



# Resolving Discrepancies in Forward and Reverse ABO Blood Group Typing

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## Abstract

**Background/Aim:** There is a need of investigation of high frequency of the cases of ABO discrepancy revealed by forward and reverse typing. The aim of the study was to reveal optimal methods for detection of weak subgroups of group A and B antigens on erythrocytes.

**Methods:** Sixteen persons with A blood group and anti-B antibody adsorbing ability (weak Bel, B<sub>m</sub> and B<sub>3</sub> antigens), 5 persons with B blood group and anti-A antibody adsorbing ability (weak A<sub>x</sub> antigen) and 2 persons with O blood group and anti-B antibody adsorbing ability (weak Bel antigen) were investigated as the cases of type II-III of ABO discrepancy and weak B antigen. Liquid chromatography, agglutination, inhibition of agglutination and adsorption reactions at 4 °C and 37 °C with prolonged incubation (12 hours) were used in the study.

**Results:** The persons with A blood group revealed by forward typing at 4 °C and O blood group detected by reverse typing at 37 °C showed the ability of the erythrocytes to adsorb anti-B antibody. The persons with B blood group revealed by forward typing with presence of anti-B antibodies reactive at 37 °C showed the ability of erythrocytes to adsorb anti-A antibody. Prolonged incubation at reverse typing, adsorption-elution tests, inhibition of haemagglutination and liquid chromatography were used to define blood group specificity.

**Conclusion:** Prolonged incubation during reverse typing at 4 °C and 37 °C, adsorption at 4 °C, heat elution and liquid chromatography might help to detect blood group type in cases of type II-III of ABO discrepancy.

**Key words:** Chromatography; Antigen; Adsorption; Erythrocyte; Transfusion.

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## ARTICLE INFO

Received: 18 July 2023  
Revision received: 13 October 2023  
Accepted: 13 October 2023

## Introduction

ABO typing is the most important pretransfusion study directly related to the quality of the person's safety. The complications due to mistyping and ABO mismatched mis-transfusion are known to elevate the risk of viral infection.<sup>1</sup>

ABO discrepancies are known to be classified into four groups. Group I represents missing antibodies, group II is associated with unpredicted reaction due to weak antigens, group III demonstrates rouleaux formation and pseudo-aggluti-

nation, group IV is associated with autoantibodies and polyagglutination.<sup>2, 3</sup> In haematological malignancies and epigenetic variations of group A and B transferases the erythrocyte antigen genes expression might be lost.<sup>4</sup> Tumour cells were reported to secrete serum soluble blood group substances.<sup>5</sup> Group II discrepancy has been observed in persons with solid organ malignancies and group III discrepancies were reported in Hodgkin lymphoma. Alterations of antigen expression have been observed in gastrointestinal cancer tissue with absence of group A, B, H or Le<sup>b</sup> antigens in 25 % of cases.<sup>6</sup> The causes of ABO discrepancy due to the cold reactive autoantibodies, alloantibodies (anti-M, anti-P1, anti-N and anti-E), rouleaux formation, hypogammaglobulinaemia, anti-A1 and anti-B antibodies in A2B case and influence of autoanti-A antibodies have been described. Some cases caused by erythrocytes' problem (loss of blood antigen in leukaemia, weak group A or B antigens, acquired group B antigen, mixed field agglutination followed ABO mismatched transfusion, non-classified agglutination) have been revealed.<sup>7</sup>

Weak subgroups are usually detected by agglutination with antibodies, adsorption elution method, definition of secretor status.<sup>8-13</sup> Thirty cis-AB cases with discrepant results in automated and manual methods were reported and fifteen cis-AB cases were determined due to weak reactions in automated methods.<sup>9, 10</sup> Thus, the implementation of automated methods does not necessarily guarantee reducing errors in ABO typing, especially for weak ABH subgroups as cis-AB.<sup>11, 12</sup>

Group A, B and H antigens of erythrocytes are known to be the most important for transfusion medicine. Group A antigen is considered to have multiple structural forms. The most common is considered A1, determined in approximately 80 %. Group A2 antigen is structurally different than group A1 antigen. There are fewer copies of group A2 antigen on erythrocytes as compared to group A1 antigen.<sup>13</sup> Carbohydrate antigens are considered to lead to the IgM immune response with the highest reactivity at 4 °C, whereas immune anti-A and anti-B antibodies agglutinate at 37 °C. Naturally occurring IgM anti-A and anti-B antibodies were reported to cause difficulties in incompatible blood transfusion and transplantation.

The use of different temperatures while incubation of erythrocytes with sera attracted atten-

tion of many scientists due to the different reactivity of IgM and IgG antibodies at 4 °C and 37 °C.<sup>14</sup> Thus, antibody, IgM or IgG, may be of clinical significance if reactive at 37 °C. ABO discrepancies in persons with lymphoma and solid organ tumours have been resolved by elution and antibody screening at 37 °C and 40 °C. The search of the conditions for optimal interaction of erythrocyte antigens and antibodies continues.

The aim of the study was to reveal optimal conditions for the detection of weak group A and B antigens on erythrocytes.

## Methods

Serological based ABH phenotypes were determined by agglutination testing according to standard methods and procedures described in AABB Technical Manual.<sup>15</sup> To obtain IgG antibodies the sera were heated for 30 minutes at 56 °C.

The polyclonal sera (*Tulip diagnostics* Goa, India) and group A, B and O erythrocytes were used for forward and reverse typing of the studied persons by tube method. The test erythrocytes and sera were taken from 25 volunteers aged  $71.4 \pm 1.2$  years old (15 men and 10 women). The erythrocytes were washed three times with normal saline and centrifugated at 1000 g for 10 minutes.

