Original article

Comparison of ABO antibody titers using the conventional tube technique and the automated column agglutination technique in ABO-incompatible kidney transplant

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Abstract:

Introduction: *The ABO antibody titration test is an essential part of the management and evaluation of patients with ABO-incompatible kidney transplants. High ABO antibody titers can cause hyperacute graft rejection in ABO-incompatible kidney transplantations and cause poor graft outcomes. Nowadays, conventional tube technique (CTT) is a standard method for measuring antibody titer; however, it is labor intensive, difficult to standardize and subjective in interpretation. The automated column agglutination technique (CAT) has been introduced to test antibody titers. Not only produces a stable, reproducible result and eliminates inter-reader variability, but also decreases manual workloads for the fast, accurate, and precise test result for clinicians.* ***Objective:*** *To compare the ABO antibody titers at room temperature and IAT phase between CTT and automated CAT (Ortho Vision Max) by using concordance, correlation, and agreement tests. We plan to apply an antibody titration method using an automated CAT instead of a CTT in routine blood bank laboratory tests.* ***Materials and Methods:*** *Altogether, 120 plasma samples from patients admitted for ABO incompatible kidney transplants consisted of 40 samples for each blood group A, B, and O. Antibody titrations were performed using CTT and automated CAT simultaneously.* ***Results:*** *The total concordance rate of CTT and automated CAT at room temperature and IAT phase was 76.25% and the discordance rate was 23.75%. The high titer and low titer results from CTT almost corresponded to automated CAT. There is no statistical significance in the comparison of mean difference titers between the two tests. Most of the results using automated CAT were higher than the CTT. The correlation between two tests using the Spearman rho correlation coefficient is 0.81(p-value < 0.01). The agreement has variation results from substantial to near-perfect agreement (Cohen's kappa = 0.752-0.870).* ***Conclusion:*** *This study demonstrated that the ABO antibody titers measured by CTT and automated CAT had a high concordance rate, a highly significant correlation, and good agreement, indicating reliable results. Therefore, automated CAT can be implemented to perform antibody titration in routine laboratory tests.*

Keywords : ● *ABO incompatible kidney transplant* ● *ABO antibody titration* ● *Automated column agglutination technique* ● *Conventional tube technique*

J Hematol Transfus Med. 2023;33:103-13.

Received 16 March 2023 Corrected 24 April 2023 Accepted 22 May 2023

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นิพนธ์ต้นฉบับ

การเปรียบเทียบการทำ ABO antibody titers โดยวิธี conventional tube กับวิธี automated column agglutination ในตัวอย่างผู้รับการปลูกถ่ายไตที่หมู่เลือด ABO ไม่เข้ากัน

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บทคัดย่อ

บทนำ *การตรวจ antibody titration มีความสำคัญต่อการประเมินผู้ป่วยที่ปลูกถ่ายไตข้ามหมู่เลือดเพราะค่า antibody titer ที่สูงจะทำให้เกิดภาวะ hyperacute graft rejection ได้หลังการปลูกถ่าย ปัจจุบันการทำ antibody titration สามารถทำได้โดยวิธีหลอดทดลองมาตรฐาน conventional tube test (CTT) ที่ต้องอาศัยความชำนาญ ประสบการณ์และความแม่นยำของผู้ทำ จึงเริ่มมีการนำเครื่องตรวจวิเคราะห์อัตโนมัติที่ใช้หลักการ column agglutination test (CAT) มาใช้ในการหา antibody titer เพื่อลดระยะเวลาในการทดสอบ ได้ค่าที่ถูกต้อง แน่นอนและแม่นยำ* ***วัตถุประสงค์*** *เพื่อศึกษาเปรียบเทียบผล antibody titer ที่ room temperature และ indirect antiglobulin test (IAT) phase ระหว่างวิธีหลอดทดลองมาตรฐาน และ automated column agglutination เพื่อหา concordance, median difference, correlation และ agreement เพื่อเป็นแนวทางในการนำเครื่องตรวจอัตโนมัติด้วยวิธี CAT มาใช้ในงานประจำของธนาคารเลือด* ***วัสดุและวิธีการ*** *ตัวอย่างพลาสมาจำนวน 120 ตัวอย่างจากผู้ป่วยปลูกถ่ายไตข้ามหมู่เลือด ประกอบด้วยหมู่เลือด A, B และ O อย่างละ 40 ตัวอย่าง มาทดสอบหาค่า antibody titer ของ anti-A และ anti-B ทั้งที่ room temperature และ IAT phase ด้วยวิธี CTT และวิธี automated CAT* ***ผลการศึกษา*** *ผล concordance ที่ให้ผลไม่แตกต่าง ในช่วงไม่เกิน 1 titer เท่ากับ 76.25% และ discordance เท่ากับ 23.75% ผลการตรวจ high titer และ low titer antibody จาก CTT และ automated CAT ให้ผลไปด้วยกันเป็นส่วนมาก ผล median difference ส่วนมากไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ และโดยรวมพบว่าการตรวจด้วยวิธี automated CAT จะมีค่า titer ที่เท่ากับหรือสูงกว่า titer จากการตรวจ CTT ผล correlation ได้ค่าสัมประสิทธิ์สหสัมพันธ์ของ antibody titer โดยรวมเท่ากับ 0.81 (p-value < 0.01) มี correlation สูงและผล agreement มีค่าหลากหลายตั้งแต่ต่ำจนถึงสูงมาก แต่ทุกข้อมูลมีความสัมพันธ์กันเชิงบวก* ***สรุป*** *งานวิจัยนี้เป็นการทดสอบเปรียบเทียบการทำ ABO antibody titer ด้วยวิธี CTT กับวิธี automated CAT ในตัวอย่างผู้ป่วยปลูกถ่ายไตที่หมู่เลือดไม่เข้ากัน พบว่าข้อมูลส่วนมากมีความสัมพันธ์สอดคล้องไปด้วยกันจากการตรวจทั้งสองวิธี บ่งบอกถึงความน่าเชื่อถือในการใช้วิธี automated CAT แทนวิธี CTT ในการทำ antibody titration*

