COMPARISON OF ACCURACY CELL GROUPING AND SERUM GROUPING EXAMINATION FOR ABO BLOOD TYPES BASED ON VARIATIONS IN CELL SUSPENSION CONCENTRATION

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ABSTRACT

ABO blood grouping relies on both cell grouping and serum grouping to ensure diagnostic accuracy. Differences in agglutination intensity between these two methods across varying cell suspension concentrations can impact test interpretation. This study aimed to evaluate and compare the agglutination grades between cell grouping and serum grouping using Test Cell A and Test Cell B across different concentrations (3%, 5%, and 10%). An observational comparative study was conducted using ordinal agglutination score data. The non-parametric Wilcoxon Signed-Rank test was utilized to determine significant differences between cell grouping and serum grouping for each test cell type. Statistical analysis using the Wilcoxon Signed-Rank test revealed no significant difference in agglutination scores between cell grouping and serum grouping for Test Cell A and Test Cell B (p > 0,05). In Test Cell A, a p-value of 0.750 was obtained, and in Test Cell B, a p-value of 0.625 was obtained. This indicates that both methods produce statistically comparable examination results across the concentrations of 3%, 5%, and 10%.

KEYWORDS ABO, cell grouping, serum grouping, ABO, suspension concentrations, degrees of agglutination.

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INTRODUCTION

Blood transfusion is a medical procedure that plays a vital role in saving patients' lives in cases of acute hemorrhage, severe anemia, or major surgery. Transfusion safety relies heavily on the accuracy of pre-transfusion testing particularly ABO blood typing because errors in blood type determination can trigger serious transfusion reactions that endanger the patient (Yunus et al., 2025)(Li et al., 2022). ABO blood group testing is a mandatory laboratory procedure before donor blood is administered to a recipient, as blood

group incompatibility can trigger an antigen-antibody reaction that endangers patient safety (Noviana, 2026).

The fundamental principle of ABO blood typing is the reaction between antigens on the surface of erythrocytes and antibodies present in serum or anti-serum reagents, resulting in agglutination or the clumping of red blood cells that can be visually observed (Ammariah & Kartika, 2022). This principle is implemented through two complementary testing techniques: cell grouping (forward grouping) and serum grouping (reverse grouping). Cell grouping aims to determine the presence or absence of antigens on the surface of red blood cells by reacting a suspension of the patient's red blood cells with anti-A and anti-B reagents, whereas serum grouping aims to determine the presence or absence of antibodies in the patient's serum or plasma by reacting the serum with group A, B, and O test cells (Karimah et al., 2026). Ideally, these two techniques should be performed simultaneously as a cross-check to ensure a more accurate determination of an individual's blood type.

Several technical factors are known to influence the degree of agglutination produced, including the concentration of the red blood cell suspension used and the duration of the reaction or incubation between antigen and antibody (Wulansari, 2025). According to WHO guidelines, the ratio between serum and the erythrocyte cell suspension is a critical determinant of the sensitivity of the resulting agglutination reaction; consequently, an incorrect cell suspension concentration risks either weakening the reaction or, conversely, triggering a disproportionate response (Utari et al., 2024)(Ananda et al., 2024).

Accurate reading of the degree of agglutination in tube-method blood typing is of significant clinical importance; misinterpreting the degree of agglutination whether due to an excessively weak reaction or non-standard procedures carries the risk of incorrect blood type determination, which directly impacts patient safety during transfusions (Wardhani et al., 2024)(Azzahra, 2025). Therefore, it is important to understand how variations in cell suspension concentration affect the resulting degree of agglutination in tube-method cell grouping and serum grouping tests, as this information can inform the development and evaluation of standard operating procedures in blood bank laboratories. Against this background, this study was conducted to analyze differences in the degree of agglutination based on variations in time and concentration during tube-method cell grouping and serum grouping.

