

# **Division of Blood Transfusion Services**

**Ministry of Health and Family Welfare**



# ABO Grouping Discrepancies

# ABO Grouping Discrepancies

Anomalous results in blood group testing i.e. where forward and reverse grouping fail to tally with each other. It can be due to:-

- Technical discrepancy
- Clinical Discrepancy

# 1. Technical Discrepancy

- Clerical errors
- Missed identification of blood specimen
- Mixing of blood samples
- Contaminated reagents or not following manufacturer's instructions
- Non-calibrated centrifuges
- Cells suspension either too light or too heavy
- Contaminated or dirty glass wares

## 2. Clinical Discrepancy

- Here the problem lies in the patient. To solve this type of discrepancy essential information regarding patient's age, diagnosis, transfusion history, H/o medication and pregnancy must be taken into account.
- ABO blood group discrepancies are mainly divided into 4 major groups.

# ABO Discrepancies

# Group I Discrepancy

This type of discrepancy is most common compared to the other groups. It is mainly seen in reverse grouping due to weak / missing antibodies.

Some of the common conditions associated with this type of discrepancy are:-



# Group I Discrepancy (contd...)

- Newborns
- Elderly patients
- Patient with Leukemia or Lymphoma
- Patients on immunosuppressive drugs
- Patients with immunodeficiency diseases
- Patients with bone marrow transplant



# Resolution

- For newborns, only forward grouping is done till 4 months of age.
- These discrepancies can be solved by enhancing the serum grouping reaction. This can be achieved by incubating the cells serum mixture at low temperatures ( $4^{\circ}\text{C}$  for 15-30 min) or by prolonging incubation at room temperature ( $\frac{1}{2}\text{hr}$ -1 hr at  $22^{\circ}\text{C}$ )

# Group II Discrepancy

This is due to missing or weak antigens. This type of discrepancy is seen least commonly.

The causes are:

- Subgroups of A or B
- Leukemia and Lymphoma
- Excess antigen of blood group soluble substances
- Acquired A or B antigens.

# Resolution

Subgroups of A or B can be solved by:

- Repeating blood grouping by using washed cells
- Use of anti AB antisera and anti-A<sub>1</sub>lectin
- Adsorption elution

# Adsorption and Elution Technique

Adsorb the three times washed test cells with polyclonal O and B sera (4°C for 1/2 to 1 hr)



Centrifuge at 3000 rpm for 5 min



Discard the supernatant



Wash adsorbed cells 6 times with NS and retain the last supernatant in separate test tube



Heat elution done at 56°C for 10 min with equal volume of packed RBCs and 6% BSA



# Adsorption and Elution Technique (contd...)

Centrifuge at 3000 rpm for 5 min



Separate the eluate in separate test tube and check in parallel with the last supernatant wash; using three un pooled A, B and O cells



Agglutination with A cells and no reaction with B or O cells suggest subgroups of A

- Similarly, subgroups of B are detected using O and polyclonal sera

# Group III Discrepancy

This is due to proteins or plasma abnormalities. The causes in this group are:

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

# Resolution

The main problem is due to Rouleaux formation, which is resolved by washing the cells with normal saline 6-8 times, and confirming it with microscopic examination.

If the serum/reverse grouping is affected, perform saline replacement technique:

- Reagent cells and patient serum centrifuged to allow antigen and antibody to react
- Serum is removed and replaced by an equal volume of saline (saline disperses cells)
- Tube is mixed, centrifuged, and reexamined for agglutination

# Group IV Discrepancy

- Polyagglutination: this is due to exposure of hidden erythrocytes antigens (T antigen in bacterial or viral infections)
- Patient with cold auto antibodies
- $A_2$  or  $A_2B$  individual with anti  $A_1$  antibodies
- Naturally occurring or irregular antibodies reacting at room temperature
- Cis-AB



# Resolution

## Poly agglutination

- Symptoms suggestive of infection
- Auto control negative
- DAT negative
- Use of various lectins (*Glycine soja*, *Arachis hypogea*)

# Cold auto agglutination

- Warm saline washes (at 37°C - 40°C) of auto agglutinated cells
- Pre warming of sera and reagent cells at (37°C)
- Performing the test at 37°C

Most of the auto agglutinins are removed by above techniques; if not Di Thio Threitol (DTT) 0.01M can be used to make cells free (equal quantity of 0.01 M DTT and washed packed test cells at 37°C for 15 min)