ความชุกและความจำเพาะของแอนติบอดีต่อเม็ดเลือดแดงจากการตรวจกรองแอนติบอดีซ้ำในผู้บริจาคเลือดคนไทย

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บทคัดย่อ

การตรวจกรองแอนติบอดีในพลาสมาผู้บริจาคเลือดมีความสำคัญในการป้องกันการเกิดปฏิกิริยาไม่พึงประสงค์จากการรับเลือดจากการแตกของเม็ดเลือดแดงในผู้ป่วย สำหรับการตรวจกรองแอนติบอดีจำนวนมากนิยมใช้เครื่องอัตโนมัติ ซึ่งส่งผลต่อจำนวนเลือดผู้บริจาคที่ให้ผลบวกต่อการตรวจกรองแอนติบอดีเพิ่มขึ้นเมื่อเทียบกับวิธีมาตรฐานทำให้เลือดสำรองในคลังเลือดไม่เพียงพอ ผู้วิจัยมีวัตถุประสงค์ที่จะตรวจกรองแอนติบอดีซ้ำ เพื่อสร้างแนวทางการตรวจกรองแอนติบอดีในผู้บริจาคเลือดคนไทย จากพลาสมาผู้บริจาคทั้งหมด 4,834 รายที่ตรวจกรองแอนติบอดีด้วยเครื่องอัตโนมัติมีจำนวน 136 รายให้ผลบวกจึงนำมาตรวจซ้ำด้วยวิธีหลอดทดลอง และ column agglutination test และตรวจแยกชนิดแอนติบอดี โดยวิเคราะห์ผลแบ่งตามเพศ อายุ และหมู่เลือด ผลการศึกษาพบว่า พลาสมาที่ให้ผลบวกต่อการตรวจกรองแอนติบอดีซ้ำจำนวน 81 ราย (1.68%) ผู้บริจาคเพศหญิงมีผลบวกต่อการตรวจกรองแอนติบอดีมากกว่าเพศชายอย่างมีนัยสำคัญ (p = 0.041) แต่ไม่พบความแตกต่างในแต่ละช่วงอายุ และ ผู้บริจาคหมู่ B พบแอนติบอดีได้มากกว่าหมู่อื่นอย่างมีนัยสำคัญ (p = 0.005) ส่วนพลาสมาที่ให้ผลบวกต่อการตรวจกรองแอนติบอดีซ้ำพบเป็น single antibody จำนวน 61 ราย (75.32%), multiple antibodies จำนวน 12 ราย (14.80%) และไม่สามารถระบุชนิดจำนวน 8 ราย (9.88%) แอนติบอดีต่อระบบหมู่เลือดที่พบบ่อยคือ Lewis และ MNS นอกจากนี้ anti-Mi^a ที่ตรวจพบในพลาสมา 2 ราย สามารถนำไปใช้เป็นแอนติซีรัมได้ โดยสรุปความสำคัญของการตรวจกรองแอนติบอดีซ้ำสามารถใช้เป็นแนวทางในกลุ่มผู้บริจาคเลือดคนไทย การประยุกต์ใช้แนวทางดังกล่าวเป็นประโยชน์ในการลดจำนวนการทิ้งเลือดโดยไม่จำเป็น อีกทั้งอาจนำพลาสมาผู้บริจาคที่ทราบชนิดแอนติบอดีและมีคุณสมบัติเหมาะสมไปเตรียมเป็น in-house antisera เพื่อใช้ในห้องปฏิบัติการได้ อีกทั้งสามารถประยุกต์ใช้กับห้องปฏิบัติการงานธนาคารเลือดอื่นที่มีปัญหาเช่นเดียวกัน

คำสำคัญ: อัลโลแอนติบอดี การตรวจกรองแอนติบอดี การตรวจแยกชนิดแอนติบอดี คนไทย

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Prevalence and Specificity of Red Cell Alloantibodies in Repeated Antibody Screening among Thai Blood Donors

