

## Original Research Article

# Study of discrepancies in ABO blood grouping: experience of a tertiary health-care center

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### ABSTRACT

**Background:** An accurate ABO grouping is the most important test which is done in the blood bank. Mistyping can lead to transfusion with ABO incompatible blood which results in severe intravascular haemolysis and may even result in the death of the recipient. An ABO discrepancy implies that the forward or red cell ABO grouping does not agree with the reverse or serum ABO grouping. The study was conducted to evaluate the frequency of ABO blood group discrepancies, to identify main causes of discrepancies, to avoid chances of wrong interpretation of blood group and to mitigate clinical impact associated with mismatch ABO transfusion.

**Methods:** A prospective study of ABO discrepancies and their causes was performed on 25,129 samples of the patients and 13,251 samples of blood donors at the red cell serology laboratory in tertiary care teaching hospital and blood bank over the period from February 2017 to July 2018.

**Results:** ABO group discrepancies were mainly divided in 4 different groups. Out of 51 discrepancies 32 (62.74%) were found in group-IV category, being highest amongst all; 10 (19.60%) in group-II which was second highest; other were 8 (15.69%) in group-I and 1 (1.96%) in group-III category.

**Conclusions:** All discrepancies reported on ABO cell and serum grouping must be investigated further, so that correct blood group is reported, minimizing the chances of transfusion reaction. A note of caution should be mentioned on the blood group card to prevent ABO incompatibility in case of transfusion.

**Keywords:** ABO discrepancies, Alloantibodies, Autoantibodies, Blood transfusion, Subgroups

### INTRODUCTION

The ABO blood group system is most important one with respect to blood transfusion practice, hematopoietic stem cell transplantation and solid organ transplantation. Karl Landsteiner was the first to discover human alloantigen by using conceptually simple experiments in 1900s.<sup>1</sup> ABO blood group system is the most important blood group system in transfusion medicine. This system consists of three antigens A, B and H; and four phenotypes A, B, AB, and O blood groups. These all antigens are present on red cell membrane and also found on other tissues. A feature of the ABO system is the regular occurrence of Anti-A and Anti-B in the absence of the corresponding red cell antigens.<sup>2</sup> Thus, the

individuals with blood group A possess A and H antigens on their red blood cells and demonstrate Anti-B in the serum, the individuals with blood group B possess B and H antigens on red blood cells and demonstrate Anti-A in the serum, in blood group O there is only H antigen on red cells along with Anti-A and Anti-B in the serum, and finally in persons with blood group AB there are A, B and H antigens on red cells with no any antibody (Anti-A and Anti-B) in the serum. Therefore, the ABO system is the only main system in which the reciprocal antibody is expected to be present in the serum of an individual whose red cells lack the corresponding ABO antigen. Even today, transfusion of the wrong ABO group remains the leading cause of death reported to United States Food and Drug Administration

(FDA).<sup>3</sup> Testing to detect ABO incompatibility between a donor and potential transfusion recipient is the foundation on which all other pre-transfusion testing is based.<sup>4</sup> Transfusion medicine is unique among diagnostic laboratory services because of delivery of a biologic product that saves lives but at the same time may be capable of causing death. The delivery of this vital product 'blood', involves many people at different levels and different area of the hospitals. Errors can occur at any point along the way and having check points along the way is to discover these errors before transfusion.

The most common cause of a discrepancy is a technical or clerical error. After this possibility has been ruled out, ABO discrepancies fall into four general categories: 1) Weak- reacting or missing antibodies in the reverse grouping, 2) unexpected or additional antigen reactions in the forward grouping, 3) discrepancy between forward and reverse grouping caused by protein or plasma abnormalities, rouleaux formation, 4) discrepancy in forward and reverse grouping due to miscellaneous problems.