คำสำคัญ : ● *การปลูกถ่ายไตข้ามหมู่เลือด* ● *ความแรงของแอนติบอดีหมู่เลือดเอบีโอ* ● *วิธีคอลัมน์ แอกกลูติเนชัน* ● *วิธีหลอดทดลองมาตรฐาน*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2566;33:103-13.

Introduction

Antibodies in the ABO system are the main cause of acute hemolytic transfusion reaction, hemolytic disease of the fetus and the newborn, and hemolysis in incompatible allogeneic hematopoietic stem cell transplantation. Moreover, it can cause hyperacute graft rejection in case of ABO incompatible liver, heart and kidney transplantations[1]. Therefore, it is necessary to perform ABO antibody titration to evaluate prognosis in ABO-incompatible hematopoietic stem cell transplantation or for decision making in ABO-incompatible kidney transplantation (usually ABO antibody titers should be below 16).

Clinically, we can divide the patient from antibody titers into two groups: high titer (baseline titer ≥ 512) and low titer (baseline titer ≤ 256)[2]. In a high titer patient, ABO desensitization should be performed using procedure to decrease risk of hyper acute graft rejection such as double-filtration plasmapheresis (DFPP), immunoadsorption in the preconditioning phase and immunosuppressive drugs such as tacholimus, mycophenolate mofetil and prednisolone[3] have a role in the maintenance regimen to prevent the production of new ABO antibodies. ABO antibody titers could be used for follow up patient after ABO incompatible transplant[2]. Determining accurate and correct antibody titers is essential for treatment, because patients with high antibody titer who undergo ABO-incompatible transplantation may be treated with DFPP, plasma exchange or anti-CD20 monoclonal antibody drug to reduce ABO antibody levels before the transplantation.

King Chulalongkorn Memorial Hospital had performed the ABO-incompatible renal transplant in Thailand since 2008[3]. ABO incompatible renal transplantation shorten the waiting time for a compatible kidney and decrease complications in renal dialysis during the waiting period. Therefore, the patients who receive kidneys from living donors, have better graft outcomes than the deceased donors[4].

In our hospital, we perform ABO antibody titration using the conventional tube technique (CTT), which is a low-cost standard technique. Anti-A and anti-B could be tested both IgM and IgG by using the same test[5]. However, the disadvantage of this technique is that there are many steps to perform and all of the procedures are performed manually, which is time consuming. Many manual steps are the sources of errors, for example dilution of plasma in two fold dilution, washing of red blood cells and reading the test result, which are subjective. All of these factors make it difficult to control the quality of the test. Although CTT remains the standard method, many technologies have been introduced for measuring, including column agglutination technique(CAT), flow cytometry and solid-phase red cell adherence technique[6]. Nowadays, automation technology (ORTHO VISION Analyzer, manufactured by Ortho Clinical Diagnostics, UK) has been implemented in our laboratories to increase the productivity and efficiency of testing using CAT. It is considered objective, reproducible and less time consuming. Thus, the aim of this study is to compare the anti-A and anti-B titers obtained from CTT and automated CAT by concordance, correlation, and agreement tests. This study will be used as a guide for the implementation of automated CAT for routine antibody titration instead of CTT.