RESEARCH METHOD

This study is a quasi-experimental research, which involves treating A and B, cell tests in a 0.9% NaCl solvent, each prepared from two K2EDTA blood samples of blood groups A and B. The cell tests were prepared at concentrations of 3%, 5%, and 10%, followed by ABO blood typing tests using cell grouping and serum grouping methods. The study was conducted at the Laboratory of the Faculty of Health Sciences, Universitas Duta Bangsa Surakarta. A total of 9 children meeting the inclusion criteria were enrolled in this study. The inclusion criteria for this study were willingness to participate as respondents by signing written informed consent and collecting venous blood samples with EDTA anticoagulant. The blood group of the collected venous blood samples was already known. The obtained primary data were statistically analyzed using SPSS 26.0 for Windows. The statistical test employed was the non-parametric Wilcoxon test.

RESULT AND DISCUSSION

Blood typing was performed to assess the degree of agglutination using cell suspensions at concentrations of 3%, 5%, and 10%. The following are the results of the examination and the observed agglutination for each concentration.

Table 1. Test Results of Cell B Testing Using Cell Grouping and Serum Grouping Methods

Concentration	R	Cell grouping					Serum Grouping				
		-	+1	+2	+3	+4	-	+1	+2	+3	+4
3%	A1					✓					✓
	A2					✓					✓
	A3					✓					✓
5%	A1					✓					✓
	A2				✓						✓
	A3					✓				✓	
10%	A1					✓					✓
	A2				✓					✓	
	A3					✓				✓	

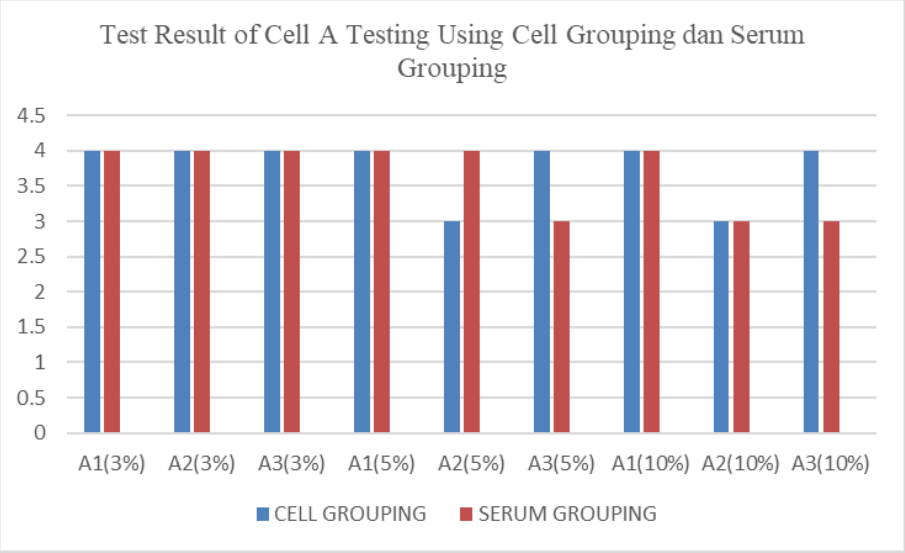


Figure 1. Comparison of Test Results for Cell A Testing Using Cell Grouping and Serum Grouping Methods

The examination results for Cell Grouping and Serum Grouping using Cell A testing demonstrated agglutination reactions across all tested concentration variations, namely 3%, 5%, and 10%. At a 3% concentration, all replications for both Cell Grouping and Serum Grouping showed a +4 agglutination degree. At a 5% concentration, Cell Grouping yielded results of +4, +3, and +4, while Serum Grouping showed results of +4, +4, and +3 across the respective replications. At a 10% concentration, Cell Grouping produced results of +4, +3, and +4, whereas Serum Grouping yielded +4, +3, and +3. Overall, the results indicate that all samples produced positive agglutination reactions with an intensity ranging from +3 to +4, with the 3% concentration displaying the most consistent results across all three replications.