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Abstract

Antibody screening test in donor plasma is important to prevent severe transfusion reactions due to immune mediated hemolysis in the recipients. An automated analyzer is widely used for mass screening; however, an increased numbers of units with positive antibody screening compared with conventional techniques may result in blood supply fluctuations. We repeated antibody screening test to establish evidence-based guidelines for antibody testing among Thai blood donors. Altogether, 136 out of 4,834 donor plasma samples with positive antibody screening test were repeat-tested using the conventional tube test and column agglutination test. Only positive samples were determined for antibody specificity and analyzed according to sex, age groups and ABO types. Unaltered positive antibody screening results were 81 (1.68%) samples. The frequencies of positive alloantibodies were significantly higher among female than male donors ($p = 0.041$) and no significant differences were found in different age groups. Positive donors were significantly higher in B blood group than other blood groups ($p = 0.005$). Antibody identification results belonged to a single antibody, 61 samples (75.32%); multiple antibodies, 12 samples (14.80%) and unidentified antibodies, 8 samples (9.88%). Antibodies in the Lewis system were the most common, followed by those of the MNS system. Potent anti-Mi^a of 2 donor plasma could be used as standard human antisera. In conclusion, the impacts of repeated antibody screening established evidence-based guidelines for antibody testing among Thai blood donors. This application was useful for not only reducing unnecessarily removed blood products but also expanding the in-house antisera. A similar strategy can be implemented in laboratories with related problems.

Keywords: Alloantibody, Antibody screening, Antibody identification, Thais

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Introduction

According to the standard guidelines of the American Association of Blood Banks, ABO and Rh(D) typing, antibody screening and infectious disease marker testing must be performed on donor units.[1] In general, two types of red cell antibodies including alloantibodies and autoantibodies can be found among blood donors. The alloantibodies consisting of naturally occurring and immune alloantibodies may be able to hemolyze the patient's red cells that possessed the corresponding antigens.[2] Alloantibodies have been detected among healthy donor populations ranging from 0.1 to 0.9%,[3-5] while, a high prevalence can occur among individuals with a history of transfusion or pregnancy. Weak cold-reactive autoanti-I are found in 0.05 to 0.1% among healthy individuals with no clinical sign of hemolysis.[1, 6, 7] In Thailand, the prevalence of alloantibodies in blood donors were high from 1.0-4.33% depending on different techniques used.[8, 9]

In Thailand, the National Blood Centre, Thai Red Cross Society (NBC-TRC) is responsible for testing donated allogeneic blood, especially in blood bank laboratories with less potential for testing. The data are sent directly to each hospital blood bank. The donor units with either positive infectious disease markers or antibody screening test will be discarded. For the positive antibody screening test, antibody specificity that has not been determined will result in an ambiguous use of red cell units for transfusions. Regarding the annual report of Thammasat University Blood Bank from October 2014 to September 2015, a total of 23,684 donor samples were sent to the NBC-TRC. Ten samples (0.04%) were positive for both antibody screening and infectious markers, 831 and 281 samples (3.51% and 1.19%) were positive for antibody screening and infectious markers, respectively. These affected the balance between blood supply and use in the hospital. Related studies revealed that false positive results of antibody screening by automated column agglutination technology (CAT) were higher than conventional tube technique (CTT).[10, 11] Therefore, repeated red cell antibody screening test and antibody identification may be helpful regarding blood supply fluctuations. We aimed to repeat the antibody screening test to establish evidence-based guidelines for antibody testing among Thai blood donors.

Materials and Methods

Samples

Blood samples was collected from 4,834 unrelated healthy donors at the Blood Bank, Thammasat University Hospital, Pathumthani, Thailand between January 2017 and June 2017, with the approval of the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumthani, Thailand. They comprised 2,275 males (age range from 17 to 61 years) and 2,559 females (age range

from 17 to 63 years). All donors had been screened stringently concerning donated allogeneic blood testing at the NBC-TRC, Bangkok, Thailand and any positive infectious disease marker result was permanently excluded from this study. Data on sex, age, and ABO blood group were also collected.

Antibody screening test

All samples underwent antibody screening test by the CAT using LISS Coombs and neutral gels on a fully automated ORTHO VISION MAX Analyser (Ortho Clinical Diagnostics, NJ, USA) at the NBC-TRC, Bangkok, Thailand. The in-house screening cells (O_1 and O_2, NBC-TRC, Bangkok, Thailand) were used, including D, C, E, c, e, M, N, S, s, Mi^a, P1, Le^a, Le^b, Jk^a, Jk^b, Fy^a, Fy^b, K, k, Di^a, Di^b and Xg^a antigens. Only donor samples with positive antibody screening results obtained from NBC-TRC received repeated antibody screening using the CTT and CAT.