Materials and Methods

1. Subjects

This prospective analytical observational study was conducted in King Chulalongkorn Memorial Hospital blood bank laboratory from August 2021 to August 2022. The minimum sample size for method comparison is 40 samples for each blood groups. Samples in EDTA tubes from 28 patients admitted for ABO incompatible renal transplant at King Chulalongkorn Memorial Hospital, Bangkok, Thailand were sent to the blood bank for antibody titration daily and were centrifuged to separate plasma. The samples were sent every day from each patient to monitor antibody titers during preconditioning

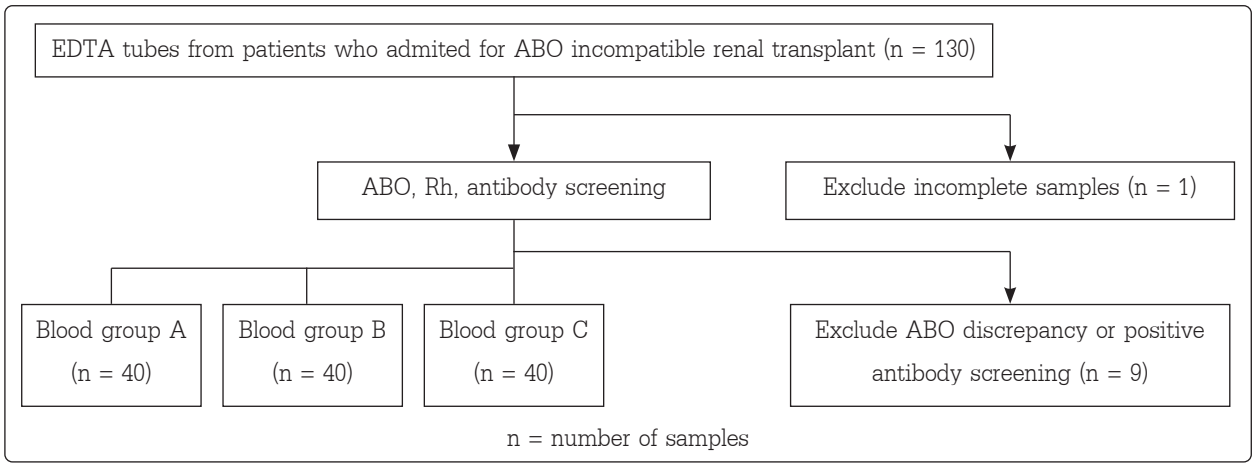

Figure 1 Flow chart of the study design

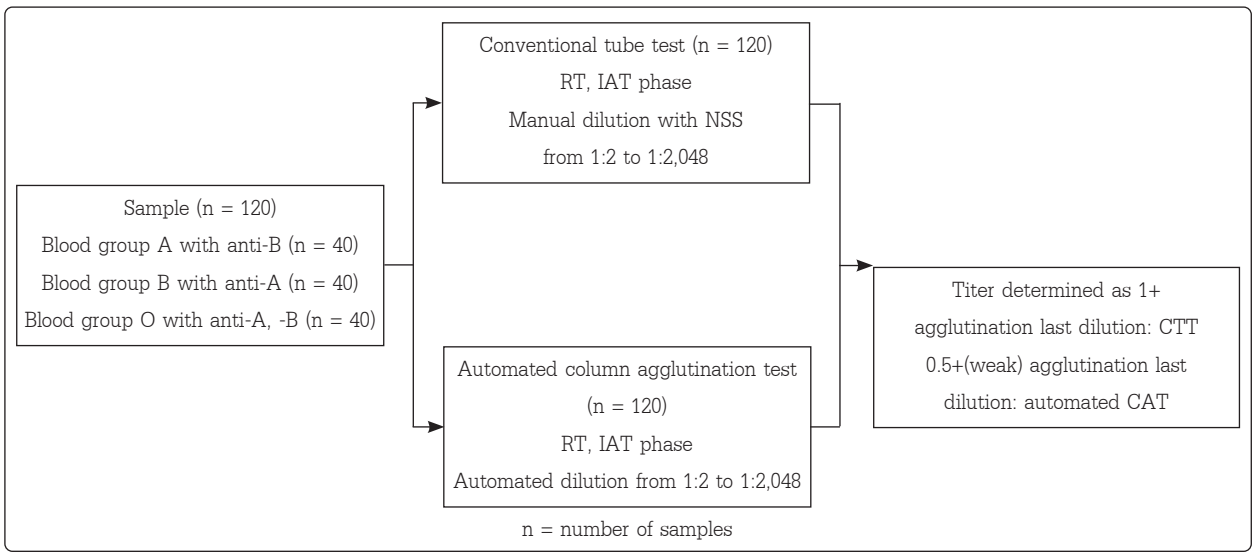

Figure 2 Flowchart of the study method

period. EDTA tubes which were not capped, low plasma level, unable to separate plasma from red blood cells, hemolysis were excluded from this study. First,we tested the samples for ABO, Rh blood group and antibody screening (Reagent and screening cells manufactured by Thai National Blood Centre). The samples which had ABO discrepancy or positive antibody screening were excluded from this study. Second, CTT was performed as a routine method for antibody titration,and then leftover samples were tested by automated CAT immediately. This process is shown in Figure 1.