## METHODS

A retrospective study was conducted over period from February 2017 to July 2018, at Blood Bank New Civil Hospital, Surat, Gujarat. In this study, 25,129 samples of the patients who were in need of blood transfusion services and 13,251 samples of blood donors who donated blood in the blood bank (camp and in-house) were included. Blood samples were collected in plain and EDTA vacuette. All specimens were analyzed as soon as possible, or stored at 2-6°C to minimize deterioration of weak antibodies or false reaction due to contamination of the specimen. ABO blood groups of patients and donors were determined by conventional tube method and the cases of discrepancies were recorded and analyzed along with clinical history to classify the discrepancies and resolve them with suitable steps. For complete ABO grouping (forward and reverse) monoclonal anti-A, anti-A1, anti-B, anti-AB, anti-H antisera and A, B, O pooled cells were used.

If discrepancy was seen, demand for new and fresh sample was done and repeat blood grouping done by tube method and column agglutination method; furthermore discrepancies were remain unsolved antihuman globulin test (direct and indirect) and also antibody screening was done using 3 cell panel and if screen came positive than antibody identification was done with 11 cell panel. Monoclonal antisera anti-A, anti-B (ERYCLONE® IgM monoclonal antibody by Tulip diagnostic private limited, India) and anti-AB (BHAT BIO SCAN® IgM monoclonal antibody by BHAT Bio tech India private limited) and Lectin antisera anti-A1 (ERYBANK® DOLICUS BIFLORUS LECTIN by Tulip diagnostic private limited, India) and Anti-H (ERYBANK® ULEX EUROPAEUS LECTIN by Tulip diagnostic private limited, India) were used for forward grouping and in-house pooled A1 cell, B

cell and O cell were used for Reverse grouping for tube method. Whereas, Matrix™ OCTOPLUS forward and reverse grouping card with auto control provided by Tulip diagnostic private limited, India used for column agglutination method. Direct anti-globulin test/Indirect anti-globulin test (coombs), Antibody screening (Matrix™ ERYGEN AS I, II, III by Tulip diagnostic private limited, India) and identification (REAGENS Kft. I to XI Wysocki, Hungary), adsorption, heat elution, saliva testing, enzyme treatment of cells. (LIQUIPAP stabilized, activated papain enzyme by Tulip diagnostic private limited, India) and saline replacement technique are the special techniques used for resolving ABO discrepancy. Various statistics were calculated using Microsoft Excel 2010 software. Data on frequency of ABO blood group were reported in simple percentage and numerical and compared with the similar studies by other authors.

## Inclusion criteria

All the patients and donors samples including EDTA anti-coagulated blood samples collected in EDTA vacuette for forward grouping and clotted blood samples collected in plain vacuette for reverse grouping.

## Exclusion criteria

Haemolysed sample, deferred donor blood samples, clerical error like WBIT (wrong blood in tube), errors due to technical problems (sample insufficiency, reagent errors, equipment errors, sample identification error).

## RESULTS

A total of 13251 donors and 25129 patients were included in the present study. Age of the donors included in the study ranged from 18 to 60 years and for patients it's from 01 day to 90 years. Out of 51 cases of discrepancies; 3 (0.0227%) were healthy donor and 48 (0.1910%) were patients. In the present study, 22 (43.14%) cases were of male gender and 29 (56.86%) were of female gender. In total 29 females; among them 17 females were have pregnancy. Out of 17 cases, 9 were having alloantibody anti-D formation, 2 females were of Bombay blood group, 1 female have subgroup of A was (blood group was A2B), 4 other female patients had alloantibody formation of anti-Leb; anti-E; anti-Jka,K, anti-E,c,Cw,K,S,Fya and 1 female was having cold autoantibody formation. Remaining 12 females had other histories; like sickle cell disease, multiple myeloma, chronic myeloid leukemia (CML), carcinoma of cervix, autoimmune hemolytic anemia, severe anemia, etc.

In present study, out of 51 discrepancies; 9 patients were having significant drug history. Out of 9 patients, 5 (55.56%) patients were having history of anti-D (Rh) immunoglobulin injection given, while 4 (44.44%) patients were having history of steroids which could lead to low antibody titer in reverse typing. Among total 51

discrepancies; 7 patients were having direct anti-globulin test (DAT) positive found, from these all were having cold autoantibody except 1 patient having cold+warm autoantibodies and 1 having allo+autoantibody. Indirect

anti-globulin test (IAT) positive in 28 patients; from these 3 having Bombay blood group, 1 having cold+warm autoantibody and rest all having alloantibodies development.