Antibody screening test by CTT was initially performed using the saline indirect antiglobulin test (IAT).[1] Briefly, two drops of each plasma sample was mixed with one drop of the in-house screening cells (O_1 and O_2, NBC-TRC, Bangkok, Thailand), mixed, centrifuged and observed at immediate spin phase for agglutination and/or hemolysis. All reactions were read macroscopically. The results were graded and recorded. Then the tubes were incubated at 37°C for 30 min, centrifuged and observed for agglutination. Next, the red cells were washed three times with normal saline and completely decanted in the final wash. Two drops of antihuman globulin reagent (CE-Immunodiagnostika GmbH, Germany) were added, mixed, centrifuged and observed for agglutination. The results are graded and recorded. All reactions were read macroscopically. Negative or weak agglutination reactions were examined under a microscope (×10). The grading of agglutination reactions were 4+, 3+, 2+, 1+, w+ and negative, according to standard guidelines and the validity of a negative test was confirmed by adding IgG-coated RBCs.[1]

Antibody screening test by CAT was performed using O_1 and O_2 cells (NBC-TRC, Bangkok, Thailand) and suspended modified LISS solution (Commercial A&B, Santiago, Chile). Fifty microliters of 0.8% screening cell suspension was added to the appropriate microtube of Bio-Type LISS Coombs card (Commercial A&B, Santiago, Chile). Then 25 microliters of donor plasma was also added to each microtube and incubated at 37°C for 15 min. The card was centrifuged for 5 min in the centrifuge (Commercial A&B, Santiago, Chile), and the results were read and recorded. A positive result was indicated when the agglutinated cells formed a red line on the surface of the gel or agglutinates were dispensed in the gel. On the other hand, a negative result was expressed by a compact cell button on the microtube bottom indicating negative antibody

screening test, according to manufacturer instructions.

Antibody identification

In the case of a positive antibody screening test result, antibody identification was performed using 11 in-house panel cells (NBC-TRC, Bangkok, Thailand) together with auto control. The antibody specificities were identified using CTT and CAT according to the above mentioned procedures. Additionally, other extra panel cells from commercial (ID-DiaPanel and ID-DiaPanel-P, Bio-Rad Laboratories, Cressier sur Morat, Switzerland) were used when the antibody specificity was inconclusive. The presence of identified alloantibodies was confirmed by antigen typing to determine all antigen negative status on corresponding donor's red cells.

Statistical analysis

Descriptive analysis of antibody screening results obtained from repeated testing by CTT and CAT were performed according to sex, age groups and ABO types. The Chi-square test and Fisher's exact test were used to compare categorical variables. The analysis was performed using SPSS 16.0 Software (SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered statistically significant.

Results

A total of 4,834 donor samples were screened for the presence of unexpected antibodies at the NBC-TRC; 136 (2.81%) samples were reported as positive for antibody screening. Among 136 donor samples, the antibody screening test was repeatedly performed by CTT and CAT. Unaltered positive antibody screening results were found in 81 (1.68%) donor samples; whereas, the remaining 55 samples showed negative results in both CTT and CAT. Antibody screening results were categorized and compared according to different sex and age groups (Table 1). The frequencies of positive alloantibodies were significantly higher among female than among male donors (OR 1.606; 95% CI 1.016-2.539, $p = 0.041$) and their frequencies in different age groups showed no significant difference. Additionally, the distribution of ABO blood types and the frequencies of positive alloantibodies were evaluated. Among 4,834 donors, the most common was group O (38.46%), followed by group B (33.59%), group A (19.44%) and group AB (8.50%). The positive donors were significantly higher in group B than in other blood groups (OR 0.539; 95% CI 0.347-0.837, $p = 0.005$), as shown in Table 2.

Of those 81 donors, 48 donor samples showed positive antibody screening test results regarding both CTT and CAT. The remaining

Table 1 Distribution of antibody screening test results of 4,834 donors according to sex and age groups

Antibody screening	Sex	Numbers of donors in different age groups (%)				Total
		< 30	30 – 39	40 – 49	⩾ 50	
Positive	Male	17 (0.35%)	6 (0.13%)	5 (0.10%)	1 (0.02%)	29 (0.60%)
	Female*	21 (0.43%)	12 (0.25%)	17 (0.36%)	2 (0.04%)	52 (1.08%)
Negative	Male	882 (18.25%)	659 (13.63%)	499 (10.32%)	206 (4.26%)	2,246 (46.46%)
	Female	1,159 (23.98%)	670 (13.86%)	498 (10.30%)	180 (3.72%)	2,507 (51.86%)
Total		2,079 (43.01%)	1,347 (27.87%)	1,019 (21.08%)	389 (8.04%)	4,834 (100.0%)

*OR 1.606; 95% CI 1.016-2.539, p = 0.041

Table 2 Distribution of antibody screening test results of 4,834 donors according to ABO types

