ABO Blood Group Discrepancies: Causes And resolution

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Introduction
The ABO system contains four major ABO phenotypes: A, B, O and AB. The four phenotypes are determined by the presence or absence of two antigens (A and B) on red cells. ABO system is also characterized by the presence or absence of naturally occurring antibodies termed iso-hemagglutinins, directed against missing A and B antigens. It is believed that the immunizing source for such naturally occurring antibodies is gut and environmental bacteria which have been shown to possess ABO like structures on their lipopolysaccharide coats1. Donor blood samples are routinely grouped for ABO at the time of donation. Recipient blood samples are grouped for ABO before transfusion. ABO grouping requires both antigen typing of red cells for A and B antigen (forward grouping or direct grouping) and screening of serum or plasma for the presence of Anti-A or Anti-B isoagglutinins (reverse grouping or plasma grouping). Both direct and reverse grouping are required for donors and patients because each grouping serves as a check on the other. A discrepancy exists when the results of red cell antigen grouping do not agree with serum grouping. The discrepancy may arise because of technical errors or clinical conditions of the patients. All technical factors that may have given rise to the ABO discrepancy should be reviewed and corrected. It is also essential to obtain information regarding the patient’s age, diagnosis, transfusion history, medications, and history of pregnancy. If the discrepancy appears to be due to an error in specimen collection or identification, a new sample should be drawn from the patient and the forward and reverse grouping repeated.

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

**ABO discrepancies may be arbitrarily divided into four major categories -**

**Group I Discrepancies -**
These are associated with unexpected reactions in the reverse grouping due to weakly reacting or missing antibodies. Discrepancies in this group includes

I. **Infants less than 4-6 month of age**\(^2,3\): Anti-A and anti-B agglutinins (IgM) produced by the infant can first be demonstrated at 3–6 months\(^1,4\). Anti-A and anti-B if present in cord sera are usually IgG and are of maternal origin. Reverse ABO grouping in infants is not warranted before 6 months of age\(^2\).

II. **Elderly patients**\(^5,6\): Earlier studies showed a progressive decrease in anti-A and -B agglutinin titers with age, with low levels (titer 4 or less) being common in subjects aged 80 years or more\(^5,7\). However in a study by Maur et al\(^8\) decline in titres was not observed.

III. **Severe hypogammaglobulinemia:** Anti A and Anti B are often present in very low concentration in patients with inherited immunodeficiency and in rare X-linked Wiskott- Aldrich syndrome\(^9\). Anti-A and anti-B may also be present in very low concentration in patients immunosuppressed by therapy or disease and in patients undergoing intensive plasma exchange.

IV. **ABO incompatible HPC transplantation:** ABO incompatible HPC transplantation with induction of tolerance e.g. group A patient receiving group O bone marrow will have circulating group O red cell but will only produce Anti B antibody.
V. In chimerism: That is, a person with dual population of cells from more than one zygote. Presence of two populations of red cells of different ABO group may lead to absence of antibodies\(^1\). Twin chimerism occurs when hematopoietic stem cells migrate between vascular bridges which allow mixing of blood between two fetuses. Chimeric twins have immune tolerance; they do not make against A or B antigens that are absent from their own red cells but present on cells of engrafted twins.

VI. Pediatric patients receiving long term parenteral and enteral nutrition which is sterile and free of bacteria\(^1\). It is believed that the immunizing source for such naturally occurring antibodies is gut and environmental bacteria which have been shown to possess ABO like structures on their lipopolysaccharide coats\(^1\).

**Resolution of group I discrepancies**

a. It can be resolved by enhancing weak or missing reaction by incubating the patient’s serum with reagent A\(_1\) and B cells at room temperature for 15-30 minutes

b. If there is still no reaction after centrifugation, serum cell mixture is incubated at 40\(\text{C}\) for 15-30 minutes

**Note:** an autocontrol and O cell control must always be tested concurrently to detect reactivity of other commonly occurring cold agglutinins e.g. anti I

**Group II discrepancies**
These are associated with unexpected reactions in forward grouping due to weakly reacting or missing antigen. Discrepancies in this group includes

I. **Subgroups of A or B**\(^{11}\): subgroups of A antigen (A\(_x\), A\(_m\), A\(_y\), A\(_e\)) which are not agglutinated or weakly agglutinated by most anti A. subgroups of B antigen (B\(_x\), B\(_m\), B\(_e\)) which are not agglutinated or weakly agglutinated by most anti B. Both can present as group II discrepancies.

II. **Leukaemia may yield weakened A or B antigen**\(^{12}\): In acute leukaemia, the A antigen may be weakened\(^{13}\). Sometimes the blood appears to contain a mixture of group A and group O cells\(^{14,15}\) or of A\(_1\) and weak A\(^{14}\). In other cases the red cells react weakly with anti-A, even behaving like A\(_3\) or A\(_m\)\(^{15}\). In a patient with
erythroleukaemia, of group B, 60% of the cells were not agglutinated by anti-B and appeared to be group O, but were really very weak B, when separated from the normal B cells they would absorb anti-B\textsuperscript{12}.

**III. Acquired B\textsuperscript{16, 17}, B(A) and A(B) phenotypes:** Acquired B’ results from the action of bacterial deacetylase, which converts N-acetylgalactosamine to \(\beta\)-galactosamine, which is very similar to galactose, the chief determinant of B. The second type of acquired B that may be called the ‘passenger antigen’ type is caused by adsorption of B-like bacterial products on to O or A cells but occurs only in vitro\textsuperscript{18}.