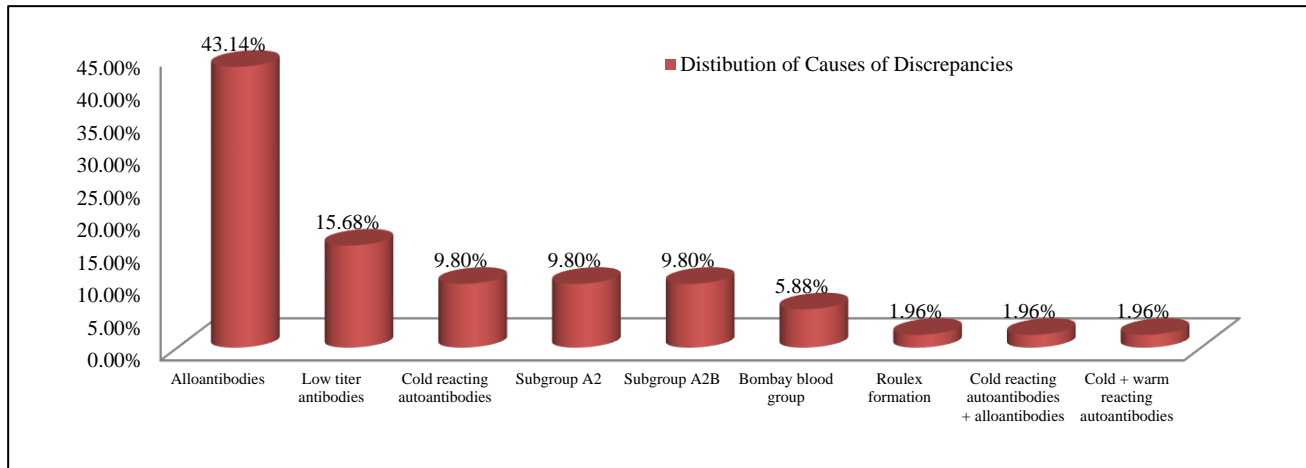


Figure 1: Causes of discrepancies.

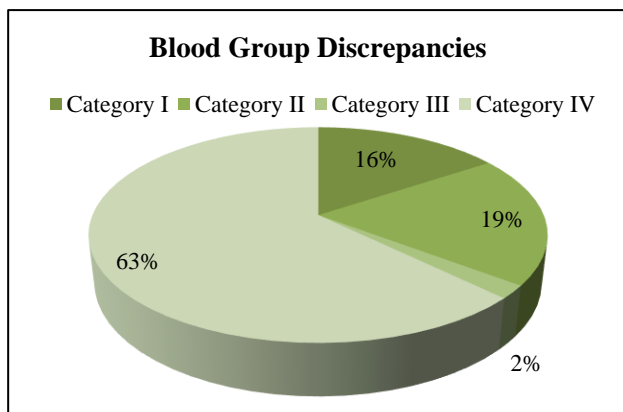


Figure 2: Comparison of category (group) wise distribution of discrepancies.

ABO group discrepancies mainly divided in 4 different groups. Maximum causes of discrepancies were found in group IV category, which were 32 (62.74%); these include 22 due to alloantibodies, 6 due to cold autoantibodies, 1 due to warm autoantibodies and 3 of Bombay blood group. Out of 51 discrepancies 10 (19.60%) were found in group II which was second highest; these include subgroups (A<sub>2</sub>, A<sub>2</sub>B) And other were 8 (15.69%) in group I; included low titer antibodies and 1 (1.96%) in group III category; included rouleaux formation. Among total 51 discrepancies, 12 were having “A” blood group, 13 having “B” blood group, 8 having “O” blood group, 5 having “AB” blood group, 5 having “A<sub>2</sub>B” blood group, 5 having “A<sub>2</sub>” blood group and 3 having “Bombay” blood group.