The first results of blood typing showed 16 persons with A blood group, 5 persons with B blood group and 4 persons with O blood group.

The study of the persons' sera on the presence of anti-A and anti-B antibodies was performed after the contact with group A and B erythrocytes at 4 °C, 8 °C and 37 °C. Fifty  $\mu$ L of test erythrocytes were added to 100  $\mu$ L of the studied serum in different dilutions. The results were recorded after microscopic investigation.<sup>16</sup> The strength of agglutination was graded according to the standards.<sup>17</sup> The studied sera showed the presence of unexpected antibodies. Thus, 11 persons with A blood group showed the presence of anti-A antibodies, active at 4 °C (9 persons) and at 37 °C (8 persons). Among them those having Bel antigen demonstrated presence of anti-B antibodies, active at 4 °C (6 persons) and at 37 °C (6 persons).

Meanwhile, persons with B blood group showed

the presence of anti-B antibodies (3 persons), active at 4 °C (2 persons) and at 37 °C (2 persons).

An attempt to resolve the observed discrepancy led to the repeating testing using adsorption method.<sup>18</sup> Polyclonal antisera of human origin (O blood group) were used for adsorption. The adsorption reaction was performed after the contact of the studied erythrocytes (50 µL) with anti-A, B serum (100 µL) for 12 hours at 4 °C (1:2 volume ratio of erythrocytes and serum accordingly). Heat elution of antibodies was performed after adsorption of the studied erythrocytes with anti-A, B serum at 56 °C for 10 minutes. The technique was performed following AABB Technical Manual.<sup>19</sup> Thus, 13 persons with A blood group demonstrated anti-B antibody adsorbing ability, 5 persons with A blood group demonstrated anti-A antibody adsorbing ability and 2 persons with O blood group showed anti-B antibody adsorbing ability.

The analysis of the erythrocytes sample on N-acetyl-D-glucosamine presence was performed by liquid chromatography. Sample: 10 mL 10-3; column: Shodex Silica 5 NH 4D; eluent buffer (pH 7.5)/ CH<sub>3</sub>CN=30/70. 3.5 g of KH<sub>2</sub>PO<sub>4</sub> was dissolved in water in 1 L of volumetric flask, 0.25 mL of ammonium hydroxide 25 % was added, diluted with water and mixed, adjusted to pH 7.5 with H<sub>3</sub>PO<sub>4</sub>. Flow rate: 1.1 mL/min. Detector: UV 195 nm. Column temperature: 35 °C.

Fifteen persons with A blood group showed type II-III of blood group discrepancy (7 with weak Bel antigen, 7 with Bm antigen, 1 with weak B3), 5 persons with B blood group showed type II discrepancy (weak Ax antigen), 2 persons with O blood group showed II type of discrepancy (weak Bel antigen).

The analysis of the erythrocytes sample on N-acetyl-D-glucosamine presence was performed by liquid chromatography. Sample: 10 mL 10-3; column: Shodex Silica 5 NH 4D; eluent buffer (pH 7.5)/ CH<sub>3</sub>CN=30/70. 3.5 g of KH<sub>2</sub>PO<sub>4</sub> was dissolved in water in 1 L of volumetric flask, 0.25 mL of ammonium hydroxide 25 % was added, diluted with water and mixed, adjusted to pH 7.5 with H<sub>3</sub>PO<sub>4</sub>. Flow rate: 1.1 mL/min. Detector: UV 195 nm. Column temperature: 35 °C.

The study was approved by the Ethical committee of Kharkiv National Medical University (protocol 4).

Statistical analysis was performed by Statistica 10.0. The Student's and the Mann Whitney U tests were used for analysis of the mean values of agglutination. Correlation of adsorption (the degree of inhibition of haemagglutination after adsorption of the sera with studied erythrocytes) and liquid chromatography data (the concentration of N-acetyl-D-galactosamine in erythrocyte's sample) was performed using Pearson's coefficient.

## Results

### The cases of ABO discrepancy of the persons typed as A blood group with unexpected anti-A antibodies (II-III type of discrepancy)

1. The person of A blood group with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption decreased the expression of agglutination of group B erythrocytes by anti-A, B serum in 1:32 titre (from 3+ to 1+), whereas the studied serum agglutinated group A erythrocytes after 12 hours of the contact at 4 °C (w+) and 37 °C (+). Interestingly the serum did not agglutinate group B erythrocytes at 4 °C and decreased their quantity at 37 °C (Table 1). Type II discrepancy (Bm antigen) was observed. Liquid chromatography of the erythrocytes sample determined the presence of N acetyl-D-galactosamine in 0.095 mg/mL concentration (Figure 1).

2. The person of A blood group with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decrease of agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre). Elution reaction after adsorption of the studied erythrocytes showed the presence of anti-B antibodies.

Meanwhile, the studied plasma agglutinated group A erythrocytes after 12 hours of contact at 4 °C and at 37 °C, as well as group B erythrocytes at 4 °C and 37 °C (decreased their quantity) (type II discrepancy, Bel antigen).

3. The erythrocytes of A blood group with anti-B antibody adsorbing ability after adsorption led to the decrease of agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:4 titre). The studied serum agglutinated group A erythrocytes at 4 °C and 37 °C (type II discrepancy, Bm antigen). Elution after adsorption of the studied erythrocytes with anti-A, B serum showed the presence of anti-B antibodies.



