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ORIGINAL ARTICLE

Preparation of a Heat-Elution Solution GSG for A/B/H Weak Antigen Test

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SUMMARY

Background: **Our study aimed to enhance the efficiency and sensitivity of the 56°C heat-elution test, minimize hemolysis in the eluate, and improve detection of A/B/H weak antigens. To achieve this, we developed a novel, simplified heat-elution solution: the glycine/sodium chloride/glycerin mixture (GSG).**
Methods: **We monitored the osmotic pressure, pH changes, and bacterial growth of GSG over a 10-week period, comparing it to fresh solution and 6% calf serum. Additionally, we assessed GSG's antibody concentration, sensitivity, specificity, hemolysis degree, and antibody preservation against 6% calf serum and normal saline. The elution efficacy of GSG was also compared with that of glycine-HCl/EDTA.**
Results: **GSG stored at 4°C for 9 weeks maintained osmotic pressure and pH values comparable to fresh preparations, demonstrating superior stability over 6% calf serum. GSG outperformed 6% calf serum and normal saline in agglutination intensity, antibody titer, sensitivity, and hemolysis degree, without yielding false positives. Agglutination strength and antibody titer remained stable 24 hours post-preparation. The sensitivity of antibodies reached 100% after 48 hours, significantly higher than that of 6% calf serum and normal saline. Moreover, GSG's elution efficacy surpassed the glycine-HCl/EDTA method.**
Conclusions: **The GSG method is superior to 6% calf serum, normal saline and glycine-HCL /EDTA elution techniques in terms of sensitivity and other elution efficiency. This breakthrough significantly improves the detection of ABH weak antigens and sets new standards for blood grouping and transfusion protocols.**
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Supplementary Data

Table S1. Factors and levels.

	A: glycine	B: sodium chloride	C: glycerinum
Level 1 (g)	0.5	0.25	1
Level 2 (g)	0.75	0.5	5.5
Level 3 (g)	1	0.75	10

According to the results of the pre-experiment, orthogonal experiment was carried out, with the concentration of glycine, sodium chloride and glycerinum as the factors to be investigated, each factor was divided into 3 levels, and the orthogonal table L9 (3^3) was selected.

Table S2. The results of L9 (3^3) orthogonal experiment.

Orthogonal experiment scheme and results				
Experimental sequence number	Factors			Results
	A	B	C	
1	1	1	1	7
2	1	2	3	10
3	1	3	2	13
4	2	1	3	37
5	2	2	2	49
6	2	3	1	40
7	3	1	2	20
8	3	2	1	33
9	3	3	3	25
K1	10	21.33	26.67	
K2	42	30.67	27.33	
K3	26	26	24	
R	32	9.34	3.33	
Optimal level	A2	B2	C2	
Order of merit	A > B > C			

Heat-elution solution was prepared by dissolving sterilization water for injection in the corresponding ratio of orthogonal table and constant volume to 100 mL. 56°C heat-elution test was performed on the same sample. The results were observed and the antibody titer integrals in the elution liquid were calculated.

Table S3. ANOVA table.

Factors	Degree of freedom	Mean square	F	p
A	2	768.444	38.210	0.026
B	2	75.111	3.735	0.211
C	2	11.111	.552	0.644

As can be seen from Table 2 and Table 3, the order of factors affecting antibody titer integrals was as follows: A > B > C, and the optimal level was $A_2B_2C_2$, those was, 0.75 g glycine, 0.5 g sodium chloride, and 5.5 g glycerinum dissolved in sterile water for injection and set to 100 mL.