Table 1: Clinical diagnosis where discrepancies found.

Diagnosis	Numbers	Percentage
Severe anemia	25	56.82
AIHA (autoimmune hemolytic anemia)	7	14.58
Multiple myeloma	3	6.25
Sickle cell disease	2	4.17
HIV positive	2	4.17
Tibia fracture	2	4.17
CML (chronic myeloid leukemia)	1	2.08
Snake bite	1	2.08
Carcinoma of cervix	1	2.08
Liver cirrhosis	1	2.08
Intestinal perforation	1	2.08
Meningioma	1	2.08
Hip fracture with total hip replacement	1	2.08
<b>Total</b>	<b>48</b>	<b>100</b>

Out of 51 cases of discrepancies; 3 were healthy donor and 48 were patients. Among 48 patients’ cases of discrepancies, 25 (56.82%) cases of severe anemia was the most common cause. The second common cause was diagnosis of AIHA (autoimmune hemolytic anemia) with 7(14.58%) cases, and other various diagnoses were seen in cases of ABO discrepancies (displayed in Table 1 above). Form total 29 female patients with ABO discrepancies; 17 females were having obstetric history and remaining 12 females had other histories; like sickle cell disease, multiple myeloma, chronic myeloid

leukemia (CML), carcinoma of cervix, autoimmune hemolytic anemia, severe anemia etc.

**DISCUSSION**

The ABO system is the most important blood group system. ABO grouping should include both forward (cell typing) and reverse (serum/plasma typing) procedures and results of both methods must agree each other. In patients, an ABO discrepancy must be resolved before transfusion of any blood components, and in donors, the discrepancy must be resolved before any blood is labelled with a blood type. An ABO discrepancy implies that the forward typing does not agree with the reverse method. It is important to identify an ABO discrepancy and resolve that before transfusion of any blood component. An ABO incompatible red blood cells transfusion is a leading cause of death from transfusion.<sup>5</sup> The subgroup typing is usually carried out when there is a discrepancy in blood group typing based on adsorptions elution studies, presence of A, B, H substances in the saliva and family studies. Moreover, ABO discrepancies may also be resolved using patient’s age, diagnosis, medication, history of pregnancy, or recent transfusion.

In the present study total 51 (0.133%) samples found with ABO discrepancies, out of total 38,380 samples received. In the study done by Heo et al, total 55 (0.143%) samples were found as discrepancies, out of 38,559 samples received.<sup>6</sup> In the study done by Sharma et al, total 51 (0.049%) samples of discrepancies found, out of total 1,04,010 samples received.<sup>7</sup> In study done by Rahgozar et al, total 41 (0.054%) samples were found with discrepancies, out of 75,066 total samples received.<sup>8</sup> In the study done by Shanthi et al, total 1331 (4.73%) samples were found with discrepancies, out of total

28,024 samples received.<sup>9</sup> The present study correlated with Heo et al, and also with study done by Sharma et al and Rahgozar et al; but not agreed with Shanthi et al which was having high rate.<sup>7-9</sup>

In present study, out of 51 total discrepancies’ samples; 8 (15.69%) samples were of category I, 10 (19.60%) samples were of category II, 1 (1.96%) sample was of category III and 32 (62.74%) samples were of category IV (displayed in Table 2). In the study by Arumugam et al, out of 21 total discrepancies’ samples; 1 (4.76%) sample was of category I, 2 (9.53%) samples were of category II, 1 (4.76%) sample was of category III and 15 (71.42%) samples were of category IV.<sup>10</sup> In the study by Shanthi et al, out of 1331 total discrepancies’ samples; 0 (0.00%) samples was of category I, 1089 (81.81%) samples were of category II, 0 (0.00%) sample was of category III and 246 (18.33%) samples were of category IV.<sup>9</sup> In the study by Heo et al, out of 55 total discrepancies’ samples; 8 (14.55%) samples were of category I, 17 (30.90%) samples were of category II, 0 (0.00%) samples was of category III and 27 (49.09%) samples were of category IV.<sup>6</sup> In the study by Esmaili et al, out of 100 total discrepancies; 82 (82.00%) samples were of category I, 2 (2.00%) samples were of category II, 10 (10.00%) samples were of category III and 6 (6.00%) samples were of category IV.<sup>11</sup> In the study by Sharma et al, out of 51 total discrepancies; 30 (58.82%) samples were of category I, 12 (23.53%) samples were of category II, 0 (0.00%) sample was of category III and 6 (11.76%) samples were of category IV.<sup>7</sup> In the study by Rahgozar et al, out of 41 total discrepancies; 17 (41.46%) samples were of category I, 14 (34.16%) samples were of category II, 0 (0.00%) sample was of category III and 5 (12.19%) samples were of category IV.<sup>8</sup>