**Table 1:** The study of erythrocytes and serum of the persons with discrepancy in forward and reverse typing

Erythrocytes	Adsorption with S anti-A, B at +4 °C for 12 h after contact with S from AB sample for 1 h	The studied serum or plasma with A er. at +4 °C	The studied serum or plasma with A er. at +37 °C	The studied serum with B er. at +4 °C	The studied serum with B er. at +37 °C	Elution after adsorption	Inhibition of agglutination
1 A (Bm+) (from S)	S anti-A, B: 1:32:3+; after adsorption 1:32:1+.	w+	1+	-	-(↓ n)		
2 A (Bel+) (from S)	S anti-A, B 1:32-1:16 with B er.	3+	+ aggl.	2+	1+ (↓ n)	with B er.: 1+	
3 A (Bm+) (from S)	S anti-A, B 1:32-1:4, S anti-B 1:32-1:32	+	+ strong			with B er.: 1+ with anti-B S: -	
4 A (Bel+) weak (citratd)	S anti-A, B < aggl. 1:32	1 + after 5 min - 12 h	-12 h	with unwashed: 1+	1+		↓ S anti-A, B 1:20 in 1:2 titre
5 A (Bm+) weak	S anti-A, B 1:32 (1:16)	-	-				↓ S anti-A, B in 1:2 – 1:4 titre
6 A (Bel++) (citratd)	S anti-A, B + B 1:32-1:16	2 + 5 min 1+	+ (no ↓ n)	w+			↓ S anti-A, B (1:20) 30 in
7 A (Bel+) weak		3+	- no ↓ n	1+	+(↓ n)		↓ S anti-A, B + A er.: ↓ in 1:2-1:16 titre, ↑ with B er.: in 1:2 titre
8 A (Bm+) weak	S anti-A, B + B er. < aggl.	2 + 5 min 1+	+ (no ↓ n)			+(aggl. of B er.)	
9 A (Bm+)	S anti-A, B + B er.: 1:32-1:16, 1:32: < aggl.	+m	-				-↓ S anti-A, B + B er.: + in 1:2-1:4 titre
10 A		-12 h	- 12 h			- with saline - with S AB	
11 A (Bm++) (citratd)		-12 h	- 12 h				
12 A (Bm+) (citratd)	S anti-A, B + B er.: 1:32-1:16	30 min + 12 h	- 30 min -12 h				↓ S anti-A + A er.: in 1:2-1:16 titre
13 A (Bel+)	S anti-A, B + B er.: 1:32-1:16	3+	+	+	-		
14 A (Bel+)	S anti-A, B+B er.: 1:32-1:8	-	-	3+	w+		
15 A (B3+) (citratd)	S anti-B + B er.: 1:32-1:16	1 h: 3+	+ + 1 h	1+	+ ↓n		

16	A (Bel-) (citrated)	S anti-B + B er.: no decrease	w+	+, w +	3+	-, ↓n +	
17	O (Bel+)	S anti-B + B er.: no decrease				+	aggl. of B er. (elution with saline, AB S)
18	O (A-Bel++) (from S)	S anti-A, B + A er.: < aggl. in 1:32 titre; + B er.: 1:32-1:16	+	↓n	+	+ no ↓n	S anti-A, B (2:20) + A er.: -
19	O						S anti-A, B + A er.: -
20	O						S anti-A, B + A er.: - with heated: -
21	B (Ax+) (citrated) weak	S anti-A, B + A er.: < aggl. in 1:32 titre			-	+	- (no aggl of A er.)
22	B (Ax+) weak (from S)	S anti-A, B: < aggl. in 1:32 titre	+	+↓n	w+ 30 min	-12 h	- citrated anti-B (2:20) + B er. +8°C
23	B (Ax+) (from S)	S anti-A, B + A er.: 1:8-1:4	3+	+ no ↓n + no ↓n	w+	w+	↓ S anti-A, B + A er.: in 1:2-1:4 titre ↓ +B er.: in 1:2-1:4 titre ↓ S anti-B + B er.: in 1:2-1:4 titre
24	B (Ax+) (from S)	S anti-A, B + A er.: 1:32: +; after adsorption: 1:32:1+.	3+	1+	2+	w+, ↓n	
25	B (Ax+) (from S)	S anti-A, B + A er.: 1:32: +; after adsorption: 1:16.	2+	1+	-	+ 12 h	↓ S anti-A + A er. and B er.: in 1:2 titre

Note: S - serum, h - hour, AB - AB blood group, aggl. - agglutination, er. - erythrocytes, n- quantity of erythrocytes.

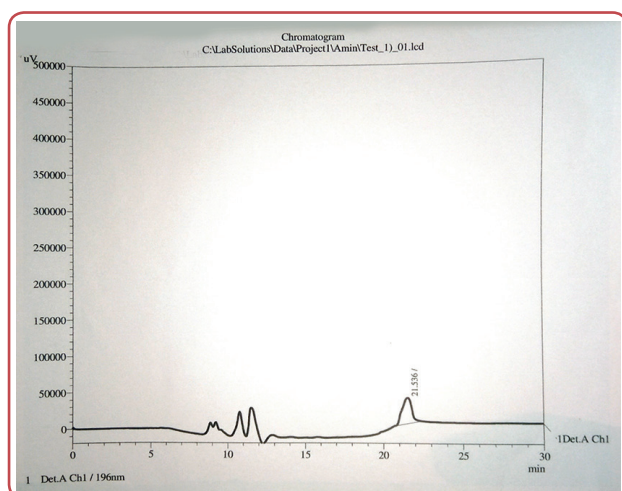


Figure 1: Chromatogram of the person with A blood group and weak group Bm antigen

4. The sample of A blood group with anti-B antibody adsorbing ability. The serum agglutinated group A erythrocytes at 4 °C after 5 minutes of contact and group B erythrocytes at 4 °C and 37 °C (type II discrepancy, Bel antigen). The serum inhibited agglutination of group A erythrocytes by anti-A, B serum in 1:2 titre. The studied serum did not agglutinate group A erythrocytes at 37 °C after 12 hours contact. The studied serum weakened the agglutinating ability of anti-A, B serum with group B erythrocytes in 1:2-1:4 titre.