2. Methods

CTT and automated CAT techniques were performed for antibody titration. The anti-A and anti-B titers were tested by two methods simultaneously. This process is shown in Figure 2.

Conventional tube test (Routine blood bank laboratory method)

Anti-A and anti-B were titrated using CTT according to the method in the AABB technical manual[7]. Normal-ionic-strength saline was used as a diluent to dilute serum. Transfer 100 μL from each serially diluted serum to a set of 1 to 12 tubes for testing at room temperature phase and transferred to another set of 1 to 12 tubes for the testing IAT phase, respectively. Then added 100 μL of 3% A1 cells or B cells according to the anti-A or anti-B in plasma (manufactured by National Blood Centre) to all tubes, mixed all the tubes well, incubated one set at room temperature (22-25°C) for 15 minutes and at the same time, incubated the second set at 37°C in an incubator for 30 minutes. After the end of the incubation time, all tubes in the first set were

centrifuged at 3,400 rpm for 15 seconds, then examined for agglutination. The last dilution tube that gave 1+ agglutination macrospically indicated the titer of the sample. Report titer in antibody titration report form. For the second set, after finishing incubation, washed the cells in each tube 3 times using an automated cell washing machine. Then added 2 drops of antihuman globulin from National Blood Centre (AHG) to each tube and centrifuged at 3,400 rpm for 15 seconds. The agglutination was examined and reported anti-A or anti-B titers in a similar way as mentioned above.

Automated column agglutination test

Automated CAT was performed by ORTHO VISION Analyzer (Ortho Clinical Diagnostics, UK). This equipment automatically performed a serial two-fold dilution plasma and tested the diluted plasma 40 µL with 50 µL 0.8% Affirmagen (A1/B) in each glass beads microcolumns. After selected dilution factor by touching the screen (1:2 to 1:2048), the machine will scan barcodes of samples, reagents and cassettes. For room temperature phase testing, the test was performed on reverse diluent cassette and immediately centrifuged for result reading. For IAT phase testing, the test was performed on anti-IgG,-C3d; polyspecific cassette and incubated at 37°C for 15 minutes, then centrifuged for the result reading. The result will be shown as a figure for the titers in the glass beads microcolumns of both anti-A and anti-B. The last column that gives 0.5+ (weak) is considered as the titer of the sample. Rechecked the results on the screen and confirmed the results. Reporting titers in the antibody titration report form compare with those of the previous CTT.

3. Statistical analysis

We compared the last 1+ titer from CTT and the 0.5+ (weak) titer from automated CAT. Both room temperature and IAT phase antibodies from 2 methods were compared and correlated. Data were presented as numbers (percentage) or medians (range), after checking the normality of the distribution by Skewness & Kurtosis and the Shapiro-Wilk test. Both results were evaluated for concordance, median difference, correlation, and agreement. To evaluate concordance, correlation anti-A and anti-B titers were transformed into base 2 logs.

The median difference between two tests was compared by inference statistic using the Wilcoxon signed rank test and calculated *p-value*. The correlation between CTT and automated CAT was analyzed using Spearman's correlation coefficient (ρ, Rho) with a 95% confidence interval (CI). It was interpreted as follows: < 0.30, negligible; 0.30-0.50, low; 0.50-0.70, moderate; 0.70-0.90, high and 0.90-1.00, very high. The agreement (interrater reliability) between CTT and automated CAT was evaluated by Cohen's kappa with a 95% confidence interval (CI) for testing two double blinded methods as follows: < 0.20, slight; 0.201-0.40, fair; 0.401-0.60, moderate; 0.601-0.80, substantial and 0.801-1.0, almost perfect. Statistical analysis was performed using IBM SPSS Statistic Base Version 22.0 (SPSS Inc., Chicago, IL, USA), and the p-value < 0.05 was considered statistically significant. Each sample was given to two different technicians for different techniques to perform the test to reduce inter-observer bias between two methods.

Results

A total of 120 blood samples were taken from patients admitted for ABO incompatible kidney transplant, 40 of each were A, B, and O blood groups. After checking distribution of data normality and homogeneous variation by Skewness & Kurtosis and the Shapiro-Wilk test, the result was non-normal distribution. In order to statistical evaluated the concordance/discordance and correlation between anti-A, anti-B titre with the base 2 log for statistical calculation.