ABO type	Numbers of donors	Numbers of antibody screening test results (%)	
		Positive	Negative
A	940 (19.44%)	19 (0.39%)	921 (19.05%)
B*	1,624 (33.60%)	39 (0.81%)	1,585 (32.79%)
O	1,859 (38.46%)	15 (0.31%)	1,844 (38.15%)
AB	411 (8.50%)	8 (0.17%)	403 (8.33%)
Total	4,834 (100.00%)	81 (1.68%)	4,753 (98.32%)

*OR 0.539; 95% CI 0.347-0.837, p = 0.005

samples showed positive results, either CTT (N=26) or CAT (N=7). Among 81 donor samples belonged to a single antibody for 61 samples (75.32%), multiple antibodies for 12 samples (14.80%) and unidentified antibodies for 8 samples (9.88%). Red cell antibody frequencies and specificities detected in 81 samples are shown in Table 3. For single antibody, anti-Le^a was the most common (21.00%), followed by anti-I (16.05%) and anti-Mi^a (11.11%). Of these samples with anti-Mi^a, 2 samples of groups O and B donors showed strong agglutination reactions (3+ to 4+) at immediate spin and IAT by CTT and CAT. In addition, IgG anti-E and IgM anti-S were found female and male donor samples. Regarding multiple antibodies, all were anti-Le^a combined with anti-Le^b and other antibodies. Interestingly, anti-Le^a + -Le^b + -E was found in a female donor sample.

Discussion

Different technologies for antibody detection have been implemented in blood bank laboratories. Although CTT is a standard method, it is unsuitable for mass screening among blood donors. Currently, automated technologies such as CAT, solid phase red cell adherence assay and erythrocyte-magnetized techniques have been used to reduce human errors and to improve the quality of testing and the reproducibility of results.[12-15] In Thailand, the majority of donor samples are tested at the NBC-TRC to ensure standardized approach and cost-effectiveness. Generally, unexpected alloantibodies have been reported in up to 0.9% of healthy blood donors.[3-5] The prevalence of alloantibodies among Thai donors varied from 1.0% to 4.3% depending on different technique used, screening cells and populations.[8, 9, 16] In this study, the prevalence of alloantibodies was reduced from 2.81% to 1.68% after repeated antibody screening testing using NBC-TRC screening cells by manual CTT and CAT. The numbers of transfusable blood products were increased in the in-stock inventory. Even though, CAT is more sensitive than CTT, the high incidence of false positive antibody screening test using automated CAT was due to the increased detection rate of benign cold-reacting antibodies.[10]

Notably, the presence of alloantibodies among Thai blood donors was statistically higher among females than among males but no significant difference was found among different age groups, similar to related reports among Delhi blood donors.[3, 17] The frequencies of ABO blood groups were determined. Group O was the most common, followed by groups B, A and AB, comparable with other studies among Thai and Southeast Asian populations.[18-20] Significant associations of positive alloantibodies among Thai blood donors increased more in group B than in other blood groups; while, no correlation was observed among patients of other populations between alloimmunization and different ABO blood types.[21, 22]

Table 3 Red cell antibody frequencies and specificities detected in 81 donor samples

Antibody specificity	Number	%
Single antibody	61	75.32
Anti-Le^a	17	21.00
Anti-I	13	16.05
Anti-Mi^a	9*	11.11
Anti-Le^b	8	9.88
Anti-M	8	9.88
Anti-P1	4	4.94
Anti-E	1	1.23
Anti-S	1	1.23
Multiple antibodies	12	14.80
Anti-Le^a + -Le^b	9	11.11
Anti-Le^a + -N	1	1.23
Anti-Le^a + -P1	1	1.23
Anti-Le^a + -Le^b + -E	1	1.23
Unidentified	8	9.88
Total	81	100.00

*Two samples showed strong agglutination reactions (3+ to 4+)

Among healthy donors, naturally occurring alloantibodies are frequently encountered including antibodies in the Lewis, MNS and P1PK systems. Antibodies against the MNS7 (formerly Mi^a) antigen were found in 11.11%, which may be due to the relatively high prevalence of the Mi^a antigen, about 9 to 10% in Thai and Southeast Asian populations.[18, 23] Therefore, screening cells should include certain antigens such as Mi^a and Di^a that are predominantly found among Asians. For patients who developed anti-Mi^a, screening of antigen-negative units with standard anti-Mi^a was required. To date, anti-Mi^a has been commercially marketed, but it is expensive. Potent anti-Mi^a derived from donor's plasma can be used as standard antisera but ABO compatibility remains a concern. Occasionally, IgG alloantibodies found among healthy donors, who were either previously transfused or pregnant could cause immune hemolysis in the recipients. Two female donors with a history of pregnancy had anti-E because of the common phenotype of D+ C+ E— c— e+ in Thai populations.[18] Other significant alloantibodies such as anti-D, anti-C and anti-K were found in other donor populations.[3, 24] Although the frequency of cold anti-I were found in 13 out of 4,834 donor samples (0.27%), which was higher than previously reported.[6, 7] This may be due to the antibody detection at room temperature phase; however, the agglutination strength was less than 2+ resulting in the increasingly usable red cell units. In addition, IgM anti-S was found in a male blood donor with an inconclusive history of blood transfusion. This antibody is infrequently found among S—s+ individuals as both IgM and IgG forms, which are caused mild to moderate adverse reactions. In this case, only red cell unit can be added to the in-stock inventory.[25]