5. The sample of group A erythrocytes with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decrease of agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre).



The serum did not agglutinate group A erythrocytes at 4 °C and 37 °C, however inhibited agglutinating ability of anti-A, B serum in 1:2-1:4 titre (weak Bm antigen).

6. The sample of A blood group with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption decreased agglutination of B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre).

The serum agglutinated citrated unwashed group A erythrocytes after 5 minutes of contact at 4 °C and 37 °C, as well as after 12 hours of contact and weakly agglutinated group B erythrocytes at 4 °C. The serum significantly inhibited agglutination of group A erythrocytes in 1:2-1:4 titre by anti-A, B serum (in 1:20 dilution) (type III discrepancy, Bel).

Anti-B serum agglutinated the studied erythrocytes after 30 minutes of incubation at 4 °C with further centrifugation. The studied serum in 1:2-1:4 titre inhibited agglutination of group B erythrocytes by serum from O blood type.

7. The person of A blood group with weak anti-B antibody adsorbing ability. The studied serum in 1:2-1:16 titre inhibited agglutination of group A erythrocytes by anti-A, B serum at 8 °C after 20 minutes of contact. The studied serum agglutinated group A erythrocytes at 4 °C, however did not agglutinate nor decrease the quantity of group A erythrocytes at 37 °C after 12 hours of contact. The serum agglutinated group B erythrocytes at 4 °C and 37 °C. Bel antigen was determined.

8. The person with A blood group and weak anti-B antibody adsorbing ability. The erythrocytes after adsorption led to the decreased agglutination of group B erythrocytes by anti-A, B serum. Elution after adsorption of anti-A, B serum with the studied erythrocytes revealed the presence of anti-B antibodies (weak Bm antigen).

9. The person with A blood group and anti-B antibody adsorbing ability. Anti-A, B serum after adsorption with the studied erythrocytes decreased agglutination of group B erythrocytes (from 1:32 to 1:16 titre). The studied serum inhibited agglutination of group B erythrocytes by anti-A, B serum in 1:2-1:4 titre (weak Bm antigen).

10. The person with A blood group. The studied serum did not agglutinate group A erythrocytes

after 12 hours of contact at 37 °C and 4 °C. The elution (with saline and serum from AB blood group) after adsorption of anti-A, B serum with the studied erythrocytes did not reveal the presence of anti-B antibodies. A blood group was determined.

11. The sample of A blood group with anti-B antibody adsorbing ability. The studied citrated plasma agglutinated group A erythrocytes at 37 °C on the contrary to the reaction at 4 °C (type II discrepancy, Bm antigen).

12. The person with A blood group and anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the weakened agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre). The studied plasma in 1:2-1:16 titre inhibited agglutination of group A erythrocytes by anti-A serum at 8 °C after 30 minutes of contact. The studied citrated plasma did not agglutinate group A erythrocytes at 37 °C after 30 minutes of contact, however agglutinated group A erythrocytes at 4 °C after 12 hours of contact (type II discrepancy, Bm).

13. The erythrocytes of the person with A blood group and anti-B antibody adsorbing ability after adsorption led to the weakened agglutinating ability of anti-A, B serum with group B erythrocytes (from 1:32 to 1:16 titre). The serum agglutinated group A erythrocytes at 37 °C and 4 °C and B erythrocytes at 4 °C on the contrary to 37 °C (type II discrepancy, Bel antigen).

14. The person with A blood group and anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre). The studied serum did not agglutinate group A erythrocytes at 4 °C and 37 °C, however weak agglutination was observed with group B erythrocytes at 4 °C and 37 °C. The serum in 1:2-1:4 titre inhibited agglutination of group A erythrocytes by anti-A, B serum (weak Bel antigen).

15. The person with A blood group and anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased agglutination of group B erythrocytes by anti-B serum (from 1:32 to 1:16 titre). Citrated anti-B plasma agglutinated the studied erythrocytes at 37 °C after 1 hour of contact, however did not agglutinate at 4 °C. The studied citrated plasma

agglutinated group A erythrocytes at 4 °C and 37 °C (with decrease of their quantity) and group B erythrocytes at 4 °C and 37 °C (with decrease of their quantity) (type III discrepancy, antigen B<sub>3</sub>).

16. The person with A blood group. The studied erythrocytes after adsorption did not lead to decrease of agglutination of group B erythrocytes by anti-B serum. Anti-B serum did not agglutinate the studied erythrocytes at 37 °C after 1 hour contact. The studied citrated plasma agglutinated group A and B erythrocytes at 37 °C and 4 °C (with decrease of their quantity) (Bel antigen, type II discrepancy).

Thus, persons of A blood group and presence of anti-A antibodies reactive at 37 °C or 4 °C (II type of discrepancy, presence of B<sub>3</sub>, Bm and Bel antigens) showed the ability of the erythrocytes to adsorb anti-B antibody. The persons with weak B antigen (type II discrepancy) demonstrated ability of the sera to inhibit haemagglutination of specific sera. The persons with type III discrepancy demonstrated haemolysing activity of the serum towards A and B erythrocyte antigen.

### The persons with O blood group type (weak Bel antigen)

17. The elution with saline and serum from AB group after adsorption of O blood group erythrocytes with anti-B antibody adsorbing ability showed the presence of anti-B antibodies (presence of weak Bel antigen, II type of discrepancy).

