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Comparison of agglutination and adsorption activities of blood group typing antibodies under different pH and temperature conditions



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ABSTRACT

Blood group compatibility is essential for the success of organ transplantation and for reducing the risk of rejection. Studying the optimal conditions for the interaction between blood group-specific monoclonal antibodies and erythrocytes plays a crucial role in accurate blood typing for cell and organ transplantation.

AIM. This study is aimed to evaluate the agglutination and adsorption activities of blood group-specific IgM and IgG antibodies under varying pH and temperature conditions.

MATERIALS AND METHODS. The agglutinating and adsorbing properties of monoclonal antibodies of IgM and IgG isotypes with different isoelectric points were analyzed after incubation with red blood cells (RBCs) under various pH values (6.0, 7.4, 7.8) and temperatures (4 °C and 37 °C). Washed group A red blood cells (RBCs) from healthy volunteers (n = 99) were incubated with a panel of IgM and IgG monoclonal antibodies, including 2-8, 2-10, 2-19 (acid-type anti-A mAbs), 2-23 (alkaline-type anti-A mAbs), and anti-H BRIC-231. Agglutination strength was evaluated microscopically and graded on a scale from 0 to 4. Adsorption activity was assessed by analyzing residual antibody activity in the supernatant after incubation.

RESULTS. For IgM of alkaline type and IgG3 antibodies, with optimal adsorbing activity in alkaline medium at 4 °C, maximal adsorbing activity at 37 °C was detected in acidic medium. IgM mAbs exhibited stronger agglutination at 4 °C compared to IgG3 mAbs. Polyclonal antibodies, conventional IgM, and acid-type IgM mAbs demonstrated higher agglutination at pH 7.8, in contrast to alkaline-type IgM and IgG3 mAbs. The optimal temperature for maximal adsorption activity corresponded to the temperature that yielded the strongest agglutination. However, the optimal pH for adsorption was generally opposite to that for agglutination.

The adsorption of IgM antibodies was pH-dependent. Specifically, at 4 °C, the highest adsorption of conventional and acid-type IgM antibodies occurred at pH 6.0, while at 37 °C, it was observed at pH 7.8. For alkaline-type IgM and IgG3 antibodies, optimal adsorbing activity occurred in alkaline medium at 4 °C, whereas at 37 °C, maximal adsorbing activity was observed in acidic medium.

CONCLUSION. The agglutination and adsorption properties of blood group-specific IgM and IgG antibodies are influenced by both pH and temperature, with their optimal pH values differing for these two functions. For the detection of weak A and B antigen variants via adsorption assays at 4 °C, pH 6.0 is recommended for IgM antibodies, while pH 7.8 is preferable for IgG antibodies.

KEY WORDS: blood group compatibility; red blood cells; agglutination; adsorption; IgM; IgG.

Human red blood cells (RBCs) possess over 300 different antigens. The ABH antigens are major alloantigens found in glycoproteins and glycolipids of the RBC surface, and are determined during blood transfusion and organ and tissue transplantation. Blood group compatibility plays a

serious role in determining the success of organ transplants and mitigating the risk of rejection, since A and B antigens are present not only on erythrocytes, but also on other cells of the body and may cause group incompatibility.

The immunological mechanisms affect graft acceptance or rejection and underline the importance of accurate blood group detection. The interactions between antibodies and antigens demonstrate how variations in blood group antigens trigger the immunological reactions and affect the success of transplants. In clinical practice, blood groups are determined using monoclonal antibodies (mAbs) [1]. However, when determining the blood group, an increasing number of cases of discrepancy between forward and reverse blood group typing has been registered. This can be observed due to both weak expression of erythrocyte antigens and non-compliance with the parameters for the optimal activity of a certain type of mAb. In this regard, the ways and means of improving the quality of blood group typing in preparing a recipient for transplantation are important to investigate.

The role of pH and temperature for antigen-antibody interactions has been clarified. However, little is known about the influence of the medium on the agglutinating and adsorbing abilities of IgG and IgM antibodies [2]. Meanwhile, the parameters of the medium have been shown to affect the synthesis of antibodies. Studies on the pH and temperature have showed that low temperature decreases the production of IgM antibodies [3]. Whereas the exposure of IgG molecules to acidic medium during immunoaffinity purification induces their antigen-binding polyreactivity [4]. Low pH destabilizes mAb structure without affecting the native structure [5,6]. Monoclonal antibodies may enter the state of unfolding and aggregation due to different ionic strength of the medium.

It was found that not only *in vitro* pH of the environment affects the activity of antibodies. Thus, in vascular, autoimmune and oncological diseases, local acidic extracellular pH demonstrated an *in vivo* effect on the complement-dependent cytotoxicity (CDC) towards the cells coated with IgG antibodies [7,8]. It has been shown that low pH does not inhibit the adsorption of mAbs: a significant increase in mAb binding was recorded at pH 5.5. It is reported that antibodies at low pH acquire new properties. Conformational changes in human IgG antibodies caused by pH were confirmed using Raman spectroscopy [9].