Regarding evidence-based repeated antibody screening testing, using only positive results by automated analyzer, to create an algorithm of management guidelines to achieve maximum blood use has been suggested (Fig. 1). The antibody screening test is performed by immediate spin CTT and IAT-CAT for IgM and IgG antibody detections, respectively. Only blood products with negative results by both tests will be returned to the in-stock inventory. For positive results from either or both tests, antibody identification is optionally suggested to be performed. The potent specific antibodies in plasma can be used for in-house antiserum. In addition, red cell units will be added to the in-stock inventory and issued as antigen-negative units.

Conclusion

Repeated antibody screening among Thai blood donors could reduce false positive results using automated CAT by 40%. This application was useful to not only reduce unnecessarily removed blood products but also to expand in-house antisera. A similar strategy could be implemented in laboratories with related problems.

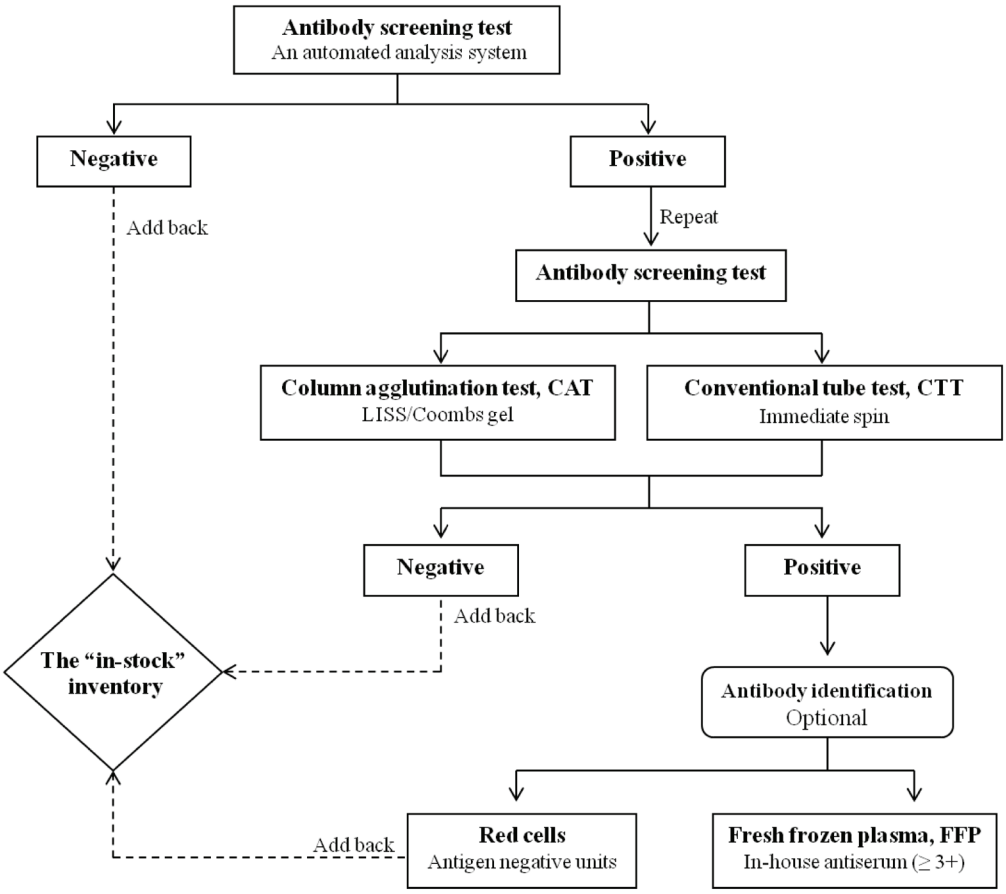

Fig. 1 An algorithm of evidence-based guidelines to manage blood donors using the positive antibody screening test to achieve maximum blood use

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