18. The sample of O blood group with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre) (presence of weak Bel antigen, II type of discrepancy).

The studied serum after 30 minutes of contact did not inhibit agglutination of group A erythrocytes by anti-A, B serum (in 2:20 dilution) at 4 °C. Anti-A serum led to the weak agglutination of the studied erythrocytes at 4 °C after 30 minutes of contact with further centrifugation. The studied serum agglutinated group A erythrocytes at 4 °C and 37 °C (with decrease of their quantity) and group B erythrocytes at 4 °C and 37 °C (without decrease of their quantity).

19. The serum from O blood group did not inhibit the agglutination of group A erythrocytes by anti-A, B serum. O blood group was determined.

20. The serum of O blood group (as well as the heated serum) did not inhibit agglutination of group A erythrocytes by anti-A, B serum. O blood group was determined.

The described cases of O blood group with anti-B antibody adsorbing ability of erythrocytes (weak Bel antigen, II type of discrepancy) demonstrated the ability of the studied serum to inhibit haemagglutination of erythrocytes by specific antisera and presence of anti-B non-haemolysing antibodies.

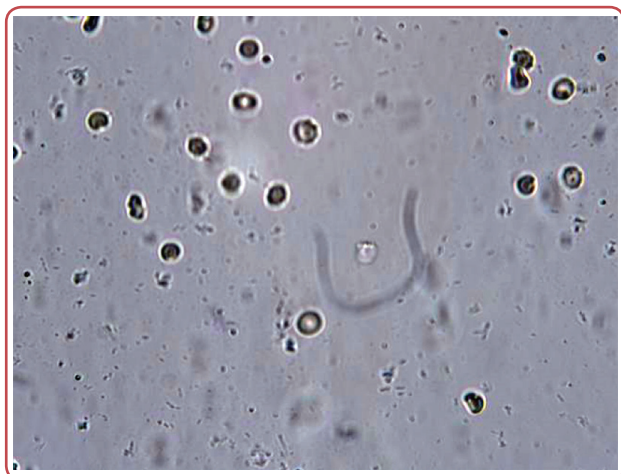
### The cases of B blood group persons with unexpected anti-B antibodies reactive at 4 °C or 37 °C (type II discrepancy, Ax antigen)

21. The person with B blood group and anti-A antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased expression of agglutination of group A erythrocytes by anti-A, B serum in 1:32 titre. Elution after adsorption of the studied erythrocytes with anti-A, B serum did not reveal the presence of anti-A antibodies. Ax antigen was determined. The citrated plasma agglutinated group B erythrocytes at 37 °C on the contrary to 4 °C.

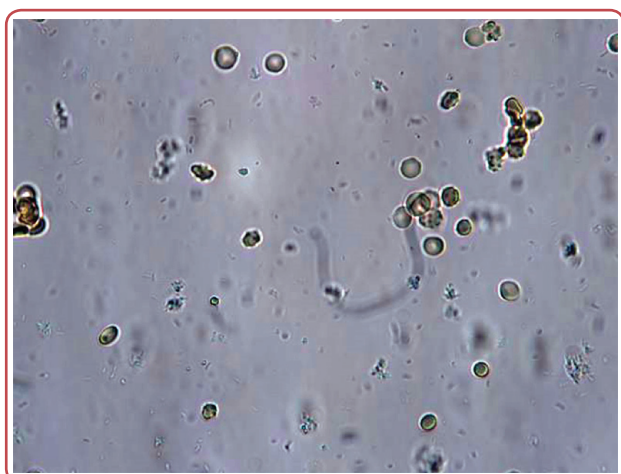
22. The person with B blood group and anti-A antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased expression of agglutination of group A erythrocytes by anti-A, B serum in 1:32 titre. The studied serum led to the weak agglutination of group B erythrocytes after 30 minutes of contact at 4 °C, with no agglutination at 37 °C after 12 hours of contact. The studied serum agglutinated group B erythrocytes at 4 °C, group A erythrocytes at 4 °C and 37 °C (with decrease of their quantity). The studied serum did not inhibit agglutination of group B erythrocytes by anti-B serum (in 2:20 dilution). Ax antigen was determined.

23. Group B erythrocytes with anti-A antibody adsorbing ability. The studied serum in 1:2-1:4 titre after 15 minutes of contact inhibited agglutinating ability of anti-A, B serum with group A and B erythrocytes at 8 °C, as well as agglutination of group B erythrocytes by anti-B citrated plasma. The studied serum agglutinated group A erythrocytes at 4 °C and 37 °C after 12 hours of contact (without decrease of the quantity of erythrocytes) and group B erythrocytes at 37 °C and 4 °C (weakly). Ax antigen was determined.

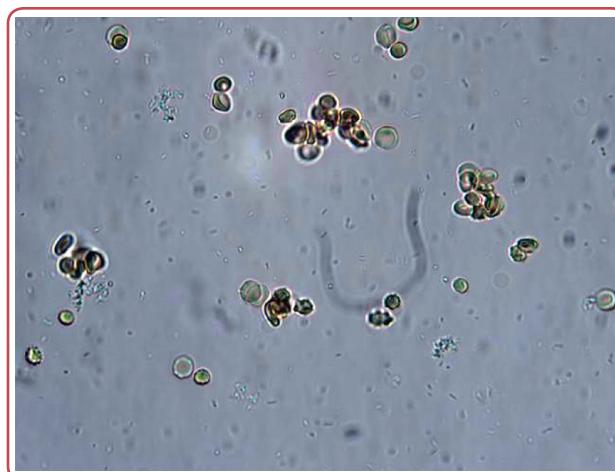
An interesting ability of the sera of the persons with A blood group and anti-B antibody adsorbing ability to inhibit agglutination of group A and B erythrocytes by anti-A, B serum was noted. The agglutination of group B erythrocytes by anti-A, B serum, previously contacted for 30 minutes with serum from A blood group person with anti-B antibody adsorbing ability was absent after adding the studied serum in 1:2 titre, however was demonstrated in 1:32 titre (Figures 2-5).



