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Determination of specimen storage conditions for measuring isoagglutinin titers

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ABSTRACT

Background: This study aimed to determine the optimal specimen storage conditions for isoagglutinin titration by changing storage time and temperature.

Study design and methods: Serum specimens from 60 individuals were stored at room temperature (RT, 25 °C) or at 4 °C and tested every 24 hours until 96 hours by the immediate spin (IS) method and the anti-human globulin method using dithiothreitol (DTT-AHG). These titer results were compared with the titers which were measured when the samples arrived. The titer endpoint was defined as the highest dilution, with clinically meaningful differences defined as more than 4-fold differences (two serial dilutions) in titer.

Results: Of the specimens stored at RT for 24, 48, 72, and 96 hours, 5%, 12%, 12%, and 12%, respectively, showed two-fold (one dilution) differences by the IS method, and 8%, 8%, 8%, and 10%, respectively, showed two-fold (one dilution) differences with the DTT-AHG method. Of the specimens refrigerated for 24, 48, 72, and 96 hours, 8%, 10%, 13%, and 13%, respectively, showed two-fold (one dilution differences) by the IS method, and 13%, 12%, 12%, and 12%, respectively, showed two-fold (one dilution) differences with the DTT-AHG method.

Conclusions: Specimens stored for up to 96 hours at RT and 4 °C showed similar titers using the IS and DTT-AHG methods. These findings suggest that tests can be scheduled regularly.

1. Introduction

Measuring of ABO antibodies titers has been used to determine treatment strategies and evaluate patient outcomes in ABO-incompatible hematopoietic stem cell and solid organ transplantation, as well as to evaluate ABO-incompatible transfusion responses [1–4]. Most immune reactions associated with transfusion and organ transplantation are caused by IgM ABO antibodies, but IgG also plays an important role [5]. IgM is the predominant immunoglobulin (Ig) class of anti-A and anti-B antibodies (Abs) produced by group B and A individuals, respectively, whereas IgG is the dominant class of anti-A and anti-B Abs in group O serum. Because the immediate spin (IS) method has low sensitivity for the detection of IgG, the anti-human globulin method, involving treatment with dithiothreitol (DTT-AHG), can be used to measure IgG titers [6].

Pre- and post-transplantation treatment protocols that include rituximab and plasma exchange were recently shown to improve outcomes of ABO-incompatible organ transplantation [3,7,8]. The Korean Network for Organ Sharing has estimated that the number of ABO-incompatible organ transplants in South Korea has increased 700% from 2009 (n = 46) to 2013 (n = 368) [9]. These increases in ABO-incompatible allogeneic stem cell and solid organ transplantation have increased the demand for

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isoagglutinin titer tests. The need to measure isoagglutinin titers within 24 hours has increased the workload of clinical laboratories. Although both keeping at room temperature (RT) and refrigerating have been accepted for specimen storage conditions of isoagglutinin titer measuring [10], there was no study to evaluate the optimal storage condition for isoagglutinin titer. This study therefore sought to determine the optimum specimen storage conditions for isoagglutinin titration.

2. Materials and methods

2.1. Study population

A total of 60 individuals who underwent heath examinations at the Center for Cancer Prevention & Detection from December 2013 to July 2014 were enrolled. Subjects with a median age of 50 years (range 19–65 years) were divided into three ABO groups, with 20 individuals (10 men and 10 women) each having blood groups A, B and O. Their sera were used for antibody titration. All study protocols were approved by the National Cancer Center Institutional Review Board (NCCNCS13811).

2.2. Storage conditions

Blood group test and isoagglutinin test were performed the day when samples arrived and the remained samples were divided into two aliquots. One aliquot was stored at RT (25 °C) and the other one was stored in the refrigerator (4 °C) up to 96 hours. Titers of all aliquots were measured in every 24 hours up to 96 hours. Titers of refrigerated aliquots were measured after keeping in RT for 30 minutes.

2.3. ABO antibody titration

2.3.1. Immediate spin (IS) tube method

Titers were obtained using the IS method as described previously [10]. Serum samples were serially diluted two-fold in saline. To each 100 μL sample was added 3% Affirmagen A1, B cell (Ortho-Clinical Diagnostics, USA), followed by incubation at ambient RT (25 °C) and centrifugation at 1000 *g* for 15 s. The titer endpoint was the reciprocal of the highest dilution yielding 1+ or weak agglutination by eye. The titer endpoint was defined as the highest dilution, with clinically meaningful differences defined as >4-fold differences (two serial dilutions) in titer [10].

2.3.2. DTT-AHG tube method

Serum samples were serially diluted into saline solution. To each 50 μL aliquot was added 0.01 M dithiothreitol (DTT). The tubes were incubated at 37 °C for 30 min, followed by the addition of 3% group A or group B red blood cells (RBCs) suspended in saline and incubation at 37 °C for 30 min. Samples were washed three times with normal saline, and 1 drop of polyspecific AHG (Millipore, Livingston, UK) was added to each. The tubes were centrifuged at 1000 *g* for 15 s. The titer endpoint and definition of clinically meaningful differences was the same as that for the IS method.

2.4. Statistical analysis

The distribution of antibody titers according to storage temperature and duration was assessed using Mann–Whitney U-tests. The difference of titers analyzed with IS method and DTT-AHG method according to gender was assessed by Wilcoxon signed rank tests respectively. All statistical analyses were performed using Stata version 12 software (StataCorp LP, Texas, USA), with a P value <0.05 considered statistically significant.

3. Results

3.1. Distribution of antibody titers

The medians (interquartile range) using the IS method were 8 (8–24) in males and 8 (8–24) in females and that using the DTT-AHG method was 4 (2–8) in males and 4 (1–12) in females. Similar titers were observed in men and women using the IS method ($P = 0.539$) and the DTT-AHG method ($P = 0.613$) (Table 1).

3.2. Comparison of antibody titers between the IS and DTT-AHG method

The medians (interquartile range) of anti-B titers in blood group A subjects were 8 (4–16) by the IS method and 1 (0–2) by the DTT-AHG method ($P < 0.001$), whereas the medians of anti-A titers in group B subjects using these two methods were 8 (8–16) and 2 (0–4), respectively ($P < 0.001$). In subjects with blood group O, the anti-B titers by the IS and DTT-AHG methods were 8 (6–24) and 4 (4–20), respectively ($P = 0.031$), while the anti-A titers by these two methods were 24 (8–32) and 16 (8–48), respectively ($P = 0.821$) (Table 2, Fig. 1).

3.3. Assessment of isoagglutinin in specimens stored for up to 96 hours

Antibody titers in samples stored at RT and 4 °C for up to 96 hours were similar at all time points ($P = 0.083$–1.000) (Table 2). A two-fold difference (one serial dilution) in titers during storage was observed in 20 of the 60

Table 1
Isoagglutinin antibody titers by the IS and DTT-AHG methods according to ABO blood type and gender.

ABO group	Antibody	Gender[a]	Antibody titer interquartile range (median)	
			IS	DTT-AHG
Group A	Anti-B	Female	8 (8–16)	0 (0–2)
		Male	6 (4–32)	2 (0–8)
Group B	Anti-A	Female	8 (4–16)	2 (0–4)
		Male	8 (8–16)	2 (0–8)
Group O	Anti-B	Female	16 (8–32)	6 (4–32)
		Male	8 (4–16)	4 (4–8)
	Anti-A	Female	32 (8–32)	16 (8–64)
		Male	16 (8–32)	12 (8–32)

IS: immediate spin method, DTT-AHG: anti-human globulin method using dithiothreitol.
[a] Ten females and ten males in each blood group.

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