OBJECTIVE. The present study is aimed to investigate the influence of pH and temperature of the medium on adsorbing and agglutinating properties of blood group specific polyclonal and monoclonal antibodies depending on their type (acid IgM, alkaline IgM or IgG).

MATERIALS AND METHODS

Blood processing. The blood samples (2 mL) from healthy volunteers aged 43.2 ± 5.3 years (52 females, 47 males) were drawn into EDTA tubes. RBCs of blood group A were selected for the study of the corresponding mAbs and were in-house prepared. Briefly, 1 mL of EDTA blood was mixed with 10 mL of 0.9 % saline solution, their contents were mixed and centrifuged for 5 min at a rotor speed of 1500 rpm. The supernatant was discarded, the washing was repeated two more times and 0.9 % saline solution was added to the initial volume of blood and used for the investigation.

Monoclonal antibodies characteristics. The mAbs of different classes were chosen with a purpose to compare abilities of IgM and IgG antibodies. To assess the antibody properties, the methods of agglutination and adsorption were applied. Polyclonal sera, anti-A and anti-B mAbs (*Tulip diagnostics*, India), and group A, B and O RBCs were used for forward and reverse blood group typing.

The study used standard anti-A monoclonal antibodies (mAbs) and acid- or alkaline-type anti-A mAbs (as specified by the manufacturer): 2-8, 2-10, 2-19 (acid-type mAb), 2-23 (alkaline-type mAb), and anti-H BRIC-231. Anti-A mAbs were obtained due to the participation in the program of the second section of the IV International Workshop on mAbs against human RBCs and related antigens (on July 19-20, 2002, in Paris, France) as a gift from International Laboratories (Gamma Biologicals Inc, USA; Ortho Clinical Diagnostics, USA; International Blood Group Reference Laboratory, England) [10].

Study design. The study was conducted in five steps.

1. The investigation of agglutinating abilities of polyclonal and monoclonal (IgM 2-10, 2-18, 2-19, 2-23 and IgG 2-8, BRIC-231) antibodies depending on pH. Three studied groups ($n = 10$; $n = 7$; $n = 7$) included the samples of 50 μL of 2 % suspension of washed RBCs contacted with corresponding antibody (100 μL) for one hour at room temperature. The agglutination was estimated under microscope.

2. The investigation of agglutinating abilities of IgM and IgG antibodies depending on the temperature. Two studied groups ($n = 7$) included the samples of erythrocytes (50 μL) contacted with specific mAb (IgM 2-10 and IgG 2-8) (100 μL) at 4 °C and 37 °C for one hour. The strength of agglutination between groups was compared.

3. The investigation of adsorbing abilities of polyclonal and monoclonal (IgM 2-19 and IgG 2-8) antibodies depending on pH. Four studied groups (total $n = 28$) included the samples of erythrocytes (50 μL) contacted with antibodies (100 μL) for one hour (adsorption phase) at pH 7.4, 6.0 and 7.8, accordingly. After incubation, the tubes were centrifuged for 1 minute at a rotor speed of 1500 rpm at room temperature. The supernatant was investigated on agglutination strength after the contact with erythrocytes at room temperature for one hour (agglutination phase). The control group included the samples of erythrocytes contacted with antibodies without previous adsorption.

4. The investigation of adsorbing abilities of mAbs IgM (2-10, 2-19) and IgG (BRIC-231) at different temperature and pH. Two studied groups ($n = 11$; $n = 11$) included the samples of erythrocytes (50 μL) contacted with mAbs (100 μL) for one hour at 37 °C and 4 °C (adsorption phase) at different pH. The mixtures were centrifuged at 1000 rpm for 1 minute. The supernatant was investigated on the strength of agglutination after the contact with erythrocytes for one hour (agglutination phase). The control group included the samples of erythrocytes contacted with mAbs without previous adsorption.

5. The investigation of agglutinating abilities of antibodies according to the temperature and pH. Six studied groups included the samples of erythrocytes (total $n = 42$) contacted with mAbs IgM (2-19 and 2-23) and IgG (2-8) at 37 °C and 4 °C at different pH (7.4, 6.0 and 7.8). The results were compared according to pH and temperature regimes.

Agglutination and adsorption testing. Antiglobulin test, agglutination and adsorption reactions were performed at pH 6.0, 7.4, 7.8, using buffer solutions and a pH meter (*Avi Scientific*, India) according to the AABB Technical Manual [11]. The results were registered after microscopic investigation. Agglutination was estimated under the microscope XS-3330 (*MicroMed*, China) and graded according to the degree, from strongly positive (4) to negative (-). Five levels from non-agglutination (level 0) to strong agglutination (level 4) were registered [12]. The adsorption of polyclonal and monoclonal antibodies was performed with erythrocytes at 37 °C in thermostat TC-20 (*MicroMed*, China) and 4 °C in refrigerator (*Infitek*, USA).