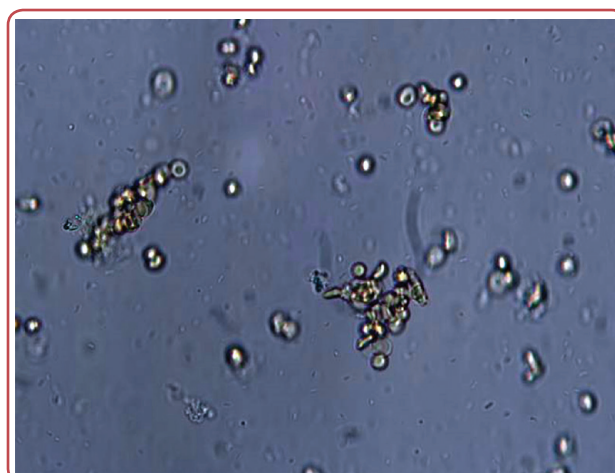
**Figure 2:** Group B erythrocytes with anti-A, B serum after the contact with serum from A blood group person with anti-B antibody adsorbing ability in 1:2 titre (no agglutination)



**Figure 3:** Group B erythrocytes with anti-A, B serum after the contact with serum from A blood group person with anti-B antibody adsorbing ability in 1:4 titre



**Figure 4:** Group B erythrocytes with anti-A, B serum after the contact with serum from A blood group person with anti-B antibody adsorbing ability in 1:8 titre

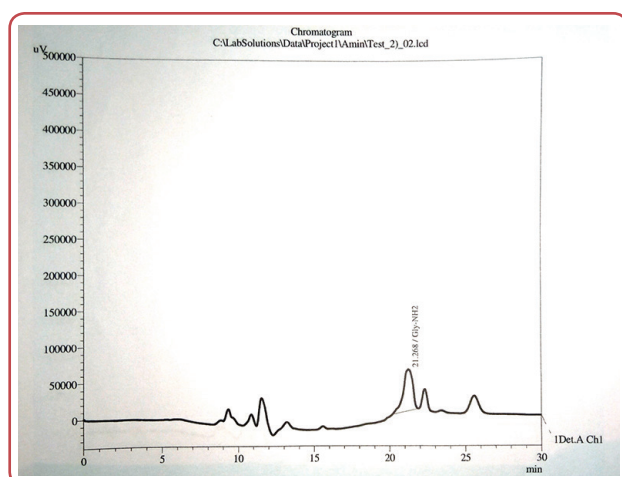


**Figure 5:** Group B erythrocytes with anti-A, B serum after the contact with serum from A blood group person with anti-B antibody adsorbing ability in 1:32 titre (presence of agglutination)

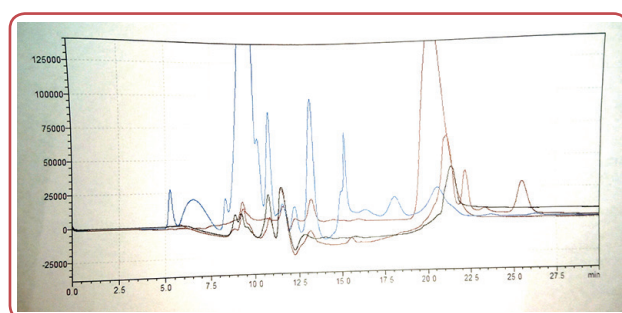
24. The person with B blood group and anti-A antibody adsorbing ability. The studied erythrocytes after adsorption decreased the expression of agglutination of group A erythrocytes by anti-A, B serum (in 1:32 titre: from 2+ to 1+). Liquid chromatography of erythrocytes' sample revealed the presence of N-acetyl-D-galactosamine in 0.056 mg/mL concentration (Figures 6, 7). Importantly, the concentration of group A antigen correlated with the degree of haemagglutination inhibition ( $r = 0.32$ ) (presence of weak antigen). II type of discrepancy with Ax antigen was determined.

25. The person with B blood group and anti-A antibody adsorbing ability demonstrated the presence of anti-B antibodies reactive at 37 °C.





**Figure 6:** Chromatogram of the person with B blood group and weak Ax antigen



**Figure 7:** Chromatograms of the persons with A blood group and weak group Ax antigen

The studied erythrocytes after adsorption with anti-A, B serum at 4 °C for 12 hours decreased the titre of the serum (from 1:32 to 1:16) (type II discrepancy). The erythrocytes' sample showed presence of N-acetyl-D-galactosamine in 0.051 mg/mL concentration by liquid chromatography (Figure 7). The person's EDTA plasma inhibited agglutination of group A and B erythrocytes by anti-A, B serum. Ax antigen was determined.

Thus, the studied B blood group type persons with presence of serum anti-B antibodies reactive at 4 °C or 37 °C (type II discrepancy, Ax antigen) showed an ability of erythrocytes to adsorb anti-A antibody.

## Discussion

The discrepancies in ABO typing are the reasons to investigate the mechanism of transfusion reactions. These discrepancies can be avoided through the analysis of the methods for the blood

typing. The study demonstrated 20 cases of blood group discrepancy type II and 2 cases of blood group discrepancy type III. The persons showing unexpected antibodies in the serum demonstrated the presence of weak subgroup of A or B antigen on erythrocytes. The person's erythrocytes were analysed by commercial antisera for blood grouping, whereas adsorption studies were performed with polyclonal sera. The data of forward (known antisera) and reverse (known antigen) typing were not complimentary. The characteristics of the studied sera were explored with test erythrocytes of group A and B at 4 °C and 37 °C. Unexpected antibodies showed activity at 4 °C and 37 °C.