The concordance rate of the total samples tested for ABO antibodies between two techniques was 76.25% (titers with 0, -1 and +1 titer differences were considered concordance) and the discordance rate was 23.75%. IAT phase antibody titers had a high concordance rate more than room temperature phase antibody titers. Furthermore, anti-B at IAT phase of blood group A and

Table 1 Concordance between CTT and automated CAT. The overall concordance rate between CTT and automated CAT is 76.25%

Blood group	Antibody	Phase (number samples)	Concordance (%)				Discordance (%)		
			CTT = CAT	CTT > CAT	CTT < CAT	Total	CTT > CAT	CTT < CAT	Total
A	Anti-B	RT (40)	8 (20)	9 (22.5)	10 (25)	27 (67.5)	3 (7.5)	10 (25)	13 (32.5)
		IAT (40)	13 (32.5)	9 (22.5)	13 (32.5)	35 (87.5)	2 (5)	3 (7.5)	5 (12.5)
B	Anti-A	RT (40)	7 (17.5)	7 (17.5)	11 (27.5)	25 (62.5)	3 (7.5)	12 (30)	15 (37.5)
		IAT (40)	11 (27.5)	6 (15)	10 (25)	27 (67.5)	1 (2.5)	12 (30)	13 (32.5)
O	Anti-A	RT (40)	12 (30)	7 (17.5)	12 (30)	31 (77.5)	2 (5)	7 (17.5)	9 (22.5)
		IAT (40)	14 (35)	7 (17.5)	14 (35)	35 (87.5)	1 (2.5)	4 (10)	5 (12.5)
O	Anti-B	RT (40)	12 (30)	6 (15)	12 (30)	30 (75)	2 (5)	8 (20)	10 (25)
		IAT (40)	15 (37.5)	9 (22.5)	10 (25)	34 (85)	3 (7.5)	3 (7.5)	6 (15)

CTT = conventional tube test; CAT = column agglutination test; RT = room temperature; IAT = indirect antiglobulin test

Table 2 Median difference of titer between CTT and automated CAT using Wilcoxon signed rank test to calculate the *p-value*

Blood group and antibody (n)	Median (Min-Max) of antibody titer in each method					
	CTT at RT	CAT at RT	*p-value*	CTT at IAT	CAT at IAT	*p-value*
Anti-B in group A (40)	8 (1-512)	16 (2-256)	0.429	16 (1-512)	16 (2-1024)	0.603
Anti-A in group B (40)	8 (2-64)	16 (2-128)	0.042	32 (2-1024)	64 (2-1024)	0.350
Anti-A in group O (40)	8 (1-256)	32 (2-512)	0.751	32 (4-512)	64 (2-1024)	0.290
Anti-B in group O (40)	16 (1-256)	32 (1-512)	0.561	64 (2-1024)	128 (2-1024)	0.727

CTT = conventional tube test; CAT = column agglutination test; RT = room temperature; IAT = indirect antiglobulin test

anti-A at IAT phase from blood group O had the highest concordance rate (87.5%). Anti-A at room temperature phase from blood group B had the lowest concordance rate (62.5%). Most ABO antibody titers from automated CAT had equal and higher titer score than titers of CTT as shown in Table 1.

The median difference between two techniques showed a mostly non significant statistical difference except anti-A at room temperature phase from blood group B showed a statistically significant difference (p-value < 0.05). Furthermore,overall titers from automated CAT had the same or higher titers (1-2 titer) than CTT and the IAT phase antibody had a higher titer score than the room temperature phase antibody as shown in Table 2.

The room temperature phase and IAT phase of anti-A and anti-B titers of total samples were tested by CTT and automated CAT and showed a high correlation in the same direction with a significant difference. Spearman correlation coefficient; rho of room temperature phase anti-A and anti-B were high (Rho = 0.70-0.90), IAT phase of anti-A and anti-B rho were high to very high, the anti-B of blood group A had the highest correlation (Rho = 0.94) and the anti-A of blood group B had the lowest in high correlation (Rho = 0.75) with p-value < 0.01. The results were shown in Table 3 and the scatterplots of the correlation between two techniques, which were in a positive trend, were shown in Figure 3.

The interrater agreement between measurements referred to the degree of concordance between two or more sets of measurements. The general agreement between CTT and automated CAT ranged from moderate to almost perfect when analyzed by Cohen's kappa with a 95% confidence interval (CI). Anti-A IAT phase antibody from blood group O had almost perfect agreement (Cohen's kappa and 95%CI: 0.870). Furthermore, all agreements were still in a positive trend as shown in Table 4.

Table 3 The correlation of ABO titers between CTT and automated CAT calculated by Spearman correlation coefficient, Rho.

