltransferase</th>
<th>Immunodominant Sugar</th>
<th>Ag sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>α-2-L-fucosyl transferase</td>
<td>Fucose</td>
<td>H</td>
</tr>
<tr>
<td>A</td>
<td>α-3-N-acetylgalactosaminy transferase</td>
<td>N-acetyl-D-galactosamine</td>
<td>810k-1170k</td>
</tr>
<tr>
<td>B</td>
<td>α-2-D-galactosyl transferase</td>
<td>D-galactose</td>
<td>610k-830k</td>
</tr>
</tbody>
</table>

**O gene:** amorph
- does not elicit production of catalytically active polypeptide transferase
- H substance remains unmodified
- has the highest H antigen concentration

**hh gene:** rare.
- doesn’t elicit production of α-2-L-fucosyltransferase
- not added to type 2 chain
- no H substance on RBC

- “Bombay” phenotype that lacks normal expression of ABH antigen because of hh genotype.

**FORMATION OF A, B, H Soluble Antigens**
- **ABH Antigen:** RBC, endothelial cell, platelet, lymphocyte, epithelial cell
- **ABH Soluble antigen:** body secretion
  → dependent on ABO genes and Sese gene (secretor gene)
  • Se
  **Secretor (secrete glycoprotein):**
  1) produce α-2-L-fucosyltransferase
  2) to modify type 1 precursor in secretion
  3) to form H substance
  4) can be modified to express A and B substance
  - Doesn’t affect the formation of ABH antigen on RBC
  - determine ABH soluble substance secretion
  • sese
  **Non secretor**

**Oligosaccharide:**
- Type 1 & 3 → more abundant
- Type 2 & 4 → RBC membrane
- **Diff:** linkage position of galactose (gal) to N-acetylgalcosamine (Glcnac)
  - **Type 1:** beta 1→3 linkage
  - **Type 2:** beta 1→4 linkage

**ABH Antigen on RBC**
- **ABH Antigen:**
  - Glycolipid, glycoprotein, glycosphingolipids
  - Synthesized on type 2 precursor chain
  - **Type 2 chain** (beta 1→4 linkage)
  - **Type 1 chain** (beta 1→3 linkage)
  - Enzyme produced by H gene:
    - α-2-L-fucosyltransferase
    - acts on Type 2 chain in RBC membrane
    - Secreted substance:
      - Glycoprotein
      - Synthesized on type 1 precursor chain
      - Type 1 chain (beta 1→3 linkage)
      - Enzyme produced by Se gene:
        - α-2-L-fucosyltransferase
        - acts on type 1 chain in secretory tissue

**Fluids of ABH Substance:**
- Saliva, tears, urine, digestive juice, bile, milk, amniotic fluid, pathological fluid

**ABO SUBGROUPS**
- A subgroup
  - more common than B subgroup

<table>
<thead>
<tr>
<th>A1</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>elicit high concentration of enzyme</td>
<td>240-290k</td>
</tr>
<tr>
<td>α-3-N-acetylgalactosaminy transferase</td>
<td>Immunodominant sugar:</td>
</tr>
<tr>
<td>810-1170k</td>
<td>N-acetyl-D-galactosamine</td>
</tr>
<tr>
<td>Immunodominant sugar:</td>
<td></td>
</tr>
<tr>
<td>N-acetyl-D-galactosamine</td>
<td></td>
</tr>
</tbody>
</table>

**Anti-Aa**
- naturally occurring IgM cold-reacting
- unlikely to cause transfusion reaction
- reacts below 37°C

**Blood group** | **Antigen present** | **Antibody** | **Anti- Aa Lectin** | **Substance in Secretion** | **Structural char** |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>A1, A</td>
<td>Anti-B</td>
<td>+</td>
<td>A, H</td>
<td>A1, Aa, A2, A3, A4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hg Ag site</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>A</td>
<td>Anti-B, Anti-A1</td>
<td>(1-8%)</td>
<td>O</td>
<td>A, H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hg Ag</td>
<td></td>
</tr>
</tbody>
</table>