Thus, a person with B<sub>3</sub> antigen, 7 persons with Bm and 7 persons with Bel antigen and 5 persons with Ax antigen were described. Two persons of O blood group and Bel antigen were revealed. The analysis demonstrated the presence of unexpected antibodies at prolonged incubation period (12 hours) at 4 °C and 37 °C with the presence of adsorbing group specific antigens by adsorption with polyclonal sera. This demonstrates the necessity to perform blood group typing in type II-III of discrepancy with prolonged incubation time both at 4 °C and 37 °C and importance of the use of polyclonal sera for adsorption reaction to avoid mistyping. Activity of the antibodies at 37 °C indicated the presence of IgG antibodies and in all studied samples we used prolonged incubation at 4 °C and 37 °C.

The unusual ability of the studied A and B blood group persons to agglutinate erythrocytes of the same specificity was determined at the incubation at 4 °C and at 37 °C. The studied erythrocytes showed additional ability to adsorb anti-A and anti-B antibodies at prolonged incubation at cold temperature (4 °C). Group II ABO discrepancy among the studied persons was the predominant type.

The described observations have been reported in various studies. The discrepancies are known to be divided into four major types. Type I discrepancies are revealed in reverse typing due to weakly reacting or missing antibodies. Type II discrepancies are observed in forward grouping due to weakly reacting or missing antigens. Type III discrepancy is observed due to excess plasma proteins. Type IV discrepancies were demonstrated in miscellaneous causes like cold auto-antibodies, cold alloantibodies and Bombay

phenotype. Type III discrepancy were observed in persons with Hodgkin lymphoma, cord blood and plasma exchange with dextran or polyvinyl pyrrolidone.

In plasma cell myeloma the discrepancy was observed due to the loss of isoagglutinin. B blood group was determined, however anti-A antibodies were absent in serum.<sup>20</sup> Washing erythrocytes with saline has been found to resolve discrepancy.<sup>21, 22</sup> A case of a person with O blood group and weak anti-A1 as compared to anti-B antibodies was reported.<sup>23</sup> The case of the person with B blood group without anti-A antibodies in the serum due to the low level of gamma-globulin has been described.<sup>19, 24</sup>

Type II discrepancy was reported, when anti-B antibodies were revealed by serum grouping, however erythrocytes were not agglutinated by anti-A antibody at room temperature and 37 °C, nevertheless erythrocytes were agglutinated by anti-A1 lectin.<sup>20, 21</sup> Similarly, the researchers reported of a sample, that on cell typing demonstrated O blood group type, whereas the serum contained anti-A antibodies with weak anti-B antibodies revealed at 4 °C. ABO genotyping showed an O/O genotype and the serum showed reduced levels of IgG and IgM.<sup>19</sup> Group II ABO discrepancy was found in stage IV Hodgkin lymphoma person. The person showed AB Rh positive blood group, however there was weak (1+) reaction with anti-A antibody and anti-A1 lectin. The sample was incubated at 40 °C for 30 minutes and 3+ reaction was revealed.

The researchers reported of the person of A blood group, however the serum agglutinated group A, B and O erythrocytes (type III discrepancy).<sup>25</sup>

Group B positive sample and unexpected agglutination of group B erythrocytes (Ewing sarcoma)<sup>6</sup> after recent group O Rh positive whole blood transfusion from relative was demonstrated. The antibodies transfused with group O plasma were responsible for the spurious result and both IgG and IgM anti-A and anti-B antibodies were detected.

The microtube column agglutination technique using anti-IgG and anti-C3d helped to find additional group B antigen in A group type: AB+ (the forward type: A cell 4+, B cell 2+; the reverse type: absence of anti-A and anti-B antibodies). The erythrocytes were negative for both Coombs test and irregular antibody screening.<sup>20, 22</sup>

Although blood typing techniques have been well described, occurrence of weak variants of erythrocyte antigens causes enigma for transfusionologist. The detection of weak antigens has been reported to be achieved by increasing the time of incubation, modifying the temperature of reaction, processing of erythrocytes with enzymes (stronger interaction of group A and B antigens with antibodies), molecular analysis, adsorption-elution methods.<sup>14, 24, 25</sup>

Usually ABO discrepancies in forward and reverse typing are revealed at room temperature. Nevertheless, the authors underlined the necessity of using different temperatures in blood typing.

Warm auto-antibodies directed against person's antigens may complicate the identification of erythrocyte allo-antibodies (type IV discrepancy).<sup>26</sup> The person demonstrated O blood group type and presence of anti-A antibodies at room temperature typing, whereas forward typing at cold temperature revealed the presence of weak anti-B antibodies.<sup>27</sup>

In accordance with these studies, unexpected antibodies was found in the sera of the persons with ABO discrepancies active at 37 °C and 4 °C on reverse typing.

The conducted study revealed, the serum of the persons with A blood group and presence of weak subgroup of B antigen agglutinated group A erythrocytes at 37 °C and 4 °C after 12 hours of contact. The contact of the studied sera (containing unexpected anti-A antibodies) with group A erythrocytes at 37 °C did not lead to their decreased quantity on the contrary to the usual serum from O blood group (containing usual anti-A antibodies), able to haemolyse group A erythrocytes. Moreover, the sera of the studied persons agglutinated group B erythrocytes at 4 °C and 37 °C, decreasing their quantity at 37 °C. Therefore, in persons of group A with B subgroup, II type of ABO blood group discrepancy, anti-A non-haemolysing and anti-B haemolysing antibodies were revealed. Thus, persons with type II discrepancy should be investigated on adsorbing ability of erythrocytes and agglutinating and haemolysing ability of the sera at 4 °C and 37 °C at prolonged incubation with erythrocytes. The studied sera are recommended to be analysed on agglutination inhibiting ability with other sera.

