Introduction to
ABO & Rh Blood Group System

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The discovery of ABO(C>O) blood group system in 1900 by Karl Landsteiner leads to safe blood transfusion.

O = German word “Ohne” which means “without”

Rhesus blood group system discovered in 1939 by Levine & Stetson.

Chromosome location of ABO & Rh Blood Group alleles.
Hence, Blood groups are inherited.
Chromosomal Location of Some Blood Groups

- **ABO Allele** - 9q
- **Se, H allele** - 19q
- **Le allele** - 19p
- **Rh allele** - 1p

Photo: Adapted from M A Ferguson-Smith 1985
ABO **Histo**-Blood Group system (Hakomori et al)

Presence of blood group antigens on organs and tissues other than blood → important for tissue transplantation.

Current Practice – Organ transplant must be ABO compatible in standard practice because of the powerful complement fixing effect of ABO antibodies on endothelial cells.

**Technical note**: Wash red cells if weak reaction encountered in blood group testing because of soluble blood group substances in plasma.

Remarks: Wash away matrix e.g. Wharton’s jelly.
To date, there are 33 blood group systems found on human red cell surface.

ABO & Rh(D) remain clinically the most significant blood groups -- Why?
- Highly immunogenic

Accurate typing of ABO & Rh(D) blood groups is of utmost importance in blood transfusion

-- The importance of typing ABO & Rh (D) 2 times ensures correct patient blood group;

Importance of Blood Bank LIS & BTNS blood group records

ABO & Rh (D) typing in the Type & Screen (T&S) test.

T = ABO & Rh(D) typing
S = Antibody screen
Blood Group & Blood Transfusion

Blood transfusion complement with anaesthesia and antibiotics & aseptic technique lead to the advancement of surgery in the last century.


# Basics of ABO blood group system

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Antigen on RBC Surface</th>
<th>Antibody in plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O/O</td>
<td>H</td>
<td>Anti-A, B</td>
</tr>
<tr>
<td>A</td>
<td>A/A or A/O</td>
<td>A</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>B/B or B/O</td>
<td>B</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>A/B</td>
<td>A &amp; B</td>
<td>None</td>
</tr>
</tbody>
</table>
Normal ABO blood group reactions

<table>
<thead>
<tr>
<th>ABO Group</th>
<th>α</th>
<th>αβ</th>
<th>β</th>
<th>A Cells</th>
<th>B Cells</th>
<th>O Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AB</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Range of Reaction Score = 0-12
Configuration of ABO/Rh(D) Cassette/Gel card

• ABO/Rh(D) **Cassette/Gel Card**

• $\alpha/\beta$ /anti-D /Ctl /A cells/ B cells

• **Newborn Cassette**
  $\alpha/\beta$ / $\alpha/\beta$ / anti-D /Ctl / **IgG**

• **Newborn Gel Card**
  $\alpha/\beta$ / $\alpha/\beta$ / anti-D /Ctl / **DAT**
Forward & reverse reactions -- counter-checking
Anti-AB ? Redundant; Not a must; double checking

Why O cells used in reverse plasma grouping?
The EQAPs include O cells;

**Pre-requisite** of ABO & Rh(D) typing:

--Patient identification (Patient name in English & HKID no.)

Why this is important?
# ABO Blood Group Distribution in Different Races

<table>
<thead>
<tr>
<th></th>
<th>HK Chinese</th>
<th>Japanese</th>
<th>Indians</th>
<th>Caucasian</th>
<th>Blacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>40.6%</td>
<td>29.4%</td>
<td>38.5%</td>
<td>45%</td>
<td>49%</td>
</tr>
<tr>
<td>A</td>
<td>26.0%</td>
<td>39.1%</td>
<td>22.9%</td>
<td>40%</td>
<td>27%</td>
</tr>
<tr>
<td>B</td>
<td>26.7%</td>
<td>21.5%</td>
<td>32%</td>
<td>11%</td>
<td>20%</td>
</tr>
<tr>
<td>AB</td>
<td>6.7%</td>
<td>10.0%</td>
<td>6.6%</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Rh(D)</td>
<td>99.7%</td>
<td>99.5%</td>
<td>95.9%</td>
<td>85%</td>
<td>92%</td>
</tr>
</tbody>
</table>

Use of group AB FFPs & O neg red cells  
For what?

Mechanism affecting population frequency of alleles  
- Natural selection  
- Random genetic drift  
- Inter-locus sequence exchange

From Strachan T Human Molecular Genetics 2
Patient Identification

Name & HKID number (2 major independent identifiers)

-- Procedure of double checking

Pre-analytic phase - Why Ward UPI system is so important ?