Blood group and antibody (n)	Spearman correlation coefficient between 2 technique (*p-value*)	
	CTT-CAT at RT	CTT-CAT at IAT
Anti-B in group A (40)	0.86 (*p-value* < 0.01)	0.94 (*p-value* < 0.01)
Anti-A in group B (40)	0.75 (*p-value* < 0.01)	0.88 (*p-value* < 0.01)
Anti-A in group O (40)	0.85 (*p-value* < 0.01)	0.80 (*p-value* < 0.01)
Anti-B in group O (40)	0.87 (*p-value* < 0.01)	0.89 (*p-value* < 0.01)

CTT = conventional tube test; CAT = column agglutination test; RT = room temperature; IAT = indirect antiglobulin test

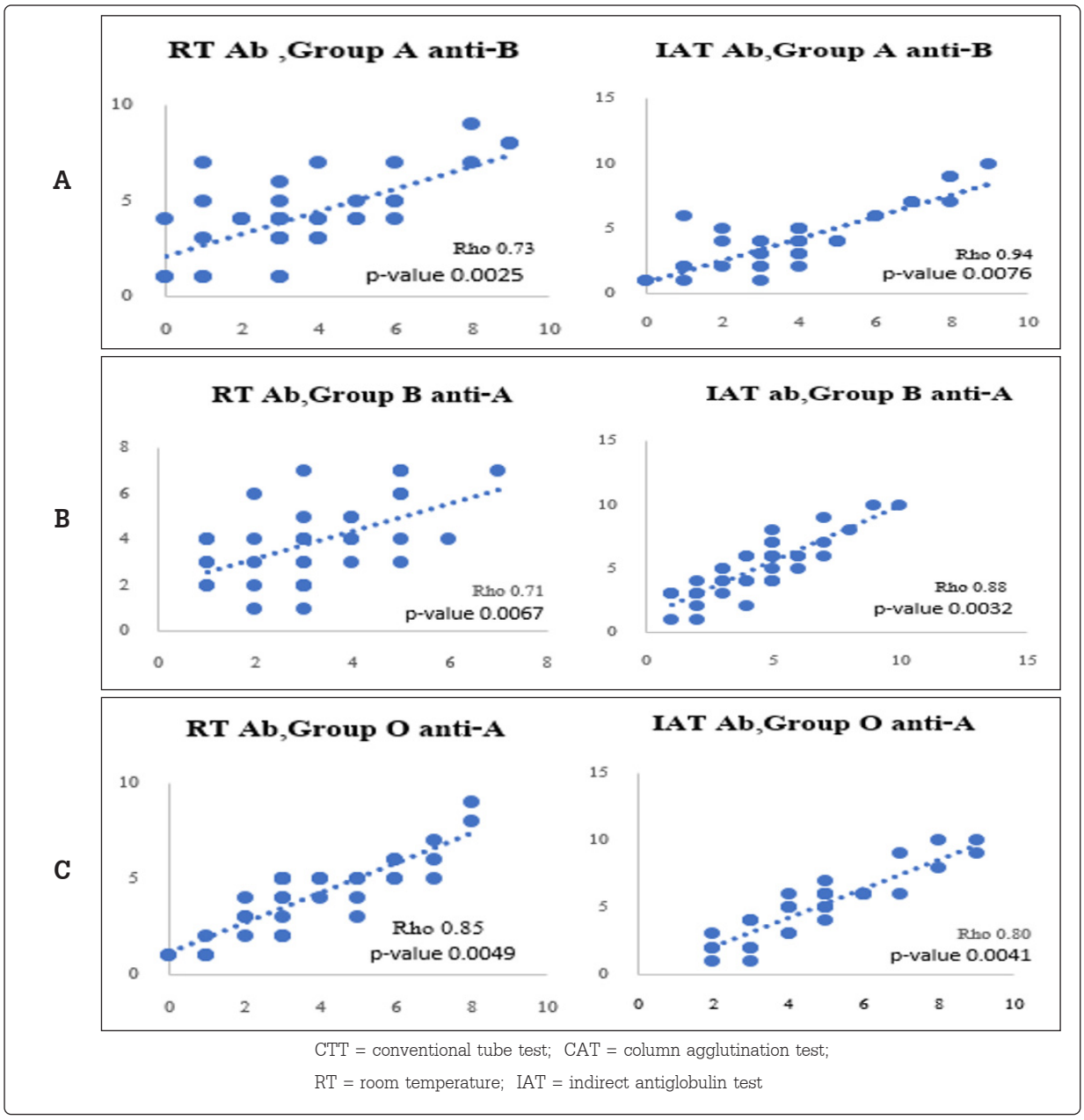

Figure 3 Scatterplots of the correlation between CTT and automated CAT, X axis = antibody titer (log2) of CTT, Y axis = antibody titer (log2) of automated CAT. A = blood group A, B = blood group B, C = blood group O