In II type of discrepancy of the persons with B

blood group and anti-A antibody adsorbing ability the ability of the sera to agglutinate group B erythrocytes at 37 °C and 4 °C after 12 hours of contact was revealed.

These findings indicate the importance to perform prolonged incubation at reverse typing at 4 °C and 37 °C in persons with B<sub>3</sub>, Bm, Bel and Ax antigen (II and III types of ABO discrepancy). The clinical significance of unexpected agglutination at 37 °C by the studied sera deserves attention in transfusion therapy.

In addition, the sera of the studied persons with ABO discrepancies showed an ability to inhibit haemagglutination of erythrocytes by specific antibodies. Thus, the sera from A blood type with presence of anti-A antibodies in 1:2-1:4 titre showed the ability to inhibit agglutination of group A erythrocytes under the influence of anti-A, B serum. The studied sera of group B persons with anti-A antibody adsorbing ability (Ax antigen) in 1:2-1:4 titre decreased agglutination of group B erythrocytes under the influence of anti-A, B serum.

This observation was mentioned by Brazilian scientists. Thus, Subramanian et al concluded that rarely patients with carcinoma and lymphoma can develop excess serum blood group substances, able to deactivate the typing antisera.<sup>28,29</sup>

Since the pretransplant antibody removal and standard immunosuppressive drug medicine as the only additional pretreatment was postulated to be insufficient when using group A1 and B donors,<sup>30</sup> the use of donor plasma able to inhibit agglutination and haemolysis of erythrocytes (as was revealed in the present study) might be important in ABO incompatible transplantation. Whether the use of the plasma of the donors with type II-III of ABO discrepancy will increase the long-term graft survival in ABO incompatible transplantation remains to be shown.

The use of the plasma of the persons with blood group discrepancies able to inhibit haemagglutination of erythrocytes by anti-A and anti-B antibodies (from the persons with blood group discrepancies) might be important for the treatment of autoimmune pathology, especially in autoimmune haemolytic anaemia, when the auto-antibodies of the person's serum react with all normal erythrocytes.

Blood group antigens are known as an expression of human individuality and in pathological conditions a reduction of the expression of group A and B antigens is accompanied by an increase of the expression of precursor molecules.<sup>14, 28, 29</sup> Type III discrepancies were found in persons with modification of blood group type: a person of O blood group with anti-A (1:8 titre) and anti-B antibodies (1:4 titre) after allogeneic group AB transplantation for juvenile myelomonocytic leukaemia showed modification of blood group type: the forward typing demonstrated conversion to AB blood group (anti-A and anti-B reagents showed weak mixed-field agglutination).<sup>17, 30</sup> Type III discrepancy was observed in persons with Hodgkin lymphoma, cord blood and plasma exchange with dextran or polyvinyl pyrrolidone.

After receiving a liver transplant from B group in the forward typing the recipient demonstrated AB, Rh (D) positive blood type, however reverse typing demonstrated group O with anti-E antibodies.<sup>31</sup>

The investigators reported, erythrocytes of A blood type person showed lost group A antigen and were agglutinated by anti-H lectin. Anti-H IgM cold autoantibody agglutinated group O adult cells, however not cord blood cells, only a weak agglutination was noted with group A erythrocytes. Liver transplantation resulted in appearance of ABO discrepancy.<sup>32</sup>

Heal and coworkers have noted, transfusion of plasma across ABH molecules can lead to generation of immune complexes of anti-A and anti-B antibodies with plasma glycoproteins or glycolipids bearing group A and B antigens.<sup>33-35</sup> These can bind to both transfused cells and cells of the recipient. The fact that glycoproteins IIIa, Ia/IIa, IV, PECAM and Ib are constitutively expressed in tissues, other than megakaryocytes (in endothelium) could have implications for transplantation of bone marrow, where ABH compatibility between donor and recipient is not usually observed.<sup>36</sup>

The studied cases of type II-III of ABO discrepancy in forward and reverse typing were associated with additional ability of erythrocytes to adsorb other specificity of antibody as revealed by prolonged incubation at 4 °C temperature. The use of 4 °C and 37 °C temperatures while reverse blood typing increased the possibility of revealing these cases. Methods of inhibition of aggluti-



nation revealed the agglutination inhibiting ability of the serum of the studied persons with type II ABO discrepancy, that might be useful for the management of autoimmune haemolytic disorders. Liquid chromatography may help to reveal the specific erythrocyte antigen.

## Conclusion

Identification of adsorbing erythrocyte antigens is important since persons might be mistyped and show decreased survival due to presence of unexpected anti-A and anti-B antibodies.

Investigation of the sera for anti-A and anti-B antibodies at 37 °C and 4 °C with 12 hours of incubation with test erythrocytes increased the possibility of detection of type II-III discrepancy. Adsorption of erythrocytes with polyclonal serum at 4 °C helped to reveal weak erythrocyte antigen and further heat elution allowed to obtain specific antibodies. Inhibition of haemagglutination helped to reveal agglutination inhibiting ability of the serum in persons with type II-III of discrepancy, whereas liquid chromatography allowed to determine the presence of erythrocyte antigen.

## Acknowledgement

None.

## Conflict of interest

None.

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