Analytic phase ? Full Automation

Post-analytic phase of the ABO & Rh(D) typing

-- Result Checking
Reagent Requirements:

2\textsuperscript{nd} ABO & Rh(D) typing: different companies grouping anti-sera

Anti-D does not detect partial D VI ; check package insert whether control shall be included

Anti-B avoid using ES4 clone because it detects \textbf{Acquired B}

\textbf{B(A) phenomenon} avoid using anti-A made with MH04 clone
Acquired B Phenomenon

- RBCs react weakly with anti-B (ES-4 clone) due to Microbial deacetylase which converts A immunodominant sugar N-acetylgalactosamine to galactosamine.
B(A) Phenomenon

- Detects by anti-A produced by MH04 clone; usu < 2+ reaction;

- Resolution – Test with non-MH04 anti-A;

- Autosomal dominant phenotype associated with polymorphism (Pro234Ala) and (Ser235Gly) in the B transferase;

- Capable of attaching A determining sugar to fucosylated molecule.
**Blood Sample** -- Clotted blood Vs EDTA blood; which is better? What is the difference between the two?

Consider automation and clotting time of clotted blood affect TAT of T&S test

Automation – use EDTA blood sample primarily

Use different brands of monoclonal ABO/D reagents in the typing ABO & Rh (D) 2 times -Why?

If manual method is used, perform ABO & Rh typing by 2 different technical staff.

What is the advantage of automated ABO/Rh typing?
ABO reagent cells for reverse blood group

1) Home made ABO reagent cells are usually Rh(D) positive. If use clotted blood, resuspend your ABO cells with EDTA-saline. Why?

2) Commercial ABO reagent cells are usually Rh(D) negative

3) Which one is better?

A2 cells
Anti-A1 occurs in approx 20% of group A2B & 2% group A2 sera. This will react with group A1 cells but not group A2 cells.
Commonly Used Methods for ABO/Rh(D) Typing

1) Perspex tile technique (still in use; good for donor group check);

2) Tube technique (plastic or glass);

3) Automated Gel card/ Cassette ABO/Rh(D) typing. (Both are CAT)

Which technique(s) to use?

CAT = Column Agglutination Technology
QC materials

ABO group - Use weak ABO group cells; commercial kit available

Rh(D) group - R₁r cells; weaker expression of D antigens

EQAP consider join both local & foreign EQAPs -- why?

Aims: To achieve accurate ABO/Rh(D) blood group results
Neonate ABO blood grouping

Baby < 4 months of age; what to type in neonate ABO group?

If baby ABO & Rh(D) type is Group AB+, what further test you would do?

Reading & Scoring of ABO & Rh(D) blood grouping (This is very important.)

Recording of results -- Write the reaction scores first and then interpret the blood group. Why?
Rh (D) Reporting

- D +ve
- D neg

- The following send to Reference Lab for confirmation:
  - Weak D pos
  - Partial D (Partial DVI common in Chinese; other commonly Partial D seen in HK include Partial DV & DFR)
  - DEL phenotype (Seldom encounter in Blood Bank)

- Crossmatch Recommendations:
  - If weak D/ Partial D, give Dneg blood.
ABH Variants

Consider A/B variants if red cell A/B antigens react with anti-A/-B with reaction score not equal to 12 or 0.

A variants – A2, A3, Ax, Am, Ael

B variants – B3, Bx, Bv, Bm, Bel

Cis-AB
Use of anti-A1 lectin (Dolichos biflorus)

Anti-A hel – differentiate between A1, A1B, A2, A2B from A3, A3B or weaker variants.

H variants – ParaBombay A,B,AB,O
Use of Anti-H lectins (Ulex europaeus)

Crossmatch Recommendations:
A variants with anti-A, give O red cells.
B variants with anti-B, give O red cells.
A/B variants without anti-A/-B, give A/B red cells respectively.
Common Abnormal/ Wrong ABO & Rh(D) results

1) Check equipment esp centrifugation setting, QC, reagent expiry etc;

2) Patient uses other person HKID;

3) Wrong patient samples;

4) Leukaemia patient;

5) After BMT;
6) Cold agglutinins;

7) transfusion of group O blood or AB patient transfused with A/B/O blood;

8) IVIg infusion;

9) Incorrect Cell suspension concentration;

10) Forget to add reagents;

11) Wrong recording of results.
First step of Resolution of ABO &Rh(D) typing anomalies:

1) Repeat blood typing

2) Wash patient's red cells times and re-type

3) Weak reverse ABO groups -- use 4:1 serum: cell ratio

4) Rouleaux formation

5) Use glass tubes
Typing Donor Blood Units

• Rh(D) + blood unit – Type ABO group

• Rh(D) - blood unit – Type ABO & Rh(D) group
Thank you for your attention