Ethical considerations. The study was approved by the Kharkiv National Medical University ethics committee (protocol No. 11 from 3.10.23). Written consent was obtained from all participants.

Statistical analysis. The data were collected and statistically analyzed by the software programs MS Excel Ver. 2007 (*Microsoft*, USA) and Statistica 10.0 software (*StatSoft Inc.*, USA). The data normality was assessed by Shapiro-Wilk test. The mean and standard deviation (SD) were used to describe quantitative data. The parameters were compared using the t-test to compare differences between independent groups, since the data were normally distributed. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Investigation of the agglutinating activity of anti-A polyclonal and monoclonal antibodies at different pH values

Anti-A polyclonal sera showed strong agglutinating activity in alkaline medium (at pH 7.8) compared to the acidic medium (pH 6.0) (Table 1). Anti-A IgM mAbs 2-10 and 2-18 demonstrated stronger agglutinating activity in the alkaline medium compared to the acidic medium. Similarly, anti-A mAb 2-19 (IgM of acid type) showed better agglutinating activity

in the alkaline medium. In contrast, anti-A mAb 2-23 (IgM of alkaline type) demonstrated decreased agglutinating activity in the alkaline medium. The agglutinating activity of IgG3 mAb 2-8 was stronger at pH 6.0 compared to pH 7.8 (in 1:2 titre). Anti-H mAb BRIC-231 showed better agglutinating activity in physiological and alkaline medium.

Table 1. Agglutination (levels 0-4) of red blood cells by monoclonal and polyclonal antibodies at different pH (M ± SD).

	Group 1 pH 7.4 (n = 10)	Group 2 pH 6.0 (n = 7)	Group 3 pH 7.8 (n = 7)	p
Polyclonal serum	1.23 ± 0.02	1.15 ± 0.01	3.27 ± 0.04	p _{1,3} = 0.031 p _{2,3} = 0.024
IgM antibodies				
2-10	2.07 ± 0.02	1.04 ± 0.02	2.15 ± 0.02	p _{1,2} = 0.041 p _{2,3} = 0.023
2-18	1.05 ± 0.01	0	2.32 ± 0.02	p _{1,2} = 0.004 p _{1,3} = 0.003 p _{2,3} = 0.001
2-19	3.16 ± 0.04	2.24 ± 0.03	3.07 ± 0.05	p _{1,2} = 0.024 p _{2,3} = 0.043
2-23	3.42 ± 0.06	2.21 ± 0.02	1.01 ± 0.01	p _{1,2} = 0.032 p _{1,3} = 0.002 p _{2,3} = 0.002
IgG antibodies				
2-8	3.11 ± 0.03	3.83 ± 0.06	3.24 ± 0.04	p _{1,2} = 0.034 p _{2,3} = 0.051
BRIC-231	1.02 ± 0.01	0	1.04 ± 0.02	p _{1,2} = 0.026 p _{2,3} = 0.044

Note: p – indicates the comparison between the data of the corresponding groups numbered 1-3.

The influence of the temperature on the agglutinating properties of IgM and IgG anti-A antibodies.

Anti-A IgM mAb 2-10 (in 1:2 titre) showed stronger agglutinating activity at 4 °C as compared to 37 °C (Table 2). Similarly, IgM 2-19 and 2-23 mAbs demonstrated high agglutinating properties at 4 °C. Whereas anti-A

IgG3 mAb 2-8 (in 1:2 titre) showed stronger agglutinating activity at 37 °C compared to 4 °C. Similarly, in an indirect antiglobulin test, stronger agglutination degree was observed at 37 °C as compared to the incubation at 4 °C (p-value = 0.002).

Table 2. Agglutination (levels 0-4) of group A red blood cells by monoclonal antibodies at different temperatures (M ± SD).

Antibodies	Group 1 4 °C (n = 7)	Group 2 37 °C (n = 7)	p
2-8	3.24 ± 0.03	3.88 ± 0.05	p _{1,2} = 0.033
2-10	3.81 ± 0.04	2.74 ± 0.03	p _{1,2} = 0.005
2-8+AGS	1.01 ± 0.01	3.11 ± 0.02	p _{1,2} = 0.002
2-10+AGS	1.23 ± 0.02	1.13 ± 0.01	p _{1,2} = 0.7

Note: p – indicates the comparison between the data of the corresponding groups numbered 1 and 2.

Investigation of the adsorbing activity of polyclonal and monoclonal antibodies at different pH regimes

Anti-A polyclonal sera showed stronger adsorption on erythrocytes at pH 7.8 compared to pH 6.0 (Table 3). The adsorbing activity of anti-A

IgG3 mAb 2-8 was stronger in alkaline medium. Similarly, the adsorbing activity of anti-A mAb 2-19 was stronger in the alkaline medium compared to the acidic medium (p < 0.05).

Table 3. Agglutinating activity (levels 0-4) of anti-A monoclonal and polyclonal antibodies after their adsorption with group A red blood cells (M ± SD).