Table 2. Test Results of Cell B Testing Using Cell Grouping and Serum Grouping Methods

Concentration	R	Cell grouping					Serum Grouping				
		-	+1	+2	+3	+4	-	+1	+2	+3	+4
3%	B1					✓				✓	
	B2				✓						✓
	B3					✓				✓	
5%	B1					✓					✓
	B2					✓					✓
	B3					✓					✓
10%	B1					✓					✓
	B2				✓					✓	
	B3					✓				✓	

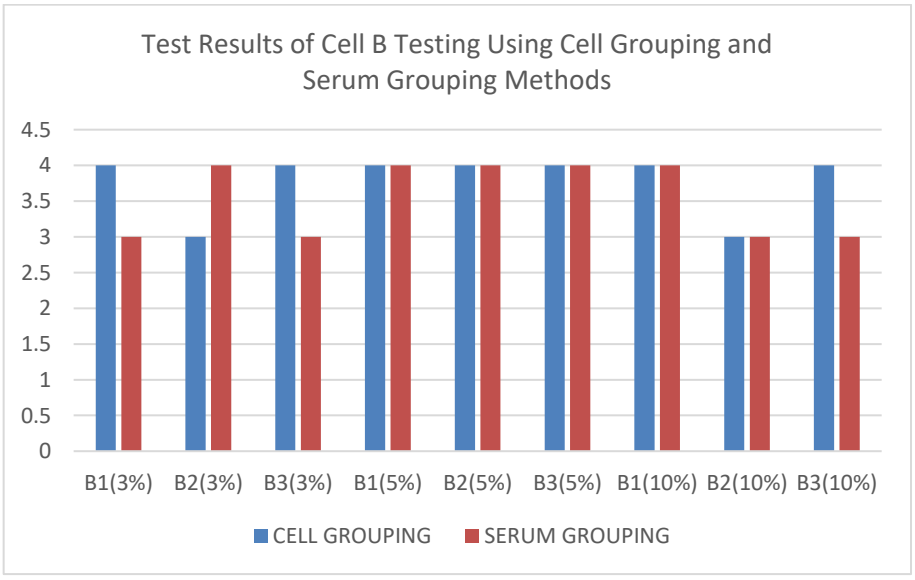


Figure 2. Comparison of Test Results for Cell B Testing Using Cell Grouping and Serum Grouping Methods

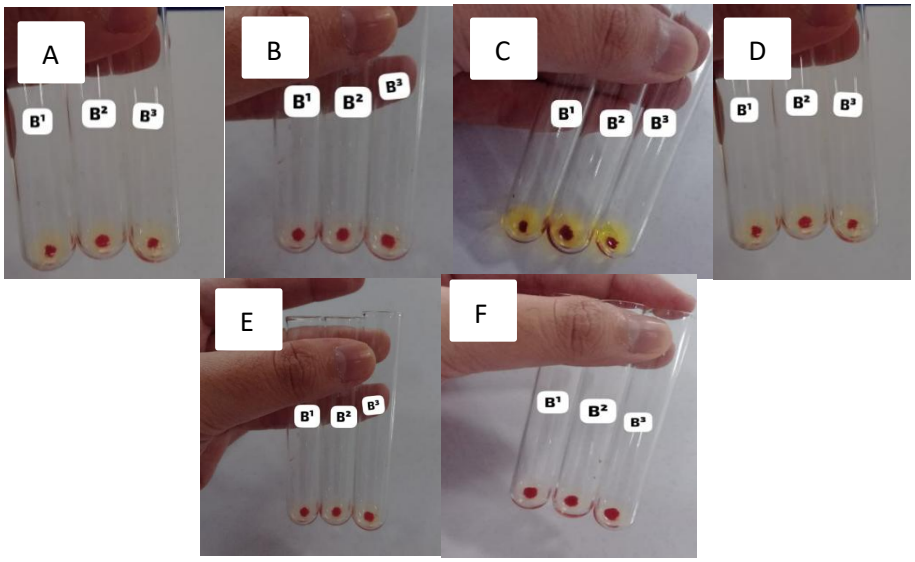


Figure 3. (A) 3% Cell B Test (B) 5% Cell B Test (C) 10% Cell B Test (D) 3% Serum B Test (E) 5% Serum B Test (F) 10% Serum B Test.