Table 4 Agreement between CTT and automated CAT calculated by Cohen's kappa and 95% confidence interval (CI)

Blood group and antibody (n)	Cohen's kappa (95%CI)	
	RT	IAT
Anti-B in group A (40)	0.832 (0.794-0.860)	0.824 (0.793-0.865)
Anti-A in group B (40)	0.752 (0.726-0.797)	0.856 (0.827-0.905)
Anti-A in group O (40)	0.773 (0.743-0.810)	0.870 (0.826-0.914)
Anti-B in group O (40)	0.802 (0.766-0.847)	0.797 (0.750-0.833)

CTT = conventional tube test; CAT = column agglutination test; RT = room temperature; IAT = indirect antiglobulin test

Discussion

Antibody titration is a semi-quantitative test to determine the concentration of the antibodies. Estimating the antibody titer appropriately is important in clinical situations for management of ABO-incompatible solid organ and hematopoietic stem cell transplant, access severity of hemolytic disease of the fetus and newborn and in the production of the "low titer" blood component. Therefore, the precise and reliable data of the report is important[8].

There are various methods for ABO antibodies titration, including CTT and CAT. CTT is the oldest and standard method from AABB[7] and the Australian Immunohematology Continuing Education (Australian NICE)[10]. King Chulalongkorn Memorial Hospital Blood Bank still uses this method for antibody titration. According to the AABB, antibody titration by manual CTT may result in a high variation of the test results[7]. As a result, the protocol accept one dilution plus or minus as no difference (concordance). However, CTT has other limitations, it is labor intensive, time consuming (75-90 minutes), has inter-observer variability, and has technical errors. Automation technique has been introduced to blood bank laboratories to increase testing productivity and efficiency since 2013[9]. The advantage of automated CAT is less time consuming, it uses smaller volumes of plasma and RBCs, objective and reproducible. The only disadvantage of automated CAT is the high cost. For automated CAT in this study, ORTHO VISION Analyzer machine was used to compare with CTT. Cassette cards with glass beads and cells from Ortho Clinical Diagnosis were used in this study to match with the machine.

Although there are no standard cut-off titers for the interpretation of antibody titer in automated CAT, several studies have researched this topic. Matsuura[12], et al. used 10 samples from each blood group A, B, O donors to test for antibody titers from the automated CAT technique and compare between two cut-offs: weak or 1+ reaction. The results were not statistically significant between the two cut-offs. Aubuchon[13] et al. compared the antibody titer between 19 laboratories, 35 samples and found that the cut-off titer between 1+ and weak showed results that were not statistically significant. Therefore, this study considered the last weak positive as the end point for the automated CAT technique.

This study compared the anti-A and anti-B titers obtained from CTT and automated CAT by concordance, correlation, and agreement testing. The information from the study will be considered for replacing automated CAT to routine anti-A and anti-B titration testing instead of CTT. The 120 samples comprised of blood groups A 40 samples, blood group B 40 samples and blood group O 40 samples. The concordance rate between the two techniques was 76.25% and the discordance rate was 23.75% for both room temperature phase and IAT phase antibodies. Furthermore, the concordance rate in the IAT phase antibody is higher than room temperature phase in all blood groups. Onpus[9], et al. had studied the comparison antibody titer between two techniques from 180 donor blood samples. The results showed that the concordance rate of the test samples between

automated CAT and CTT was very high. Moreover, the concordance rate of the IAT phase antibody titers in blood group O was as high as 98.3%. Minjeong[14], et al. also found the same result for the high concordance rate between the two tests in blood group O.

Overall the concordance, discordance titers of automated CAT is greater than or equal to CTT. Similar to other studies[7,11,12], the automated CAT technique was more sensitive than CTT. Furthermore, CAT which uses glass beads cassette may show a higher ABO antibody titer than a gel matrix cassette[4]. However, Minjeong[14], et al. found that the antibody titer from automated CAT was lower than CTT in blood group A, and blood group B but greater in blood group O. These are the reasons why there were many variations in results which came from different methods and protocols to perform the automated CAT technique such as incubation time, cassette type, and cut-off titers for interpretation. Therefore, before implementing this method, the laboratory staff of the blood bank should review their standard operating procedures and validate this method before establishing.

The variation of the medians between the two techniques was compared and evaluated by median difference and Wilcoxon signed rank test to compare. Almost all the median room temperature phase and IAT phase antibodies of the two techniques did not show significant differences (Table 2). Except anti-A at room temperature phase from blood group B showed a significant difference (p-*value* < 0.05). This observation was similar to the study by Park[15], et al. Their study revealed that the median IAT phase anti-A and IAT phase anti-B titers in blood groups A and B by CTT and automated CAT showed no significant differences. In contrast to Onpus[9], et al, statistically significant differences were found in almost all blood groups because of the high variation of the medians. However, the median and the concordance in automated CAT titer is greater than or equal to CTT.