	Before adsorption		After adsorption		p
	Group 1 (n = 7)	Group 2 At pH 7.4 (n = 7)	Group 3 At pH 6.0 (n = 7)	Group 4 At pH 7.8 (n = 7)	
Polyclonal serum	2.24 ± 0.02 (1:2 titre)	1.01 ± 0.01 (1:2 titre)	1.34 ± 0.02 (1:2 titre)	0 (1:2 titre)	p _{2,3} = 0.041 p _{2,4} = 0.003 p _{3,4} = 0.002
	1.02 ± 0.01 (1:4 titre)	0 (1:4 titre)	1.02 ± 0.01 (1:4 titre)	0 (1:4 titre)	p _{2,3} = 0.006 p _{3,4} = 0.005
2-8 (IgG3)	3.36 ± 0.04 (1:2 titre)	1.24 ± 0.02 (1:2 titre)	2.27 ± 0.02 (1:2 titre)	1.22 ± 0.01 (1:2 titre)	p _{2,3} = 0.006 p _{3,4} = 0.006
	2.65 ± 0.03 (1:4 titre)	1.1 ± 0.01 1.2 (1:4 titre)	2.0 ± 0.01 (1:4 titre)	1.0 ± 0.01 (1:4 titre)	p _{2,3} = 0.004 p _{3,4} = 0.003
2-19 (IgM)	3.85 ± 0.04 (1:2 titre)	3.63 ± 0.05 (1:2 titre)	3.72 ± 0.03 (1:2 titre)	3.11 ± 0.03 (1:2 titre)	p _{2,4} = 0.032 p _{3,4} = 0.024
	3.64 ± 0.03 (1:4 titre)	3.32 ± 0.04 (1:4 titre)	3.27 ± 0.02 (1:4 titre)	2.17 ± 0.02 (1:4 titre)	p _{2,4} = 0.002 p _{3,4} = 0.016

Note: p – indicates the comparison between the data of the corresponding groups numbered 2-4.

Investigation of adsorbing properties of anti-A mAbs at different values of temperature

mAb 2-10 showed stronger adsorption on erythrocytes at 4 °C compared to 37 °C (Table 4). The adsorption of mAb 2-10 on group A RBCs at various temperature values appeared to be dependent on the pH level. Thus, at pH 6.0, the adsorption was stronger at 4 °C compared to 37 °C (p-value = 0.002), whereas at pH 7.8 stronger adsorption was revealed at 37 °C compared to 4 °C (p-value = 0.053). Adsorption of mAb 2-19 in acid

or alkaline medium was also dependent on pH level. At pH 6.0, the adsorption of mAb 2-19 on erythrocytes was stronger at 4 °C compared to 37 °C (p-value = 0.002). In contrast, at pH 7.8 the adsorption of mAb 2-19 was stronger at 37 °C compared to 4 °C (p-value = 0.045). The adsorption of anti-H mAb BRIC-231 appeared to be stronger at 4 °C compared to 37 °C (p-value = 0.047). The adsorption of BRIC-231 was stronger at pH 7.8 and 4 °C, as well as at pH 6.0 and 37 °C (p = 0.002). The adsorption of IgG3 mAb 2-8 was also stronger at 4 °C and pH 7.8 (p = 0.023).

Table 4. Adsorbing ability (levels 0-4) of anti-A mAbs by group A red blood cells at different pH and temperature values.

	Agglutination level			p	
	pH	Group 1 adsorption at 37 °C (n = 11)	Group 2 adsorption at 4 °C (n = 11)		Before adsorption (n = 11)
2-10 (IgM)	6.0	2.34 ± 0.02(1:2 titre) 2.01 ± 0.02(1:4 titre)	2.28 ± 0.02 0		p _{1,2} = 0.571 p _{1,2} = 0.002
	7.8	1.27 ± 0.02(1:2 titre) 1.05 ± 0.01(1:4 titre)	3.24 ± 0.04 1.26 ± 0.02		p _{1,2} = 0.001 p _{1,2} = 0.053
	7.4	2.75 ± 0.04(1:2 titre) 2.24 ± 0.03(1:4 titre)	2.32 ± 0.03 1.13 ± 0.01	3.2 ± 0.03 2.7 ± 0.02	
2-19 (IgM of acid type)	6.0	3.27 ± 0.03(1:2 titre) 2.71 ± 0.02(1:4 titre)	1.28 ± 0.02 1.15 ± 0.01		p _{1,2} = 0.003 p _{1,2} = 0.002
	7.8	2.43 ± 0.03(1:2 titre) 2.11 ± 0.02(1:4 titre)	2.84 ± 0.04 2.63 ± 0.03		p _{1,2} = 0.053 p _{1,2} = 0.045
	7.4	3.13 ± 0.04(1:2 titre) 2.45 ± 0.03(1:4 titre)	3.03 ± 0.03 2.76 ± 0.02	3.43 ± 0.03 3.24 ± 0.02	
BRIC-231 (IgG3)	6.0	0(1:2 titre) 0(1:4 titre)	1.05 ± 0.01 0		p _{1,2} = 0.002
	7.8	1.24 ± 0.02(1:2 titre) 1.07 ± 0.01(1:4 titre)	0 0		p _{1,2} = 0.034 p _{1,2} = 0.023
	7.4	1.06 ± 0.01(1:2 titre) 0(1:4 titre)	0 0	1.24 ± 0.02 1.05 ± 0.01	

Note: p – indicates the comparison between the data of the corresponding groups numbered 1 and 2.