**IV. Out of group transfusion or ABO mismatched hematopoietic progenitor stem cell transplantation:** ABO compatible but not identical transfusion of red cell (e.g. group O red cells transfused to group A or B person) results in artificially induced chimerism. ABO mismatched hematopoietic stem cell transplant (e.g. group O person transplanted with group A or B marrow, group A or B person transplanted with group O marrow).

**V. Neutralization of anti A and anti B typing reagent by high concentration of A or B soluble substances in serum with serum or plasma suspended red cell**

**VI. Chimerism in fraternal twins, mosaicism arising from dispermy:** Tetra-gametic or dispermic chimeras present with chimerism in all tissues and are more frequently identified because of infertility and rarely because of mixed populations of red cells

**Resolution of group II discrepancies**

a. Weaker reactions with antisera can be resolved by enhancing reaction of antigen with respective antisera by incubating test mixture at room temperature for 15-30 minutes

b. Sub groups causing group discrepancies can be resolved by adsorption elution studies

c. Acquired B phenomenon can be resolved by lowering P\textsuperscript{H} of monoclonal antisera. Anti B in the serum of acquired B person does not agglutinate autologous red cells (autocontrol negative). Secretor status of person can resolve acquired B, saliva of acquired B person contains A substance not B substance. Serum
of acquired B person contains A substance.

d. High concentration of A or B substance causing group discrepancies can be resolved by saline washing of red cells

**Group III discrepancies**

These are associated with protein or plasma abnormalities, rouleaux formation and pseudoagglutination. Discrepancies in this group includes

I. Elevated level of globulin from e.g. multiple myeloma, waldenstorm macroglobulinemia, Hodgkin lymphoma.

II. Elevated level of fibrinogen.

III. Small fibrin clot in plasma or incompletely clotted serum can be mistaken for red cell agglutinates of reverse grouping.

Principal: patient’s sample with abnormal concentration of serum proteins, altered serum protein ratio, or high molecular weight volume expanders can aggregate reagent red cells and can mimic agglutination. Rouleaux are red cell aggregates that adhere along their flat surfaces, giving a stacked coin appearance microscopically. Rouleaux will disperse when suspended in saline. True agglutination is stable in the presence of saline.

**Group IV discrepancies**

These discrepancies are because of miscellaneous problems. These can be due to-

I. Recent transfusion of out of group plasma containing component.

II. Cold alloantibodies (e.g. anti M) or autoantibodies (e.g. anti I), P\textsuperscript{h} dependent autoantibodies, a reagent dependent antibody (e.g. EDTA, paraben) leading to unexpected positive reaction.

III. Recent infusion of IvIg which can contain ABO isoagglutinins.

IV. Mix field agglutination with circulating red cell of more than one ABO type.

V. Polyagglutination (e.g. T activation) resulting from inherited or acquired abnormalities of red cell membrane with exposure of auto cryptantigen\textsuperscript{20}. The T determinant is normally covered by Nacetylneuraminic acid and can therefore be described as a cryptantigen. The antigen can be exposed by the action of bacterial or viral neuraminidases Anti-T and anti-Tn present in the serum of all subjects except infants, are presumably formed as a reaction to T and Tn present in many Gramnegative bacteria and
vaccines$^{20}$. Very many organisms, including pneumococci, streptococci, staphylococci, clostridia, *E. coli*, *Vibrio cholerae* and influenza viruses are capable of producing this effect *in vitro*. T activation may occur *in vivo*. Usually, this polyagglutinability occurs as a transient phenomenon, disappearing within a few weeks or months of the time when it is first observed. In the past, T activation was almost always detected by finding discrepancies between the results of testing red cells and sera in the course of ABO grouping. Nowadays, monoclonal anti-A and -B are widely used and so T activation seldom causes trouble in blood grouping.

**Resolution of group IV discrepancies**

a. Cold autoantibodies causing false positive reaction in forward grouping can be eliminated by washing of red cell with warm saline. If warm saline fails to resolve, removal of autoantibody with acid glycine EDTA or chloroquine can be used.

b. Autoagglutination causing false positive reaction in reverse grouping can be resolve by incubating at 37°C for 30-60 minutes. It can also be resolved by incubating red cells in presence of either dithiothreitol or 2-mercaptoethanol.

c. Unexpected alloantibodies in the patient’s serum other than ABO isoagglutinins causing group discrepancy is resolved by as soon as antibody is identified (e.g. anti M), reverse grouping should be repeated by A1 and B cell that are negative for that antigen.

d. Unexpected ABO isoagglutinins (e.g. anti A1 in A2 or A2B) producing group discrepancies can be resolved by repeating reverse grouping using at least 3 A1, A2, B, O cell along with autocontrol. Patient’s red cell can be typed with anti A1 lectin from dolichos biflorus to determine subgroups of A antigen.

e. A reagent dependent antibody (anti acriflavine antibody against acriflavi used in Anti B) causing group discrepancy should be resolved by washing person’s red cell with normal saline for at least 3 times.

**Conclusion**

A major reason for the clinical importance of the ABO blood group system is the obligatory presence of isoagglutinins, potent natural antibodies directed against A and B antigens lacking on an
individual’s own red cell (RBC) membranes. Therefore, an individual’s ABO phenotype must be determined both by directly testing the antigens on the RBCs and by testing the plasma for the presence of isoagglutinins. A discrepancy exists when the results of red cell antigen grouping do not agree with serum grouping. When a discrepancy is encountered, results must be recorded, but interpretation of the ABO type must be delayed until the discrepancy is resolved. If the discrepant sample is from a potential transfusion recipient and there is a clinical urgency, it is better to issue group O, Rh- compatible RBCs before the discrepancy is resolved.

**Bibliography**


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