The correlation of ABO antibody titers at room temperature phase and IAT phase in this study was shown by the Spearman correlation coefficient; rho was very high as rho = 0.81 (p-*value* < 0.01) (Table 3). Both techniques had a high correlation, which was in agreement with many previous reports[16-18]. However, only good correlation may not be enough to indicate correlation of the agreement between the two tests. Another test of agreement should be applied. Therefore, to study the agreement of antibody titers between the two techniques, we decided to use Cohen's kappa and the 95% confidence interval (CI) for interrater reliability testing. Cohen's kappa and 95%CI showed a wide range of agreement from moderate to almost perfect. In this study, a correlation between the antibody titer results using the CTT and automated CAT was in positive agreement, similar with other published studies[20,21]. However, some studies[18,19] found that the agreement between two tests showed discrepancies due to different protocols. For measurement of antibody titers, there were interlaboratory variations due to the different techniques and the absence of a standard method. As a result of the fact that the standard protocols for CTT and automated CAT have not been established and standardized yet, each blood bank laboratory uses different protocols and materials.

This study has some limitations. First, the ABO IgM antibody could react in the temperature range of 4°C to 37°C, the same as IgG antibody. Therefore, in order to titrate the IgG antibody, IgM antibody in plasma should be destroyed before testing for the IAT phase. Dithiothreitol (DTT) is mostly used for this purpose. It can inactivate the IgM antibody by destroying the disulfide bonds of the IgM antibody, allowing the identified unaffected IgG antibody[22]. Many studies[23-25] reported that the titers of IgG antibodies that did not use DTT treatment were higher than the titers of DTT treated plasma samples. However, the process for DTT treatment is difficult and could destroy some IgG

antibodies, and most blood banks, including us do not use DTT treatment in a routine method and allow the result in the IAT phase as the titer of IgG antibody. Second, many studies[14,16,17] used monospecific anti-IgG for CTT and the anti-IgG cassette for automated CAT to perform IgG antibody titration for avoiding the effect of complement, which can cause higher result than the actual result. However, according to the study by Onpus[9], et al. comparing between anti-IgG cassette and polyspecific cassette, 45% of the IgG antibody gave the same titer and 55% of the IgG antibody gave higher titers from the anti-IgG cassette which was not statistically significant difference. Third, this study used a glass beads column for the ORTHO VISION Analyzer machine, which had different results compared to a gel matrix column. Moreover, some study[6] had suggested for comparison automated CAT with flow cytometry, which is a gold-standard test for titer estimation nowadays.

This study showed that the titration of ABO antibodies by automated CAT and CTT gave high correlation and good agreement. Automated CAT has the advantage of reducing the interlaboratory variations and decreasing the turn-around time. However, due to the requirement of expensive reagents for each automated machine and financial limitations, the use of automated CAT in routine testing for the ABO antibody titer is limited in Thailand. Furthermore, an evaluation of clinical setting, such as a comparison between titration methods and patient outcomes, complications, and acute graft rejection resulting from the automated CAT technique, is necessary before implementing this new method. Therefore, the information could be applied effectively for the therapeutic use of the patient.

To reduce the variation of titration results between different methods, an antibody titration external quality assurance (EQA) program is instantaneously needed for most of the laboratories. The establishment of a standard method and participation in an EQA program could reduce variation and allow transferrable results between laboratories. Furthermore, there is a new innovation in using kodecytes[26] instead of natural red cells for ABO titration. Kodecytes have a stable level of ABO antigen. This feature allows the potential to be a global standardized quality control of cells in ABO antibody titer testing resulting in more accurate clinical interpretation of ABO titer results.

Conclusion

This study showed the comparison between CTT and automated CAT for ABO antibody titration from plasma samples obtained from ABO incompatible kidney transplant patients using concordance, correlation, and agreement tests. Automated CAT showed a high concordance rate, a high correlation, and good agreement. The median difference had no statistical significance, which reflected the reliability of automated CAT. Due to the advantages of automated CAT such as high throughput, less time performing, objective and reproducibility, this technique will allow clinicians to provide good care to patients successfully. Therefore, automated CAT can be implemented to perform antibody titration in our routine laboratory tests.

Acknowledgements

The authors sincerely thank Miss Sunisa Onpus and Associate Professor Phandee Watanaboonyongcharoen for their advice in the preparation of the manuscript. This study was approved by Chulalongkorn University Faculty of Medicine Ethics Committee IRB No. 280/64 and was funded by Ratchadapisek Research Funds 2021.

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