In some A1 individuals:
- A1 = extremely low, produce Anti-Aa
- A2 = completely lacking, produce Anti-Aa

**Lectins** – seed extracts that agglutinate human cells
- Dolichos biflorus – agglutinates A1 or A2
- Bandeiraea simplifolia – agglutinates B cells
- Ulex europaeus – agglutinates O cells (H specificity), and other blood group depending on amount of H antigen (differentiate Real O and Bombay)
WEAK A SUBGROUPS

Characteristics:
- Decreased Rf of A antigen sites per RBC (results in week/no agglutination with anti-A)
- Increased variability in detectability of H antigen
- Strong reaction with anti-A
- Presence/absence of Anti-A in serum

Test: secretor studies, adsorption-elution tests, molecular testing

Serology test:
- Forward grouping of A & H antigen with anti-A, anti-A,B, anti-H
- Reverse grouping with ABO isoagglutinin and presence of anti-A
- Adsorption-elution tests with anti-A
- saliva studies (detect & A H substance)

Molecular testing (mutation/serum glycosyltransferase studies, detect A enzyme)

<table>
<thead>
<tr>
<th>Reaction with Ab Reagents</th>
<th>Presence of Ab in serum</th>
<th>Antigen in secretion</th>
<th>A transferase in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-AB</td>
</tr>
<tr>
<td>A</td>
<td>++ref</td>
<td>3+</td>
<td>0/ wk</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>H</td>
<td>Sometimes</td>
</tr>
<tr>
<td>A</td>
<td>wk</td>
<td>with anti-A, B</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>B</td>
<td>Anti-AB</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>H</td>
<td>No agglutination</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>H</td>
<td>A, H</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>H</td>
<td>No agglutination</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>H</td>
<td>A, H</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>H</td>
<td>no agglutination</td>
</tr>
</tbody>
</table>

WEAK B SUBGROUPS

Criteria:
- Strength & type of agglutination with anti-B, anti-A,B, anti-H
- Presence of ABO isoagglutinins in serum
- A/E studies with anti-B
- Presence of B substance in saliva

Molecular testing

THE BOMBAY PHENOTYPES (O0)

Bombay - Bombay, India

- Inheritance of a double dose of the h gene (hh)
- ABO genes cannot be expressed
- ABH antigens cannot be formed
- No H antigen
- Cannot react with anti-A, anti-B, anti-H
- Don’t react with anti-H lectin (Ulex europeus)
- Can only be transfused with O, B individual
- Reacts strongly in 37°C
- IgM antibody + Complement RBC Lysis
- Autosomal recessive
- Mutation in gene FUT1 (H gene) produce silenced gene, incapable of coding α-1,2-fucosyltransferase.
- Anti-ABH present in serum

ABH ANTIGEN AND ANTIBODIES IN DISEASE

- RBC alteration → weak reaction, pseudoantigen (forward grouping)

HYPER EMA, CHROMOSOME 9 TRANSLOCATION, HEMOLYTIC DISEASE, HODKIN’S DISEASE

- Induce stress hematopoiesis → depress antigen strength (mixed field agglutination)

Weak isoagglutinins (anti-A, anti-B, anti-AB) ---- simple serum protein electrophoresis

- Leukemia (hypogammaglobulinemia)
- CLL
- Congenital agammaglobulinemia

Increased permeability of intestinal wall (allow passage of bacterial polysaccharides from E.coli O15 into circulation):

- Intestinal obstruction
- Carcinoma of colon or rectum
- Lower intestinal tract disorder

Lack of detectable ABO antigen: (same RBC, serum contains excessive BGSS that may neutralize antisera)
- Stomach cancer
- Pancreas cancer

ABO DISCREPANCIES

- When forward vs reverse
- Repeat test with RBC suspended in saline
- Unexpected reaction