**Table 2: Comparison of category wise discrepancies with various studies.**

Various studies	Category wise discrepancies (%)				
	I	II	III	IV	Total
Arumugam et al <sup>10</sup>	1 (4.76)	2 (9.53)	1 (4.76)	15 (71.42)	21
Shanthi et al <sup>9</sup>	0	1089 (81.81)	0	246 (18.46)	1331
Heo et al <sup>6</sup>	8 (14.55)	17 (30.90)	0	38 (69.18)	52
Esmaili et al <sup>11</sup>	82 (82.00)	2 (2.00)	10 (10.00)	6 (6.00)	100
Sharma et al <sup>7</sup>	30 (58.82)	12 (23.53)	0	6 (11.76)	51
Rahgozar et al <sup>8</sup>	17 (41.46)	14 (34.16)	0	5 (12.19)	41
Present study	8 (15.96)	10 (19.60)	1 (1.96)	32 (62.74)	51

ABO discrepancies may be arbitrarily divided into four major categories. Group I discrepancies are associated with unexpected reactions in the reverse grouping due to weakly reacting or missing antibodies. In present study, out of 51 total discrepancies 8 (15.96%) samples were of category I, which is similar to study done by Heo et al which was 8 (15.38%); which is much lower than study done by Esmaili et al 82 (82.00%), Sharma et al, 30 (62.50%) and Rahgozar et al 17 (41.46%); but higher

than Arumugam et al 1 (5.26%).<sup>6-8,10,11</sup> Group II discrepancies are associated with unexpected reactions in the forward grouping due to weakly reacting or missing antigens. In present study, there were 10 (19.60%) samples in category II discrepancies, which was more than study done by Arumugam et al 2 (10.53%); but lower than, Shanthi et al 1089 (81.81%), Heo et al 17 (32.69%), Rahgozar et al 14 (34.16%) and Sharma et al 12 (25.00%).<sup>6-10</sup> Another study done by Esmaili et al,

type II discrepancy was least common were 2 (2.00%) only.<sup>11</sup> Group III discrepancies between forward and reverse groupings are caused by protein or plasma abnormalities and result in rouleaux formation or pseudo-agglutination. In this study, out of total 51 discrepancies 1 (1.96%) sample was from category III, which was lower to study done by Esmaili et al 10 (10.00%) and Arumugam et al 1 (5.26%).<sup>10,11</sup> Group IV discrepancies between forward and reverse groupings are due to miscellaneous problems due to unexpected ABO isoagglutinins; unexpected non-ABO alloantibodies, etc. Study done by Arumugam et al 15 (78.95%) and Heo et al 27 (51.92%) was similar to present study in which 32 (62.74%) samples were there in category IV; which is much higher than, by Esmaili et al 6 (6.00%), Sharma et al 6 (11.76%), Rahgozar et al 5 (12.19%) and Shanthi et al 246 (18.46%).<sup>6-11</sup>

Out of 38380 samples collected; total 10 samples (0.026%) were of subgroups; including 5 samples (0.013%) of A<sub>2</sub> subgroups and 5 samples (0.013%) of A<sub>2</sub>B subgroups. In the study of the importance of weak ABO subgroups by Thakral et al, out of total 86867 samples collected; total 17 samples (0.020%) were of subgroups; including 14 samples (0.016%) of A subgroups and 3 samples (0.0036%) of B subgroups.<sup>12</sup> As per comparison of discrepancies due to ABO subgroups, results of the present study are matching with results of the study done by Thakral et al.<sup>12</sup> Also, 3 samples found to have no reactivity with anti-H and also in reverse grouping, strong reactive with all A, B and O pooled cells with presence of H antigen in saliva. These samples were found to have Bombay blood group.