The agglutinating and adsorbing properties of mAbs at 4 °C and 37 °C in acid and alkaline medium

The investigation demonstrated the differences in the agglutinating and adsorbing abilities of anti-A IgM and IgG antibodies associated with pH and temperature values. At 4 °C mAb 2-19 showed stronger agglutinating activity in alkaline medium compared to acidic medium (p-value = 0.023). The adsorbing ability at 4 °C appeared to be stronger at pH 6.0 compared to pH 7.8.

Anti-A IgM mAb of alkaline type 2-23, similarly to mAb 2-19, showed stronger agglutinating ability at 4 °C. Similarly to mAb 2-8 at 37 °C, anti-A mAb 2-23 demonstrated a strong agglutinating ability at pH 6.0 compared to pH 7.8 (p-value = 0.033) (Figures 1, 2). Stronger adsorbing ability at 37 °C was detected at pH 7.8 compared to pH 6.0 (p-value = 0.023) (Figures 3, 4).

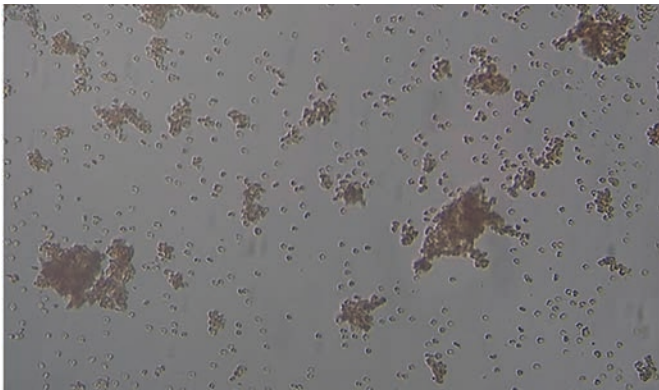


Fig. 1. Microphotography of human RBCs showing agglutination of group A RBCs with mAb anti-A 2-23 under conditions 37 °C and pH 6.0. Light microscopy, magnification x400.

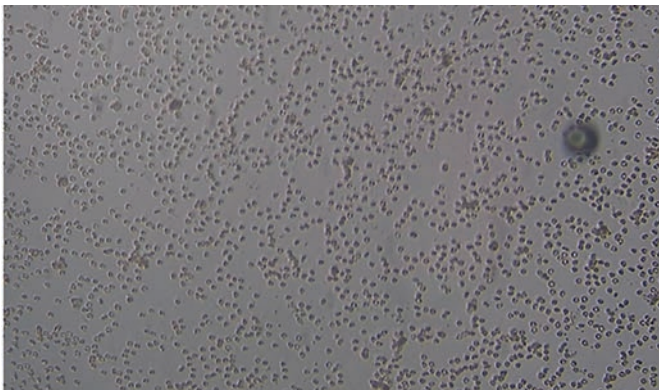


Fig. 2. Microphotography of human RBCs showing agglutination of group A RBCs with mAb anti-A 2-23 under conditions 37 °C and pH 7.8. Light microscopy, magnification x400.

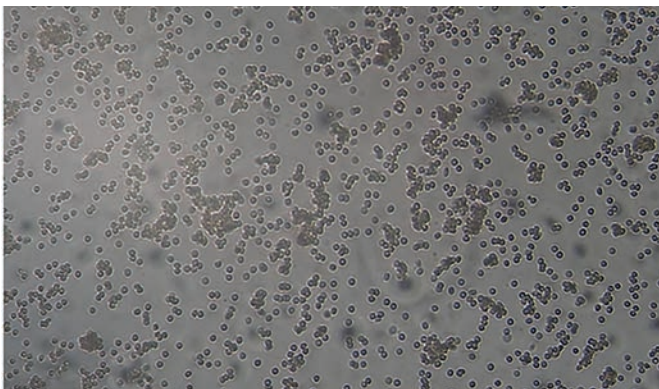


Fig. 3. Microphotography of human RBCs showing agglutination of group A RBCs with mAb anti-A 2-23, that was adsorbed under conditions 37 °C and pH 6.0. Light microscopy, magnification x400.