TECHNICAL ERROR (CLERICAL)

- Sample/px misID
- Error in recording results
- Mislabeling of test tubes
- Transcription error
- Computer entry error
- Simple mix up

CRITICAL ELEMENTS

- Serum:Cell ratio
- Media
- Temperature
- Incubation time
- Sample type
- Centrifuge rpm

REAGENT/EQPT VARIABLE

- Outdated reagents
- Inappropriate storage temperature
- Contaminated reagents
- Uncalibrated centrifuge
- Warming of samples during cent

PROCEDURAL ERROR

- Improper cell suspension
- Reagents not added
- Failure to recognize hemolysis
- Misinterpretation of results
- Don’t follow manufacture

Clotting deficiency: allow serum to clot completely, add protamine sulfate to neutralize heparin, add thrombin to induce coagulation.

# Hemolysis: maybe caused by bacterial contamination

Acquired Pk info:
- Age, Dx, Transfusion history, Medication, history of pregnancy
Group I
Reverse grouping (weak/missing Ab)
- Newborn (undetectable until 4-6 months)
- Elderly (depressed)
- Leukemia (hypogammaglobulinemia)
- Congenital/acquired agammaglobulinemia
- Immunodef. Dse
- BM/Stem cell transplantation
- Ab have been diluted by plasma/exchange transfusion
- ABO subgroups
- Newborn (undetectable until 4-6 months)
- Elderly (depressed)
- Leukemia (hypogamma globulinemia)
- Congenital/acquired agamma globulinemia
- Immunodef. Dse
- BM/Stem cell transplantation
- Ab have been diluted by plasma/exchange transfusion

Group II
Forward grouping (weak/missing Ag)
- subgroup of A/B
- leukemia
- Hodgkin's die
- acquired B phenomenon (bacterial enzyme modify N-acetyl-D-galactosamine to D-galactosamine)
- ESA/monoclonal anti-B clone * acquired B antigen
- Anti-B (pH 6-8) * acquired B antigen

Group III
Forward & Reverse (Rouleaux/pseudo agg)
- Protein plasma abnormalities
- Multiple Myeloma, Waldenstrom's macroglobulinemia, plasma cell dyscrasias
- Elevated fibrinogen
- Plasma expanders (dextran, polyvinylpyrrolidone)
- Wharton jelly in cord blood

Group IV
Forward & Reverse (MISC)
- Cold reactive Autoantibodies
- > 1 ABO group circulating RBC (RBC transfusion/ transplant)
- Unexpected ABO isoagglutinin
- Unexpected non-ABO alloantibodies

RARE GROUP II DISCREPANCIES
- Excess BGSS in plasma neutralizes anti-A & anti-B reagent
  ---- false (-) or weak reaction
- Antibodies to low incidence antigen
  ---- weak or missing reaction
- Chimerism = presence of 2 cell population in a single individual (twins)
  O mom, B dad → O + B (mixed field reaction)
  ---- True Chimerism (rare)
  ---- Vascular anastomosis → causes in utero exchange of blood
  ---- Dispermy (not twins) = 2 sperms fertilizing 1 egg (mosaicism)
  ---- Blood transfusion (O to A/B)
  ---- Transplanted BM or PB stem cell of different ABO
  ---- Exchange transfusion
  ---- Fetal maternal bleeding

RARE GROUP IV DISCREPANCIES
- Cis-AB : Inheritance of AB & O gene (Japan)

<table>
<thead>
<tr>
<th>FORWARD</th>
<th>REVERSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missing/weak Antigen</td>
<td>Extra Antigen</td>
</tr>
<tr>
<td>(II) Leukemia</td>
<td>(III) Rouleaux</td>
</tr>
<tr>
<td>(IV) Cold Auto Ab</td>
<td></td>
</tr>
<tr>
<td>(III) Wharton's jelly</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Passive acquired antibody