The examination results for Cell B testing in Cell Grouping and Serum Grouping showed agglutination reactions across all tested concentrations, namely 3%, 5%, and 10%. At a 3% concentration, Cell Grouping exhibited agglutination degrees of +4, +3, and +4, while Serum Grouping yielded results of +3, +4, and +3 for the respective replications. At a 5% concentration, all replications in both Cell Grouping and Serum Grouping showed +4 agglutination. Meanwhile, at a 10% concentration, Cell Grouping produced results of +4, +3, and +4, whereas Serum Grouping yielded +4, +3, and +3. Overall, all concentrations resulted in positive agglutination reactions with intensities ranging from +3 to +4. The 5% concentration demonstrated the most consistent results, as all replications in both Cell Grouping and Serum Grouping gave a +4 agglutination degree.

Table 3. Wilcoxon Test Results of Cell B Testing Using Cell Grouping and Serum Grouping Methods

Konsentrasi	Cell Grouping	Serum Grouping	p-value
3%	4, 4, 4	4, 4, 4	1,000
5%	4, 3, 4	4, 4, 3	1,000
10%	4, 3, 4	4, 3, 3	1,000
Wilcoxon Test			**0,750**

In Cell A testing, no statistically significant difference was detected between the results of Cell Grouping and Serum Grouping at concentrations of 3%, 5%, or 10% (all $p > 0.05$). When all concentrations were combined, $p = 0.750$.

Table 4. Wilcoxon Test Results of Cell B Testing Using Cell Grouping and Serum Grouping Methods

Konsentrasi	Cell Grouping	Serum Grouping	p-value
3%	4, 3, 4	3, 4, 3	0,750
5%	4, 4, 4	4, 4, 4	1,000
10%	4, 3, 4	4, 3, 3	1,000
Wilcoxon Test			**0,625**

In Cell B testing, no statistically significant difference was detected between Cell Grouping and Serum Grouping at concentrations of 3%, 5%, or 10% ($p > 0.05$). When all concentrations were analyzed together, $p = 0.625$.

CONCLUSION

This study compared the results of Cell Grouping and Serum Grouping using Cell A testing and Cell B testing at concentrations of 3%, 5%, and 10%. Descriptive analysis indicated that agglutination reactions were generally consistent, with most results falling within the range of +3 to +4. Only a few samples exhibited a one-grade difference in reaction between Cell Grouping and Serum Grouping. Descriptively, the average agglutination score in Cell A testing was $3{,}78 \pm 0{,}44$ for Cell Grouping and $3{,}67 \pm 0{,}50$ for Serum Grouping. Meanwhile, in Cell B testing, the average agglutination score for Cell Grouping was $3{,}78 \pm 0{,}44$ and for Serum Grouping was $3{,}56 \pm 0{,}53$. Although minor variations occurred in the form of a decrease in agglutination grade from $+4$ to $+3$ in several samples at cell concentration levels of 5% and 10%, these differences did not affect the validity or agreement between the methods.

For the statistical testing of Cell A testing, the Wilcoxon Signed-Rank test showed a p-value of 0.750, indicating no statistically significant difference between Cell Grouping and Serum Grouping ($p = 1.000$). Similarly, for Cell B testing, no statistically significant

difference was observed, with a p-value of 0.625. These findings demonstrate that both grouping methods produce comparable results for each cell test.

Thus, it can be concluded that the Cell Grouping and Serum Grouping methods produce comparable, consistent, and highly aligned agglutination degrees across all variations of cell suspension concentration. This confirms that cell concentration variations of 3%, 5%, or 10% can be used effectively without compromising the accuracy of ABO blood grouping results.

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