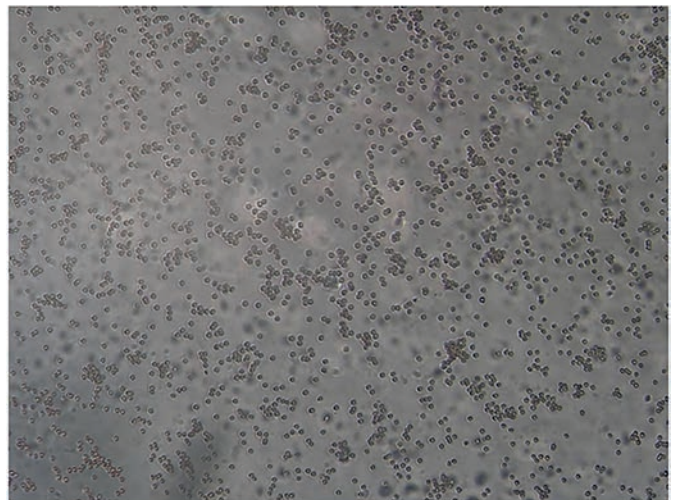


Fig. 4. Microphotography of human RBCs showing agglutination of group A RBCs with mAb anti-A 2-23, that was adsorbed by A RBCs under conditions 37 °C and pH 7.8. The agglutination is weaker compared to Figure 4, that testifies to the stronger adsorbing ability of 2-23 mAb at pH 7.8 compared to pH 6.0. Light microscopy, magnification x400.

Anti-A IgG3 mAb 2-8 showed strong agglutinating and adsorbing properties with RBCs at 37 °C (Table 5). At 4 °C mAb 2-8 demonstrated stronger agglutinating ability in acidic medium compared to alkaline medium (p-value = 0.051). Stronger adsorption at 4 °C was detected in alkaline medium compared to acidic medium (Figures 5, 6). Therefore, the expression of the agglutinating and adsorption properties of IgM and IgG mAbs at the same temperature was observed in opposite pH values of the medium.

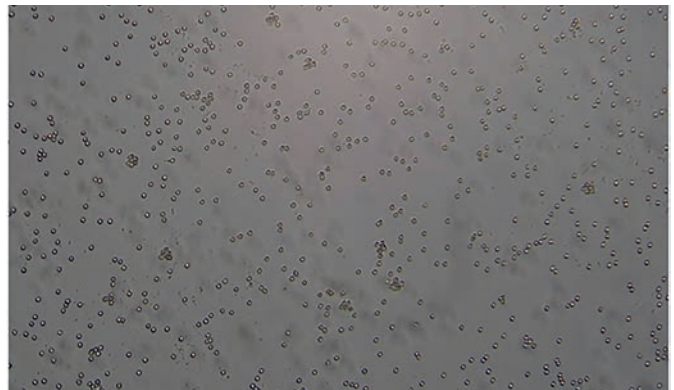


Fig. 5. Microphotography of human RBCs showing agglutination of group A RBCs with mAb anti-A 2-8 under conditions 4 °C and pH 6.0. Light microscopy, magnification x400.



Fig. 6. Microphotography of human RBCs showing agglutination of group A RBCs with mAb anti-A 2-8 under conditions 4 °C and pH 7.8. Light microscopy, magnification x400.

Table 5. Agglutination (levels) of group A red blood cells by monoclonal anti-A antibodies in acid and alkaline medium

	37 °C			4 °C			p
	Group 1 pH 7.4 (n = 7)	Group 2 pH 6.0 (n = 7)	Group 3 pH 7.8 (n = 7)	Group 4 pH 7.4 (n = 7)	Group 5 pH 6.0 (n = 7)	Group 6 pH 7.8 (n = 7)	
2-19	3.25 ± 0.02	3.13 ± 0.01	3.57 ± 0.02	3.34 ± 0.03	3.21 ± 0.02	3.83 ± 0.02	p _{1,3} = 0.042 p _{2,3} = 0.054 p _{5,6} = 0.023
2-23	2.71 ± 0.01	3.01 ± 0.01	2.61 ± 0.02	3.22 ± 0.02	3.71 ± 0.02	3.11 ± 0.02	p _{1,2} = 0.054 p _{2,3} = 0.033 p _{4,5} = 0.026 p _{5,6} = 0.002
2-8	3.64 ± 0.03	3.73 ± 0.02	3.41 ± 0.01	3.05 ± 0.03	3.54 ± 0.02	3.11 ± 0.01	p _{1,3} = 0.053 p _{2,3} = 0.034 p _{4,5} = 0.037 p _{5,6} = 0.051

Note: p – indicates the comparison between the data of the corresponding groups numbered 1-6.

IgM and IgG mAbs are widely used for the detection of ABH antigens and their weak variants. Their important role has been demonstrated in transplantation and transfusion practice [13]. However, the agglutinating and adsorbing abilities of mAbs according to their isoelectric properties (acid or alkaline type) have not been studied. Meanwhile, IgM antibody synthesis (in acidic medium) and cytotoxic activity of IgG mAbs (completely absent in acidic medium) have been proved to be pH dependent. The appropriate pH is essential for the binding of antibodies with antigens. The antigen-antibody contact is based on the presence of electrokinetic potential of epitopes and paratopes [14]. Our previous studies showed that binding of blood group specific antibodies with corresponding RBCs reduces their electrokinetic potential, since, pH modifications may induce the shift of the charge in both mAb molecules and RBC antigens [15].