The main limitation of the study could be limited availability of special antisera, identification or confirmation of blood group was not possible for every case; partly can be due to unavailability of gene or molecular typing at the center. Cell panel available at our center was not manufactured from Indian population, this can be a drawback for resolution of blood group discrepancy

## CONCLUSION

To avoid chances of wrong interpretation of blood group and clinical impact associated with mismatch ABO transfusion. It is important to resolve discrepancies.

In the present study total 51 samples found with ABO discrepancies, out of 38,380 (0.133%) samples; in which 25,129 samples were of patients and 13,251 samples were of donors. Out of 25,129 patients' sample, 48 (0.191%) samples and 3 (0.0227%) samples out of 13,251 donors' sample were found as discrepant. Causes of discrepancies were identified, mostly due to formation of alloantibodies, low titer antibodies, cold reacting autoantibodies, subgroup A<sub>2</sub>, subgroup A<sub>2</sub>B, Bombay blood group rouleaux formation, cold reacting

autoantibodies + alloantibodies and cold + warm reacting autoantibodies.

All discrepancies reported on ABO cell and serum grouping must be investigated further, so that correct blood group is reported, minimizing the chances of transfusion reaction. A note of caution should be mentioned on the blood group card to prevent ABO incompatibility in case of transfusion.

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## REFERENCES

1. Landsteiner K. To the knowledge of the antifermentative, lytic and agglutinating effects of the blood serum and the lymph. *Zentralblatt Bakteriologie.* 1900;27:357-62.
2. Hakomori SI. Blood group ABH and li antigens of human erythrocytes: chemistry, polymorphism, and their developmental change. *Semin Hematol.* 1981;18:39-62.
3. Dzik W. Emily Cooley Lecture 2002: transfusion safety in the hospital. *Transfusion.* 2003;43(9):1190-9.
4. Brecher M. technical manual. American Association of Blood Bank, Bethesda MD; 2002.
5. Rudlof B, Just B, Deitenbeck R, Ehmann T. Mismatched transfusion of 8 ABO-incompatible units of packed red blood cells in a patient with acute intermittent porphyria. *Saudi J Anaesth.* 2011;5(1):101.
6. Heo MS, Cho D, Park HR, Shin MG, Shin JH, Suh SP, et al. Analysis of ABO blood discrepancies and transfusion experiences in Chonnam National University Hospital. *Korean J Blood Transfus.* 2013;24(3):222-32.
7. Sharma T, Garg N, Singh B. ABO blood group discrepancies among blood donors in Regional Blood Transfusion Centre GTB Hospital, Delhi, India. *Transfus Apher Sci.* 2014;50(1):75-80.
8. Rahgozar S, YAVARI F, Hariri MM, Moafi AR. ABO discrepancy prevalence and qualitative study of relevant factors in blood donors of Isfahan Regional Blood Transfusion Center.
9. Shanthi B, Babu KR. Analysis of ABO group discrepancies in a tertiary care centre south India. *J Evol Med Dent Sci.* 2017;6(79):5615-8.
10. Arumugam P, Hamsavardhini S, Ravishankar J. Resolving ABO discrepancies by serological

- workup-an analysis of few cases. *Int J Res Med Sci.* 2017;5(3):893-900.
11. Esmaili HA, Taghipour H, Talghini S, Mohammadhoseyni B. An analysis of discrepancies between forward and reverse ABO blood grouping. *Res J Pharm Bio Chem Sci.* 2014;5(3):1360-6.
  12. Thakral B, Saluja K, Bajpai M, Sharma RR, Marwaha N. Importance of weak ABO subgroups. *Lab Med.* 2005;36(1):32-4.

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