The role of acid pH in cell biology has been also reported in cell biology. The acidic medium was shown to delay the rate of apoptosis in neutrophils, endothelial cells and tumor cells and to regulate cell functions [16]. The studies demonstrated the influence of pH on activation of the complement, leading to the organ damage in viral infections, autoimmune diseases, and transplantation [17, 18]. Differences in pH lead to the mAb and cell modifications [19]. The acidification of the medium modified the agglutinating abilities of anti-A mAbs and their inhibition by glycoconjugates of RBC membranes of A1 group type [20].

The temperature regime has been also shown to regulate the activity of antibodies. Shifting the pH and temperature significantly influences the cell proliferation rate and antibody productivity [21]. Naturally occurring antibodies were reported to react optimally at 4 °C with hemolytic properties at 37 °C. IgG anti-A and anti-B antibodies from O blood group persons were reported to induce hemolysis at 37 °C [22, 23].

The different regimes of agglutination and adsorption reactions for blood group typing in cases of type II-III ABO discrepancy have been investigated in our previous studies [24]. High adsorbing ability of IgM antibodies on erythrocytes and epithelial cells in alkaline medium was revealed. Our studies showed the successful use of the heated polyclonal anti-A serum and IgG3 mAb 2-8 at 37 °C in an antiglobulin test for weak blood group antigen A detection [25]. Low temperature regime in adsorption reaction was revealed to be necessary for the weak antigens typing [26].

The present study revealed strong agglutinating and adsorbing properties of polyclonal sera and IgM and IgM of acid-type mAbs in alkaline medium (at pH 7.8 compared to pH 6.0). Meanwhile, IgG3 mAb and IgM

mAb 2-23 of the alkaline type demonstrated strong agglutinating activities in acidic medium. Polyclonal and monoclonal antibodies differed in their agglutinating and adsorbing properties according to the various pH and temperature regimes. Strong agglutinating and adsorbing properties of IgM antibodies were revealed at 4 °C, whereas optimal activity of IgG antibodies was detected at 37 °C. The obtained data agree with the recommendations to perform the adsorption of IgM antibodies at 4 °C [27].

The adsorption of mAbs was also dependent on pH of the medium, since electrostatic interactions between a protein and the surface are known to play a central role in driving adsorption. The difference in adsorption may arise from subtle changes in the charged state of local patches of acidic/basic residues. For usual IgM antibodies and IgM antibodies of acid type at 4 °C higher adsorbing ability was determined at pH 6.0, while at 37 °C stronger adsorption was observed at pH 7.8. Whereas IgM of alkaline type and IgG3 antibodies at 4 °C demonstrated maximal adsorbing activity in alkaline medium and at 37 °C optimal adsorbing properties were revealed in acidic medium.

The data agree with the previous studies that showed better adsorption of alkaline mAb (with pI 8.99) at pH 7.4 compared to pH 5.5. At both pH values, mAb was expected to carry a net positive charge facilitating electrostatic attraction with the negatively charged surface [28]. The differences in the degree of agglutinating and adsorption abilities of mAbs at the same pH and temperature regimes indicate that they bind to the group-specific antigens with different isoelectric characteristics. The indirect antiglobulin test revealed a strong avidity of IgG3 antibodies to the erythrocytes at 37 °C. IgG3 antibodies showed stronger adsorption on erythrocytes at 37 °C compared to 4 °C, in contrast to the weak adsorption of IgM antibodies at 37 °C.

The obtained data supplemented the existing data on the influence of the medium on antibody activity and may be useful in resolving discrepancies between forward and reverse blood group testing in cases of weak expression of antigens on RBCs [29, 30]. The adsorption studies at 4 °C with IgM and IgM of acid type would be more accurate at pH 6.0, meanwhile, with IgM of alkaline type and IgG3 mAbs – at pH 7.8. The pH and temperature values for optimal activity of mAbs have been described in the study in the hope that it will provide a logarithm of an appropriate pH and temperature regimes for blood group typing (**Table 6**).

Table 6. The pH and temperature regimes for optimal agglutinating and adsorbing activity of polyclonal and monoclonal antibodies

	Agglutination					Adsorption				
	pol	IgM	IgM of acid type	IgM of alkaline type	IgG3	pol	IgM	IgM of acid type	IgM of alkaline type	IgG3
T, °C	4 °C	4 °C	4 °C	4 °C	4 °C	4 °C	4 °C	4 °C	4 °C	4 °C
pH	7.8	7.8	7.8	6.0	6.0	7.8	6.0	6.0	7.8	7.8

Note: pol – polyclonal serum, T – temperature.

The obtained differences in the agglutinating and adsorbing activities of mAbs at 4 °C at opposite values of pH were associated with acidic or alkaline types of IgM antibodies that testifies to the importance of appropriate pH medium control while using acidic or alkaline IgM antibodies in

blood group typing. The revealed properties of mAbs are the novelty of the study and testify to the importance of using appropriate pH and temperature regime according to the class and type (acid or alkaline) of antibody to achieve reliable results and reveal weak variants of ABH antigens.

CONCLUSION

The polyclonal antibodies, usual IgM and IgM of acid type mAbs demonstrated strong agglutinating activities in alkaline medium in contrast to IgM of alkaline type and IgG3 mAbs with strong agglutinating properties in the acidic medium. IgM and IgM of acid type antibodies showed strong agglutinating activities at 4 °C, whereas IgG3 antibodies showed better agglutinating properties at 37 °C. The optimal temperature regime for the strongest agglutinating activity of antibodies coincided with that for their best adsorbing activity. Meanwhile, the pH values for the strongest antibody adsorbing activities were opposite to those for their best agglutinating properties.

At 4 °C IgM and IgM of acid-type mAbs showed optimal agglutinating activity in alkaline medium, whereas the strongest adsorbing ability was revealed in the acidic medium. For IgM of alkaline type and IgG3 antibodies with optimal agglutinating activity in acidic medium, maximal adsorbing activity was detected in alkaline medium.

Our study highlights the different influence of the pH of the medium on the activity of mAbs (acid type IgM, alkaline type IgM and IgG), as well as on the manifestation of their agglutinating and adsorption properties in blood group typing. Detection of weak variants of A and B antigens in the adsorption reaction at 4 °C is recommended to be carried out at pH 6.0 when using IgM group specific antibodies, and at pH 7.8 when using IgG antibodies.

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Порівняння аглютинаційної та адсорбційної активності антитіл для визначення групи крові за різних умов рН і температури

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РЕЗЮМЕ

Сумісність за групами крові є необхідною умовою для визначення успіху трансплантації органів і зниження ризику відторгнення. Дослідження оптимальних умов для реакції моноклональних антитіл з еритроцитами відіграє серйозну роль для визначення груп крові при трансплантації клітин і органів.

МЕТОЮ ДОСЛІДЖЕННЯ було оцінити аглютинуючу та адсорбційну активність групспецифічних IgM та IgG антитіл при різних режимах рН та температури.

МАТЕРІАЛИ ТА МЕТОДИ. Аглютинуючі та адсорбційні властивості моноклональних антитіл ізотипів IgM та IgG з різними ізоелектричними точками було проаналізовано після інкубації з еритроцитами за різних значень рН (6,0; 7,4; 7,8) і температур (4 °C та 37 °C). Відмиті еритроцити групи А, отримані від здорових донорів (n = 99), інкубували з панеллю моноклональних антитіл IgM та IgG, зокрема 2-8, 2-10, 2-19 (anti-A антитіла кислотного типу), 2-23 (anti-A антитіла лужного типу) та anti-H BRIC-231. Силу аглютинації оцінювали мікроскопічно за шкалою від 0 до 4. Адсорбційну активність визначали шляхом аналізу залишкової активності антитіл у супернатанті після інкубації.

РЕЗУЛЬТАТИ. IgM моноклональні антитіла показали кращу аглютинуючу активність при 4 °C на відміну від IgG3 антитіл. Поліклональні антитіла, IgM та IgM кислотного типу антитіла продемонстрували високу аглютинуючу здатність при рН 7,8 на відміну від IgM лужного типу та IgG3 антитіл. Оптимальний температурний режим для найсильнішої адсорбційної активності антитіл збігався з температурним режимом їх найкращої аглютинуючої активності. Однак значення рН для найкращої адсорбуючої активності антитіл були протилежними значенням для їх найвищої аглютинуючої здатності.

Адсорбція антитіл IgM залежала від рН середовища. Так, для звичайних антитіл IgM та IgM кислотного типу при 4 °C найкраща адсорбуюча здатність спостерігалася при рН 6,0, тоді як більш сильна адсорбція була виявлена при рН 7,8. Для антитіл IgM лужного типу та IgG3 оптимальна адсорбційна активність виявлена в лужному середовищі при 4 °C, тоді як при 37 °C максимальна адсорбційна активність спостерігалася в кислому середовищі.

ВИСНОВКИ. Прояв аглютинуючої та адсорбційної здатності групспецифічних антитіл IgM та IgG при однаковій температурі спостерігався при протилежних значеннях рН середовища. Виявлення слабких варіантів антигенів А та В у реакції сорбції при 4 °C рекомендується проводити при рН 6,0 при використанні антитіл IgM та при рН 7,8 при використанні антитіл IgG.

КЛЮЧОВІ СЛОВА: сумісність за групами крові; еритроцити; аглютинація; адсорбція; IgM; IgG